Sugar palm (Arenga pinnata Wurmb Merr.): a review on plant tissue culture techniques for effective breeding

NA Muda¹ and A Awal²

¹ Senior Lecturer, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA, 26400, Bandar Tun Abdul Razak, Jengka, Pahang, Malaysia
²Research and Innovation Division, Aras 3 Bangunan FF1, Universiti Teknologi MARA Selangor, Kampus Puncak Alam, 42300 Bandar Puncak Alam, Selangor, Malaysia
²Agricultural Biotechnology Research Interest Group, Faculty of Plantation and Agrotechnology, Universiti Teknologi MARA Melaka, Kampus Jasin, 77300 Merlimau, Melaka, Malaysia.

E-mail: asmah138@uitm.edu.my

Abstract. This review paper outlines the plant tissue culture works done on sugar palm (Arenga pinnata Wurmb Merr.); a tree under the family Arecaceae and genus Arenga cultivated mainly for its sugary sap, sweetened endosperm and highly valuable industrial black fibers. Plant tissue culture technique is a method of growing plant cells, tissues and organs on an artificial nutrient medium under controlled aseptic conditions. Plant tissue culture is a revolutionary biotechnological tool which facilitates successful breeding programs and research of many incredible plant diversity particularly of species which are facing the risks of extinction, plants of valuable economic importance and plants with morphological and physiological sterility and incompatibility. The taxonomy, botanical description, population distribution, ecology and climate requirement, horticulture practice as well as the economic contributions and challenges of sugar palm were also described.

1. Introduction
This paper reviewed the botanical aspects, economical contributions and challenges of sugar palm; a stout, solitary palm scientifically known as Arenga pinnata Wurmb Merr. which are widely planted throughout South East Asia countries including Malaysia for its economically valuable products. Plant tissue culture works done in the effort to overcome the challenges of conventional propagation methods and to facilitate effective breeding programs of the palm species through biotechnological intervention were also reviewed.

1.1. Taxonomic classification of sugar palm
Sugar palm belongs to the palm (Arecales) family in the major group of Angiosperm and genus Arenga. Genus Arenga consists of 24 Asiatic palm species which includes sugar palm. The taxonomic classification of sugar palm is summarized as in Table 1 [1]. Depending on different regions of distribution, sugar palm trees are known with different vernacular names such as kabung, aren palm, palm, black-fiber sugar palm, sagwire-palmae, and toddy palm [2].
Table 1. Taxonomy classification of sugar palm.

| Classification  | Description                        |
|-----------------|------------------------------------|
| Domain          | Eukaryota                          |
| Kingdom         | Plantae                            |
| Phylum          | Spermatophyta                      |
| Subphylum       | Angiospermae                       |
| Class           | Monocotyledonae                    |
| Order           | Arecales                           |
| Family          | Arecaceae                          |
| Genus           | Arenga                             |
| Species         | *Arenga pinnata* Wurmb Merr.       |

1.2. Botanical features of sugar palm

Sugar palm is a tall, solitary, stout palm with the average height of 8.0-15.0 m and a trunk diameter of 0.4-0.5 m. The sugar palm trees can be identified with the display of tough, black fibers surrounding the trunk covered by old leaf bases. The leaves grow in ascending manner, pinnate and can reach up to 9.1 m long, 3.1 m wide with an estimate of more than 100 pairs of whitish waxy coating (beneath) and dark-green (above) leaflets. A singular leaflet can measure up to 1.0-1.5 m long, 6.0 - 8.0 cm wide, lobed tip and auricled base. The petiole (1.5-2.0 m long) is very stout while the base is covered with black fibers and weak spines [3]. The presence of two short and synchronized leaves at the top of the sugar palm’s canopy indicate the palm’s maturity. The sugar palm’s first flowering was reported to appear as early as 5 to 6 years of planting but most generally started at the age of 10-12 years. Meanwhile, an untapped sugar palm tree has an average flowering period of 4-6 years. Initial inflorescences can be found to arise from a node near the upper meristem while the following subsequent inflorescences appear from the lower nodes in a descending order. A single node can only produce a single inflorescence. In general, female inflorescence are identified to be produced at the earliest 6-8 nodes at the upper part of the stem, while the male inflorescences are the rest of the lower nodes [4].

