A Potential Study of *Synsepalumdulcificum*(Sapotaceae) *"Miracle Berry"*

Fatimah AbdolLatif
SarinaHashim
Zairus Ismail

ABSTRACT

Synsepalumdulcificum or Miracle berry plant is a slow-growing shrub belonging to Sapotaceae. The aims of the study were to identify the morphology of S. dulcificumand to examine the seed germination through in vitro propagation. Mature plants of S. dulcificum were obtained from Tekam Plantation, Jerantut, Pahang. The 20 cm stem cutting was used to study the phyllotaxis and to investigate the feature of flower and fruit. To determine the performance of seed culture, seeds sterilization done by surface-sterilization with 95% ethanol for 5 min, 20% NaOCl for 20 minutes. Then, the sterile seeds were maintained on Murashige and Skoog (MS) basal medium. The record of shoots and roots formation were taken by direct observation. This study shows that S. dulcificum have a deep green, 10-13 cm elongated thin leaves with entire margin. The flowers are born axially, with small white flowers about 1 cm length during opening, change to creamy yellow, then black when past mature. The plants produced 2-3 cm ellipsoid fruitswith a single seed. The seeds were used for in vitro propagation. Shoots and rootsstarted to form after 16 weeks of culture incubation. Propagation was successfully donethrough seeds in the MS basal medium. Anyhow, further studies on the modifications of the in vitro physical and chemical environments of the nodal culture are recommended to enhance propagation of the plants. This study indicates that S. dulcificum has a great potential in landscaping because of their evergreenand slow growth rate, so that it does not grow higher. Seeds culture can increase the number of seedlings for plant lovers, so it can overcome the problem of slow sprouting rate of the plants.

Keywords: *Synsepalumdulcificum*, morphology, *in vitro* propagation, potential.

Introduction

Synsepalumdulcificum (Miracle berry) is anevergreenshrub of the Sapotaceae family (Table 1). The plant is indigenous to tropical West Africa, distributed from Ghana to the Congo region and commonly found growing in the wild of virgin forest, coastal areas and in mountainous forest (Opeke, 1984). The plant can usually be found growing in partially shaded areas under the canopy of the rain-forest in its native habitat and is able to tolerate full sunlight and drought (Duke, 1993).

Table 1. The hierarchy of *Synsepalum*

Categories	Taxa
Kingdom	Plantae – Plants
Subkingdom	Tracheobionta - Vascular plants
Superdivision	Spermatophyta- Seed plants
Division	Magnoliophyta- Flowering plants
Class	Magnoliopsida-Dicotyledons
Subclass	Dilleniidae
Order	Ebinales
Family	Sapotaceae
Genus	*Synsepalum*
Species	*dulcificum*

Fruits are an interesting part of the plant and are actively studied by many researchers. The fruits are small berries composed of a thin layer of edible pulp surrounding a single seed(Ogunsola and Ilori, 2008). Immature berries are dark green, and become bright red when ripe. The most unusual thing about the fruit is the extraordinary effect of the fleshy pulp of the fruit has on the taste buds of the tongue that causes every sour food eaten to taste very sweet. The taste-modifying effect, changed acid food substances such as sour lime, lemon, grape fruits and even vinegar to taste sweet (Rehm and Espig, 1991). Interestingly, the active material of the berry is the protein, miraculin, which has no taste in itself(Yamamoto et al., 2006).

The interest in natural sweeteners, which do not contain carbohydrates, has been reawakened because of the healthhazards associated with the use of some artificial sweeteners like saccharine and the suspicion that these synthetic sweeteners especially the cyclamates, are carcinogenic (WHO, 1991). Health concious people always try to find out natural sweeteners especially from plants. Ogunsola and Ilori (2008) reported the natural sweeteners from plants are the extremely sweet-tasting protein, monellin found in the berries of *Dioscoreophyllum cumminsii*,thaumatin from the aril of *Thaumatococcus danielli* and the miraculin from *S. dulcificum*.

