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

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***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 grown as embryogenic compact callus within 4-8 weeks of culture. The optimum 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 ($AgNO_3$) 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 is 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, limited offshoot production, high degree heterozygosity, dioceous nature [5], inferior quality of seedlings, difficult rooting of offshoots under field condition [6], poor seed germination [7], and time-consuming germination process due to dormancy [8]. In agriculture, these issues can be overcome through plant tissue culture as it offers the included benefits of rapid mass propagation of genetically identical plants production, making clones of slow growing species which are very difficult to propagate by 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 merismetic 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 areng palm, black fiber palm, aren and toddy palm. Sugar

Manuscript received Dec. 29, 2015. This work was supported in part by research grant 600-RMI/DANA5/3/REI (1/2013) and Mybrain-15 Scholarship, Ministry of Higher Education, Malaysia.

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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 term 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 1.0 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 121^0C. Cultures were then incubated under complete darkness at the temperature of 25 ± 2^oC. 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 $AgNO_3$ (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 I: THE ESTABLISHMENT OF EMBRYOGENIC CALLUS ON BASAL STEM EXPLANT OF SUGAR PALM AFTER 8 WEEKS OF CULTURE

Treatment	Composition (mg/L)		Callus Induction Frequency (%)	Callus fresh weight (g ± SE)	Callus diameter (cm ± SE)
	2,4-D	KIN			
A1	0.1	0.5	60	0.200 ±0.08	0.500±0.12
A2	0.2	0.5	70	0.450±0.06	0.850±0.17
A3	0.3	0.5	50	0.075±0.01	0.500±0.12
A4	0.4	0.5	Nil	Nil	Nil
A5	0.5	0.5	Nil	Nil	Nil
A6	0.1	1.0	70	0.300±0.00	0.400±0.00
A7	0.2	1.0	Nil	Nil	Nil
A8	0.3	1.0	Nil	Nil	Nil
A9	0.4	1.0	70	0.075±0.03	0.225±0.09
A10	0.5	1.0	Nil	Nil	Nil

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.

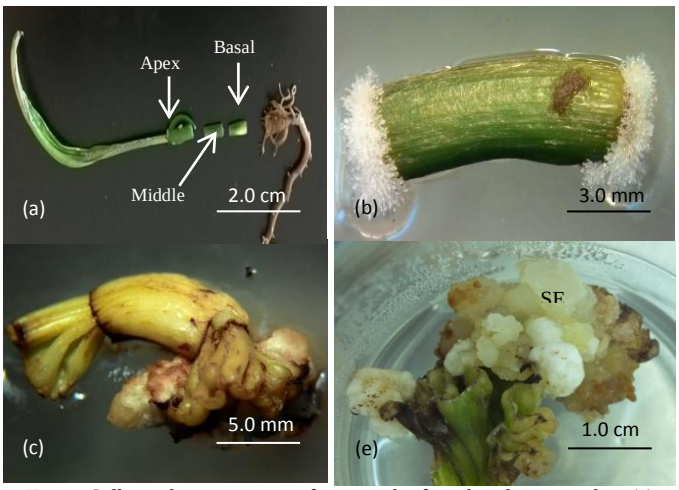

Fig. 1. Callus induction system of sugar palm from basal stem explant (a) separated single basal stem explant from 10 month old *in vitro* seedling [b] induction of friable white floss callus after 4 weeks of culture (c) compact primary callus induction after 8 weeks of culture in optimum culture medium consisted of 0.3 mg/L 2, 4-D + 0.5 mg/L Kin (d) embryogenic callus on proliferation media after 3 months of culture.

V. Conclusion

To summarize, embryogenic callus has been achieved in sugar palm, firstly reported from 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 to Kin (0.5 mg/L). Further research protocol is needed to investigate somatic embryogenesis and plant regeneration of sugar palm.

Acknowledgement

The authors gratefully acknowledge the support of this research by research grant 600-RMI/DANA5/3/REI (1/2013) and Frgs/1/2013/STWN03/UITM/02/3, Universiti Teknologi MARA (UiTM) Shah Alam, Malaysia and Mybrain15 scholarship, Ministry of Higher Education, Malaysia.

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