Sugar palm bears flowers and fruits year-round. The fruits of sugar palm are ovoid, smooth and sized of 5.0-6.0 cm in diameter. Immature fruits are green, turning yellow and gradually turning black after falling. Each fruit contains two to three grey-brown seeds with a length of 2.5-3.5 cm and 2.0-2.5 cm wide with lush endosperm [5]. The outer husk and the whitish fleshy mesocarp of the fruit contain stinging crystals of calcium oxalate (raphides) which cause intense burning sensation as it touches the skin. This part of the fruit is also poisonous [6]. The tree dies after a lifetime of 12-20 year, depending on site, climate and genotype [4].

1.3. Geographical distribution

Sugar palm trees are widespread throughout the tropical regions of Asia as a planted or semi-domesticated tree [7]. Sugar palm trees are thought to have been originated from countries including Malaysia, Indonesia, Philippines, Myanmar, Papua New Guinea and India [4]. In the rural areas of Malaysia, sugar palm trees are normal scenery since they are generally grown wild. According to [8], there is only one commercial plantation of sugar palm in Malaysia located in the district of Tawau in the state of Sabah, while smaller-scale plantations are found in the districts of Benta and Kuala Lipis in the state of Pahang and the districts of Kuala Pilah and Kuala Jempol in the state of Negeri Sembilan. As in 2015, a total 892 hectares of land area was planted with sugar palm [9].
1.4. Ecology and climate requirement
Sugar palm grows best in humid tropical rainforest at the elevation from 700 m to 1,200 m [10] with the annual temperature of 19°C - 27°C. It is reported tolerant to temperatures below freezing but damaged at -2°C [5]. It usually survives in most soil conditions such as in wasteland, rough hill-slopes, valleys of depleted soil as well as along timberland edges and mountain streams’ embankment. It develops more slowly in flat, exposed or sunny habitats [3]. In its native range, sugar palm trees are usually found grown near human populated areas where anthropic plant breeding plays an important role [11]. Sometimes, sugar palm trees are also being discovered in pristine jungle since its fruits are consumed and scattered by wild animals such as bats, boars and other small mammals [1].

1.5. Horticulture
Sugar palm is propagated from seeds which germinates in 3-12 months in its natural habitat. Sugar palm is rarely troubled by drought, pests or diseases [3]. The stem-rotting fungus *Ganoderma pseudoferreum* has been reported to affect sugar palm trees. In Southeast Asia, the rhinoceros beetle (*Oryctes rhinoceros*) has been known to feed on the foliage [5]. While some degree of regional selection has been applied to sugar palm, no rigorous breeding or cultivar evaluation program has been conducted [12].

1.6. Economic contribution
Sugar palm is widely cultivated for its economic importance in the industries of food, beverages, construction, pharmaceutical and some other trivial applications. The primary products of the tree are starchy pith, sugary sap and black fibers. The different uses of different parts of sugar palm trees are summarized in Table 2 as mentioned by several authors ([13], [4], [14], [15], [16], [17], [18], [19], [20], [21]).

Table 2. Summary of different uses of different parts of the sugar palm tree.

| Parts          | Uses                                                                 |
|----------------|----------------------------------------------------------------------|
| Sap            | Fresh beverage; sugar / palm sugar; Source of biofuel (bioethanol), palm wine, arrack, alcohol, vinegar. |
| Male Inflorescence | Tapped to collect sap.                                                 |
| Female Inflorescence | Source of nectar for honey production.                              |
| Fruit          | Edible endosperms of unripe fruits as kolang-kaling (sweetmeat); Pulps as natural cosmetic bases which proclaimed to have anti-aging effects. |
| Seed           | Black seed as toy beetle.                                             |
Fibres
- Fibres of leaf bases as rigging, brushes, covers of underwater telegraph cables, road construction, rope, mattresses stuffing, sieves, brooms, roof material;
- Fibres of trunk pith as ship cordage;
- Fine outgrows (hairs) of the leaf petiole as a material to kindle fire;
- Fibres of roots are used to make cloaks or mantles in Indonesia and hats in Indo-China.