*S. dulcificum*are usually found growing in the wild than cultivated and its sprouting rate and growth rate are very slow. Since the plant has the great potential in landscaping and for their medicinal values, there is a need for an alternative method of propagation that will overcome these growth constraints. Traditionally, miracle berry is propagated from cuttings of mature plants and is raised in polybags. The method is not economical since the collection of stem cuttings leads to arrest of growth and development of the mother plant. Moreover, the market demand for propagules is hardly met with such cuttings. Therefore, this study tried to explore the characteristics of *S. dulcificum* in order to increase the knowledge and information of this potential landscape species.

Materials and Methods

Plant materials

Mature plants of *S. dulcificum* were obtained from Tekam Plantation, Jerantut, Pahang to study the morphologyof plants (Figure 1). Other than that, seeds without the fleshy pulp of the fruitswere used as source materials for micropropagation study.

Figure 1.A mature plant of *S. dulcificum*.

Morphological Study

About 20 cm of stems were used to examine their phyllotaxis and to investigate the features of flowers and fruits of the plants. Identification and description of the species were done by the aids of Glimn-Lacy & Kaufman (1984), Jones &Luchsinger (1987) and Kubitzki et al. (2007).

*In vitro*Culture

Murashige and Skoog(MS) media in powdered form were used in the experiments. The constituent of the media were adjusted to 1 liter after dissolving the sucrose and the pH adjusted to 5.8, prior to the adding of agar technical (Agar No.3); (8 g) and activated charcoal (0.5 g) to the media. Media were autoclaved at 121° C and 1.3 or 1.5 kPa for 15 to 20 minutes. Molten media then were dispensed into sterile plastic vials containing 20-25 ml of aliquots, inside the laminar air flow cabinet.

Seeds were washed thoroughly under running tap water for 1 to 2 hours, to remove the dirt and any residue of fruit vesicles or mucilaginous cells that surrounded the seeds. Seeds were transferred to the laminar air flow cabinet and were rinsed again in sterile distilled water for three times, then surfaced sterilized by immersing in ethanol 70% for 2 min, followed by continuous shaking for 15 minutes in 25% solution of sodium hypochlorite (NaOCl) with the addition of 0.1 % Tween 20 and subsequently rinsed 5 times in sterile distilled water. The seeds were grown on the MS media and place under continuous cool-white fluorescent light (50 µmol $m^{-2}s^{-1}$) at 25 ± 2° C. The performance of seed culture was done by direct observation.

Results and Discussion

Classification

Subclass, Dilleniidae; Order, Ebinales; Sapotaceae: Sapodilla Family
Scientific name: *Synsepalumdulcificum*
Local name: *Pokokbuahajaib*, Miracle berry

Phytography

Leaves: Alternate, simple, deep green lamina 10-13 cm elongated, flat and thin with entire margin, obtuse apexs; lanceolate with short stipules about ½ cm (Figure 2a, 2b, 2c).

Flowers: Bisexual, short pedicel, gamosepalous and persistent hairy calyx, actinomorphic, polypetalous with 1 cm white flowers turn to creamy yellow then black when past mature. Stigma emerges from the center of the flower. The flowers are born axially, sometimes in a cluster of two to five (Figure 2b). It is small about 1 cm length and ½ cm width.

Fruits: Simple, fleshy, 2-3 cm ellipsoid fruit (Figure 2d). The plants produced fruits year-round. Young green fruits hide in deep green leaves. When the fruit is ripe, the berries turn red. The pulp of the fruit is tasteless, but can give great effects to the human tongue. After chewing the pulp, everything sour afterwards turns sweet.

Seed: One per fruit, small; fertile seed.