Petiole
- Material for basketry, crafting work for furniture; walking sticks, butts of blow-pipes.

Leaf
- Young leaf as salads, cigar paper;
- Mature leaf as material for thatching house, wrappers, rough brooms and woven baskets;
- Leaf and old leaf bases are used for igniting fire.

Pith of leaf’s rachis - Drinking cup.

Terminal bud or ‘cabbage’ - Salads, eaten raw or cooked.

Stem
- Stem core as sago starch and fibers;
- Pith of young stems eaten in soups or fried and pickled preserve;
- The starch is also applied as biopolymer or plasticizer;
- The hard bark and trunk are used as material to produce furniture, barrels, flooring, farming support tools (such as poles for creepers) and musical appliances.

Root
- Folk medicine as tea to cure kidney stones;
- Insect repellent;
- Subdue soil erosion for natural conservation programs (root system penetrate up 3.0 meters deep and 10.0 meters wide in soil helps to stabilize soil in place);
- Improve soil macro conditions, soil porosity, and trapping rainwater.

Sawmill waste - Edible mushroom media.

1.7. Challenges of sugar palm’s conventional breeding
Breeding of sugar palm trees at commercial scale is hindered by different challenges. Among the limitations to establish successful breeding of sugar palm conventionally are seed dormancy [22], short-lived seeds in storage and poor field growth seedlings ([16], [23]). Wild seedlings are usually collected and raised in nurseries before being cultivated as an attempt to maintain sugar palm’s population. Since natural regeneration greatly influenced the population of sugar palm, extensive harvesting of its fruits meant for human consumption had significantly reduce the seed source [10]. There is also concern of high labor requirement to process the sugar palm products. The extraction of edible endosperms of sugar palm particularly requires caution as the decomposing fruits contain stinging raphides which cause intense itching, burning sensation and irritation as it touches skin [24].

2. Plant tissue culture of sugar palm
Plant tissue culture or ‘micropropagation’ is an amazing biotechnology apparatus for stretching out regenerative cycles to a more extensive scope of cells and tissues of different plant species. The technology was fundamentally established aimed for fundamental research work of cellular and tissue differentiation, morphogenesis and identification and function of hormones [25]. Plant tissue culture generally defined as the inoculation of plant cells, tissues or organs on a formulated nutrient media under aseptic conditions [26]. The types of plant tissue culture methods which most generally recognized in
practice are organ cultures (organogenesis), callus cultures (callogenesis), suspension cultures, protoplast cultures and anther cultures [27]. Seed culture and embryo culture are another different category of plant tissue culture frequently practiced [26]. Among the benefits of plant tissue culture are including mass regeneration of clonal seedlings, somatic breeding methodologies (e.g. protoplast integration), elimination of plant diseases, genetically engineered plants as well as conservation of germplasm [28]. Work on plant tissue culture of sugar palm is still at an early stage. Established plant tissue culture protocols of sugar palm can be useful for obtaining pure line seedlings commercially to conserve its germplasm and particularly important for the establishment of crop improvement studies through biotechnological interventions. Some of plant tissue culture works of sugar palm which had been reviewed are as follows:

2.1. Embryo Culture of Sugar Palm
Embryo culture is among the earliest types of tissue culture techniques and has been verified to provide the greatest value to plant breeders [29]. Embryo culture shortens the breeding cycle of a plant species by overcoming seeds dormancy [30]. [31] established an embryo culture protocol in sugar palm with the objectives to evaluate the relationship of embryos age and different compositions of nutrient medium factors to the rate of in vitro germination and plant development within a certain period of time. Embryos excised from fresh fruits at 15 and 30 months after anthesis were used as explants. At the end of the study, it was reported that the explants obtained from the 15-month-old fruits promoted the highest rate of germination (90%), whilst explants obtained from 30-month-old fruits promoted 72% rate of germination. The cultures growth and development were uninfluenced by the different composition of nutrient medium, though abnormalities to the formation of haustorial and cotyledonary petioles were observed.

In another study, [32] reported an established methodology of aseptic seedlings production of sugar palm via embryo culture. Given the prolonged germination of sugar palm seeds under nursery planting, plant tissue culture method was observed to promote rapid regeneration of aseptic seedlings within a relatively short period of time. Immature embryos as explants were extracted from surface sterilized immature fruits and inoculated on basic MS [33] medium and MS medium modified with different concentrations of 6-benzylaminopurine (BAP). Results obtained after 8 weeks of observation showed that 60% of explants cultured on MS medium supplemented with lower concentrations of BAP promoted rapid regeneration in the forms of enclosed sheath and radicles. Adventitious shoots development was only visible after 32 weeks. The highest percentage shoots regeneration was eventually observed from the explants cultured on free-hormone MS medium with 90% shoots emergence after 24 weeks. Complete aseptic seedlings were successfully established after 32 weeks.

2.2. Organogenesis of sugar palm
The capability to develop adventitious roots and shoots through plant tissue culture (organogenesis) is regarded as the utmost importance in plant tissue culture [34]. [35] reported direct organogenesis response in sugar palm using immature zygotic embryos and basal stem segments excised from aseptic seedlings as explants. All explants were inoculated on MS medium containing different concentrations of plant growth regulators (PGRs) and organogenesis potential of explants was observed. Within 8 weeks of culture, tested explants were responsive, however the numbers of shoots and roots establishment substantially influenced by the compositions of PGRs in the culture medium. Optimum organogenesis response was observed from basal stem explants cultured on MS + 1.0 mg/L kinetin (Kin) + 2.0 mg/L 1- Naphthaleneacetic acid (NAA) with 90% rate of regeneration with a number of 9 adventitious shoots and 25 roots were established. Frequent sub-culturing of the regenerated shoots on basal MS medium (MS0) optimized further development of plantlets. In the meantime, immature zygotic embryo explants inoculated on MS + 2.0 mg/L BAP + 2.0 mg/L NAA was observed to develop an optimum rate (70%) of root regeneration. It was also reported that the embryo explants inoculated on MS + 2.0 mg/L BAP + 1.0 mg/L gibberellin acid (GA₃) + 1.0 mg/L silver nitrate (AgNO₃) was found effective to promote optimum adventitious shoots regeneration (10 shoots) in culture. MS + 3.0 mg/L Indole-3-butyric acid (IBA) promoted plentiful rooting of cultures. Complete plantlets were later acclimatized on planting medium prior to transfer under greenhouse condition.
2.3. Callogenesis and somatic embryogenesis of sugar palm

Another essential technique of plant tissue culture is the induction of callogenesis and somatic embryogenesis. Callus cultures are useful to produce genetically similar copies of plants having desirable characteristics [36]. Meanwhile, in somatic embryogenesis somatic cells develop by division to form complete embryos which function correspondingly to zygotic embryos [37]. Embryo production through somatic embryogenesis can be used to study cell differentiation in plants [38]. One of the standardized micropropagation protocols to obtain callogenesis in sugar palm was reported by [39] using basal stem explants obtained from aseptic seedlings. Optimum callogenesis potential of the selected explants was investigated in response to different concentrations and combinations of PGRs supplemented in culture media. Optimum rate of callogenesis in sugar palm was eventually observed from the explants cultured on MS medium supplemented with 0.1 - 0.5 mg/L 4-Dichlorophenoxyacetic acid (2,4-D) in combinations of similar concentrations of Kin. Friable, translucent whitish primary callus induction was regenerated from the explants 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 on MS + 0.2 mg/L 2,4-D + 0.5 mg/L Kin. Calluses were observed to form from the wounded region of explants.