Description habit: Shrubs, woody plant; seed propagate, slow growing; acid-loving plant; full-to-partiall

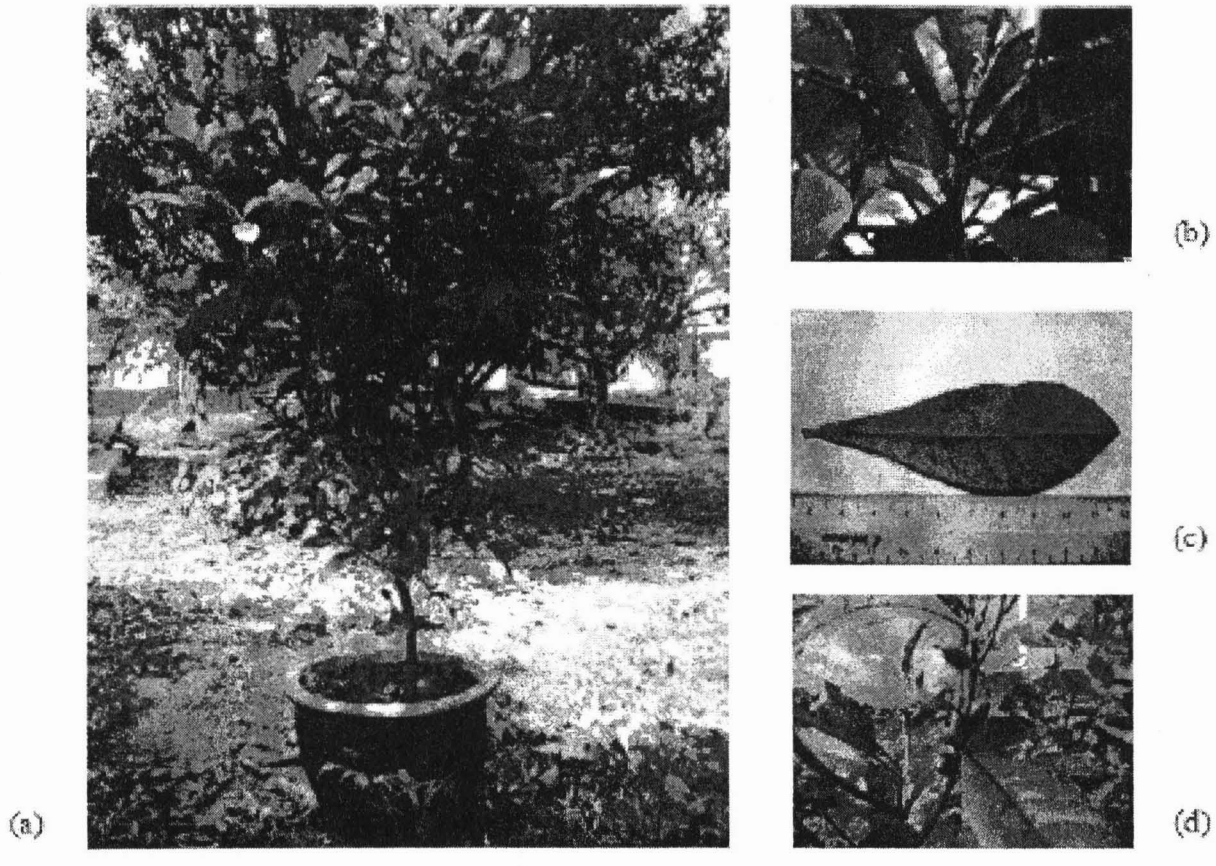

Figure 2: A photographic study of *S. dulcificum*.(a) A mature plant, (b) Patterns of leaves arrangement and the young flowers, (c) Leaf venation, and (d) Young and ripe berries.

Seed culture

Sterilization with 95 % ethanol for 5 minutes was appropriate for obtaining sterile seeds of *S. dulcificum.* 20 seeds were successful cultured in MS basal media. The seeds started to germinate after 16 weeks. The explants produced shoot, followed by the roots. Figure 3 shows the step done in seed culture technique. Generally, only certain seeds produced the seedlings and new shoots. The cotyledon explants tend to proliferate and produce callus. This study indicates that the propagation was successfully done through seeds in MS media.

The preliminary studies done indicate that the regeneration of whole plantlets of *S. dulcificum* from the mature embryo will enhance the cultivation and *in vitro* conservation of the plant. It will also reduce the breeding cycle of this slow-growing plant, promote germplasm of pathogen-free miracle berry plantlets. Further studies on the growth rate in *in vitro* physical and chemical environments of the nodal culture are recommended to enhance propagation of the plant for future use.