In another study, [40] discussed callogenesis and somatic embryogenesis response of sugar palm using immature zygotic embryo as explants. Selected explants were cultured on MS medium supplemented with different concentrations and combinations of 2,4-D + NAA to obtain callus induction. All cultures were incubated under complete darkness at the temperature of 25±2°C and the callogenesis response were observed. After 8-12 weeks, a maximum rate (100%) of embryogenic callus formation was established from the explants cultured on MS + 0.4 mg/L 2,4-D + 0.5 mg/L BAP. High concentrations of sucrose (60.0 g/L) and the addition of 2.0 g/L casein hydrolysate (CH) to the existing culture medium composition facilitate growth and development of somatic embryos at globular stage, while the incorporation of AgNO₃ promoted development of heart-shaped and torpedo somatic embryos. Sub-culturing of the established somatic embryos on maturation medium (MS + 1.0 mg/L BAP + 1.0 mg/L NAA) promoted growth of multiplied cotyledonary embryos. Clonal roots regeneration was observed as the cultures were transferred on half-strength basal MS medium after 4 months.

2.4. Synthetic seed establishment of sugar palm

Synthetic seed is a promising technique for germplasm propagation and preservation of a plant species. The establishment of synthetic seeds basically aims at converting micropropagules, mainly somatic embryos into artificial or synthetic seeds which can be grown in the field or greenhouses when required [41]. Mechanical encapsulation of somatic embryos has generated the concept of “artificial seeds”, in which they are designed to be stored, handled and planted like natural seeds [37]. A method describing the encapsulation of somatic embryos of sugar palm as synthetic seeds was carried out by [42] as a part of her doctoral dissertation. Somatic embryos of sugar palm at cotyledonary stage were extracted as individual from aseptic culture and encapsulated in a matrix solution consisting of 30.0 g/L sucrose, 1.0% - 4.0% sodium alginate and different concentrations of PGRs. Prior to encapsulation procedure, the matrix solution was autoclaved at 121°C for 20 minutes. Each somatic embryo was dropped into the prepared solutions and later pipetted into sterile solutions of calcium chloride to form synthetic beads. The synthetic beads containing somatic embryos were rinsed and dessicated on distilled paper following culture on basal MS media for germination. After 4-8 weeks of culture under dark condition at the temperature of 25±2°C, 80% germination rate of the synthetic seeds was recorded; promoting adventitious shoots and roots formation. The demonstrated synseed procedure would be a great mechanism to promote rapid multiplication of both aseptic and clonal seedlings of sugar palm intended for large-scale production whilst systematically preserving its genetic resources (germplasm) for future use.

2.5. Greenhouse acclimatization

The hardening and acclimatization process of plantlets are the most crucial steps to determine success in plant tissue culture [41]. Acclimatization stage served the conventional plant tissue culture method in which the developed plantlets or micro cuttings raised in vitro were introduced to the ex vitro
environment for growth. [43] reported the acclimatization procedure of sugar palm. Aimed to investigate the suitable components of growing media for successful acclimatization of aseptic and clonal seedlings of sugar palm prior introduction under greenhouse condition, the procedure was carried out in two phases. In the first phase, aseptic and clonal plantlets were deflasked, washed and transferred to new culture containers containing MS salt solution following incubation at the temperature of 25±2°C for 2 weeks. Later, for the second-phase procedure, the subsequent plantlets were treated with fungicide and planted in plastic pots filled with different growth media for acclimatization. The highest rate of plantlets survival (100%) at 4 weeks of acclimatization was observed on soil:peatmoss:perlite substrate mixture at 2:2:1 ratio following the end result of 80% survival rate after 4 months of transfer to soil.

3. Conclusion
Sugar palm trees are fibrous plants of thousand uses currently gaining popularity in Malaysia as emerging alternative sources of sugar, alcohol, fibers and timber. Seed dormancy, lack of quality planting material and insufficient cultivar evaluation are hindering issues for commercial-scale cultivation of sugar palm. Plant tissue culture technology provides hope to ensure successful rapid and mass propagation of sugar palm as this method shortened the long cycle of plant growth through conventional breeding. Plant tissue culture also benefits the crop improvement studies of sugar palm through biotechnological interventions.

References
[1] Lim T K 2012 Edible Medicinal and Non-Medicinal Plants vol 1 (The Netherlands: Springer) p 285-292
[2] Witt A and Luke Q 2017 Guide to The Naturalized and Invasive Plants of Eastern Africa (Africa: CABI)
[3] Duke J A 2000 Handbook of Nuts: Herbal Reference Library vol 4 (USA: CRC Press)
[4] Mogea J, Seibert B and Smits W 1991 Multipurpose palms: The sugar palm (Arenga pinnata Wurmb Merr.) Agroforestry Systems 13[2] 111-129
[5] Janick J and Paull R E 2008 The Encyclopedia of Fruit and Nuts (UK: CABI)
[6] Florido H B and De Mesa P B 2003 Sugar Palm [Arenga pinnata Wurmb Merr.]. Research Inform. Series on Ecos. 15 [2] 2-6
[7] Dransfield J and Mogea J P 1984 The flowering behaviour of arenga [Palmae: Caryotoideae]. Botanical Journal of the Linnean Society 88 [1-2] 1-10
[8] Sahari J 2011 Physico-chemical and mechanical properties of different morphological parts of sugar palm fibre reinforced polyester composites [MS Dissertation] (Malaysia: University Putra Malaysia)
[9] Malaysian Palm Oil Board 2015 Oil palm planted area by state in December 2014 (Hectares), Bangi, Selangor, Malaysia. Available online at: http://bepi.mpob.gov.my/image/area/2014/Area_summary.pdf
[10] Martini E, Roshetko J M, Van Noordwijk M, Rahmanulloh A, Mulyoutami E, Joshi L and Budidarsono S 2012 Sugar palm [Arenga pinnata Wurmb Merr.] for livelihoods and biodiversity conservation in the orang utan habitat of Batang Toru, North Sumatra, Indonesia: mixed prospects for domestication. Agroforestry Systems 86[3] 401-417
[11] Orwa C, Mutua A, Kindt R, Jammnadass R and Simons A 2009 Agroforestree Database: A Tree Species Reference and Selection Guide Version 4.0. (Kenya: World Agroforestry Centre ICRAF)
[12] Meerow A W and Broschat T K 1991 Palm seed germination Horticultural Reviews vol 42 ed Meyer M H, Reid M S and Swietlik D (USA: Wiley Blackwell)
[13] Redhead J 1989 Utilization of tropical foods: Trees. FAO Food and Nutrition 47[3] 52
[14] Johnson D V 1996 Palms: Their Conservation and Sustained Utilization: Status Survey and Conservation Action Plan vol 31. (IUCN)
[15] Smits W T M 1996 Arenga pinnata (Wurmb) Merrill PROSEA 9 53-59
[16] Soeseno S 2000 Bertanam Aren (Jakarta (ID): Penebar Swadaya)
Sastra H Y, Siregar J P, Sapuan S and Hamdan M M 2006 Tensile properties of Arenga pinnata fiber-reinforced epoxy composites Polymer-Plastics Technology and Engineering 45[1] 149-155

Ishak M R, Sapuan S M, Leman Z, Rahman M Z A, Anwar U M K and Siregar J P 2013 sugar palm [Arenga pinnata]: Its fibres, polymers and composites. Carbohydrate Polymers 91[2] 699-710

Sahari J, Sapuan S M, Zainudin E S and Maleque M A 2013 Thermo-mechanical behaviors of thermoplastic starch derived from sugar palm tree (Arenga pinnata) Carbohydrate Polymers 92[1] 1711-1716

Djarwanto D and Suprapti S 2016 Utilization of aren [Arenga Pinanata Merr.] sawmilling waste for edible mushroom cultivation media. Indonesian Journal of Forestry Research 3[1] 9-18

Yanti M and Ali S 2017 Cosmeceutical effects of galactomannan fraction from Arenga pinnata fruits in vitro Pharmacognosy Research 9[1] 39

Askin D and Puspitaningtyas D M 2000 Study on in vitro and in vivo seed germination of Arenga pinnata (Wurmb Merr) (Indonesia: Puslitbang Bioteknologi).