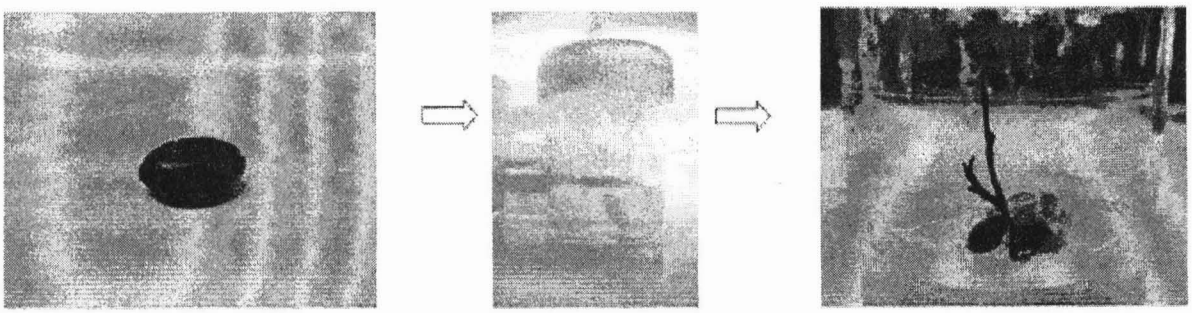

Figure 3:Seed culture of *S. dulcificum.* (a) Sterile seed, (b) Seed in MS basal medium, and (c) *In vitro* plantlet after 16 weeks.

Economic Importance of S. *dulcificum*

S. dulcificum has great potential in landscaping. *S. dulcificum*is one of theornamental plants that are easily cultivated. The plant is an evergreen tree growing to 3-5 m in its native habitat. The plants need full sunlight. It is also an acid-loving plant and need a soil pH of around 5.6. The mature plant flowers and fruits all year-round. The plant is recalcitrant to propagation both by seeds and cuttings, as the seeds dry very quickly after harvest and loses viability after drying while the rooting of the cuttings have been very difficult (Okhapkina, 2006). As the seed viability is short in natural habitat, propagation through the*in vitro* technique becomes important to ensure enough seedlings provided to plant lovers.

Nowadays, *in vitro* propagation methodshave become importantfor conservation purposes as the wild population is becoming sparse. Different types of explants including shoot tips, leaf pieces, petiole sections, peduncle sections, root pieces, inflorescences, lateral buds, or stem sections can be used to regenerate whole plants. Shoot tip culture is the method in the widest use for the mass propagation of woody species (Brichia et al., 2002). For *S. dulcificum*, the seeds and the shoot meristems usually used as an explants.

S. dulcificum is an exciting plant, therefore, called the 'miracle plant'. The miracle is in the berries. Miracle fruit can enhance the sweetness of a low-sugar dessert while limiting energy intake in comparison to a higher calorie, sucrose-sweetened popsicles (Wong and Kern, 2011). People in parts of West Africa have been using the miracle berry to sweeten sour foods and drinks for centuries, but it is only recently that the global food and pharmaceutical industries are beginning to realize its significance (Most et al., 1979).

The plant is not important as a food crop, but can be a beautiful ornamental plant with amazing fruit for dieters and health concious people.*S. dulcificum*can be commercialised for sweetener agents and also as a dried fruit product or miracle fruit tablet for the diabetic patients to enjoy the taste of sweetness in their life.

Conclusion

S. dulcificum has the great potential in landscaping and also for their sweetness values. The plant propagates by seed and the seedling is not abundance in our nurseries. Therefore, micropropation of *S. dulcificum* should be taken into consideration to increase their survival, and to ensure enough supply of the plants.

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FATIMAH ABDOL LATIF, Faculty of Plantation and Agrotechnology, UiTMCawanganPahang. fatimah@pahang.uitm.edu.my

SARINA HASHIM, Faculty of Applied Science, UiTMCawanganPahang.hsarina@pahang.uitm.edu.my

ZAIRUS IDRIS, Faculty of Applied Science, UiTMCawanganPahang. zairus@pahang.uitm.edu.my