Putih R, Satria B and Thaib R 2003 Upaya perbanyakan vegetatif enau (Arenga pinnata Wurmb Merr.) melalui regenerasi tunas secara in vitro Stigma 11 208-212

Schmidt L 2007 Seed processing Tropical Forest Seed 67-142

Robert M F and Wink M 2013 Alkaloids: biochemistry, ecology, and medicinal applications (New York: Springer Science & Business Media)

Chawla H S 2002 Introduction to Plant Biotechnology (USA: Science Publishers)

George E F, Hall M A and De Klerk G J 2008 The Components of Plant Tissue Culture Media I: Macro- and Micro-Nutrients (The Netherlands: Springer) p 65-113

Kung S D and Arntzen C J 2014 Plant Biotechnology: Biotechnology vol 11 (USA: Butterworth Publishers)

Dunwell J M 1986 Pollen, ovule and embryo culture, as tools in plant breeding. Plant Tissue Culture and its Agricultural Applications 375-404

Bridgen M P 1994 A review of plant embryo culture. HortScience 29[11] 1243-1246

Arsyad M A, Sudarsono A P and Dinarti, D D 2013 Pengaruh umur embrio dan jenis media dasar terhadap keberhasilan embryo rescue aren (Arenga pinnata Wurmb Merr.) secar a in vitro. [Dissertation] (Indonesia: School of Graduate Studies, IPB)

Abdullah N S, Aziz N A and Awal A 2015 Establishment of sugar palm [Arenga pinnata] shoot from zygotic embryo in MS medium supplemented with different concentrations of benzylaminopurine Malays. Appl. Biol. 44 [4] 31–35

Murashige T and Skoog F 1962 A revised medium for rapid growth and bioassays with tobacco tissue cultures Physiologia Plantarum 15 473-497

Dodds J H and Roberts L W 1995 Experiments in Plant Tissue Culture. (USA: Cambridge University Press)

Nazarul Asikin M, Awal A, Nor Azma, M Y and Mohd Yusoff A 2019 In vitro regeneration of sugar palm (Arenga pinnata Wurmb Merr.) International Journal on Advanced Science, Engineering and Information Technology 9[3] 888-894

Ranjan D C and Kadunlung G P 2017 Callus mediated indirect somatic embryogenesis and plant regeneration of Saurauia punduana Wallich (Actinidiaceae) from in vitro cotyledonary leaves Current Biotechnology 6[1] 69-76

Jha T B and Ghosh B 2005 Plant Tissue Culture: Basic and Applied (India: Universities Press)

Quiroz-Figueroa F R, Rojas-Herrera R, Galaz-Avalos R M and Loyola-Vargas V M 2006 Embryo production through somatic embryogenesis can be used to study cell differentiation in plants Plant Cell, Tissue and Organ Culture 86[3] 285

Muda N A, Awal A, Abdullah M Y and Abdullah S 2016 Embryogenic callus induction of Arenga pinnata Wurmb Merr. from basal stem explant. Int'l Journal of Advances in Agricultural and Environmental Enng. 3[1] 106-109
[40] Muda N A and Awal A 2017 Somatic embryogenesis in sugar palm (*Arenga pinnata* Wurmb Merr.) from zygotic embryo explants. *Pertanika Journal of Science and Technology* **25** 133-144

[41] Purohit S D 2012 *Introduction to Plant Cell Tissue and Organ Culture* (Delhi: PHI Learning Pvt. Ltd.)

[42] Nazatul Asikin M 2018 *Micropropagation of Sugar Palm (Arenga pinnata Wurmb Merr.)* [Doctoral Dissertation] (Malaysia: Universiti Teknologi MARA)

[43] Muda N A and Awal A 2018 *In vitro* germination and acclimatization of sugar palm (*Arenga pinnata* Wurmb Merr.) *Regional Conference on Science, Technology and Social Sciences* (Singapore: Springer) p 951-961