Materials Science Forum
ISSN: 1662-9752, Vol. 981, pp 316-321
doi:10.4028/www.scientific.net/MSF.981.316

Submitted: 2019-08-06
Revised: 2019-08-20
Accepted: 2019-10-12
Online: 2020-03-12

Preliminary Study on Micropropagation of *Hylocereus polyrhizus* with Waste Coconut Water and Sucrose

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Keywords: micropropagation, regeneration, sucrose, coconut water.

Abstract. Mature coconut water is always been thrown away as a waste. However, it contain a lots of nutritional elements such as amino acids, pytohormones and minerals. Plant needs some essential nutrients besides pytohormones for their growth and development. In this study, the effect of waste mature coconut water and sucrose was observed on the micropropagation of dragon fruit tree (*Hylocereus polyrhizus*). The stem was inoculated on MS medium containing Benzyleaminopurine (BAP) 0.03mg/L BAP and 0.01mg/L Naphthaleneacetic acid (NAA) supplemented with waste mature coconut water in various concentrations (0%, 2%, 4% and 6% v/v) and with various concentrations : 0%, 1%, 2% and 3% of sucrose respectively. As the concentration of the waste mature coconut water and sucrose increased up to 4 % and 3%, increase in the elongation of the stem and the number of root regenerated per explant was observed repectively. In conclusion, waste mature coconut water has enhanced the elongation of the shoots but has not promoted on the root induction with the suitable amount of sucrose.

Introduction

Dragon fruit tree known as *Hylocereus* spp. is a member of Cactaceae that is cultivated from Florida coast to Brazil. Most *Hylocereus* species that cultivated around the world are mainly *H. undatus, H. monacanthus* and *H. megalanthus*. There are only two types of species that suitable planted in Malaysia which are *H. undatus* (white flesh) and *H. polyrhizus* (red flesh). They also been known as Pitahaya, Pitaya, Buah Naga and Night Blooming Cactus. There are several species of pitahaya that commonly found in the market which is yellow pitahaya *S. megalanthus* (yellow skin with white pulp) (Schum.) and red pitahaya *Hylocereus* (red skin with white or red pulp) spp.

The dragon fruit could provide many benefits for community. The whole parts of it could be utilized such as fruit, flower and act as medicine for health. The fruit is multi-purposes as it can be utilized as jam, pastries, juice, ice cream and wine besides it also has delicious taste. The seed oils of dragon fruit act as a good source of essential fatty acids and tocopherols with high oxidative stability [1]. The flowers and vines of dragon fruit can act as vegetables and herbs while its peel can be used to extract color ingredient especially for food and cosmetic industries [2].

Recently, dragon fruit tree has attracted the attention of the public as it provides numerous benefits to humans in terms of health and people nowadays prioritize health over mostly anything. In a result, dragon fruit has become a hot topic to be discussed among the public. The health benefits provided by dragon fruit are dragon fruit could prevent dyslipedemia and hypercholesterolemia. This is because dragon fruit has certain antioxidant based on high antioxidant activity and phenolic content. Besides that, it also has the potential in lowering low-density lipoprotein (LDL) and increasing high-density lipoprotein (HDL) [3]. Dragon fruit tree also helps in controlling or lowering blood glucose level in

type 2 diabetic patients. According to [4], the ability of dragon fruit able in lowering blood glucose level in type 2 diabetic patient might be due to high content of dietary fibre. Moreover, dragon fruit contains high amount of antioxidant and this antioxidant could neutralize free radicals which is produced from oxidation process and has the potential damage transient chemical species [5].

Dragon fruit tree has possesses great potential as economic crop in extreme conditions such as high temperature, drought and poor soil. The growing areas of dragon fruit tree increasing rapidly and start getting attention in many countries due to its economic potential and nutritional benefits [6]. According to [7] that simple technology is mostly used at resident around Central America and Mexico to grow some of *Hylocereus* species. However, Malaysia, Thailand, United States and the Israel used modern technology which are producing high yield crop especially in Israel, which the yields up to 40 t ha^{-1}/ 17.8 short tons per acre of fruit are picked. Besides, Southeast Asia especially Vietnam has become the major supplier of fresh dragon fruit to other countries. They have taken over California, Florida and Hawaii where they are the only states that cultivate dragon fruit commercially last time.

Usually, the propagation of dragon fruit tree is done by using cuttings from field plants. However, multiplication rates are very low and it is difficult to obtain enough planting material because of the large size (>50 cm lengths) of the cuttings required [8]. Nevertheless, the vegetative propagation of dragon fruit tree can be enhanced via biotechnological tools such as in vitro tissue culture or plant tissue culture. This technique could produce large numbers of clonal plants in a short time period by using very small amount of starting material [9]. Tissue culture is an efficient method for rapid propagation of plants in order to obtain healthy plants without pathogen in a short time and minimal space with very few starting materials [10].

Coconut water is a very nutritional solution where it is traditionally used as supplement in micropropagation. The wide applications of coconut water can be justified by its unique chemical composition of sugars, amino acids, minerals, vitamins, and phytohormones [11]. However, the mature coconut water is usually being thrown away where the consumer only used the meat to prepare Santan. Thus, in order to reduce the waste, we are the first attempt in investigating the combination of different concentration of mature coconut water with sucrose with minimal level of NAA and BAP in tissue culture of *H. polyrhizus*.

Experimental Design

Sterile plants of *H. polyrhizus* used were obtained from the plant culture laboratory of Universiti Malaysia Pahang. The medium used for this experiment were Murashige and Skoog medium with B5 vitamin [9]. Various hormones and additives were added to this media for experiments. The waste mature coconut water was collected from the grocery who sell mature coconut. To study which concentration of coconut water paired with which concentration of sucrose could give optimum growth to the *H. polyrhizus*, MS media were prepared with 0.03mg/L BAP and 0.01mg/L NAA added to them. Various concentrations of coconut water were prepared: 0%, 2%, 4% and 6% and were added to the MS media. For each concentration of coconut water, different concentrations of sucrose: 0%, 1%, 2% and 3% w/v were added to it.

The stems of the dragon fruit tree were cut into 2 cm pieces and cultured into each media as mentioned above in triplicate. These experiments were run with three time. All of these cultured explants were kept under 16 hour cycled fluorescent light cooled incubator with temperature regulated at 25± 1˚C. Observations were done and recorded every week.

The data recorded were the numbers of roots grown and the length of shoots grown. The data was calculated into three sets of means. Then, an average mean and standard error were determined.

Results and Discussion

Effect of coconut water and sucrose on shoot length. The effects of different concentrations of coconut water (0%, 2%, 4% and 6%) and different concentrations of sucrose (0%, 1%, 2% and 3%) on the length of shoot of *H. polyrhizus* were shown in Fig 1. For the growth of length of shoot on

6 weeks, 3% sucrose had showed to have the longest shoot length in the MS media which contained 4% coconut water (2.45 ± 0.04cm), followed by 2% and 0 % coconut water with 2.44 ± 0.06cm and 2.28 ± 0.01cm, respectively. This shown that the shoot of *H. polyrhizus* elongated best in 3% sucrose with the increasing concentration of coconut water till 4%. When higher percentage of application of coconut water (6%), it seems to be have an adverse effect of the elongation of the shoot which reduce it shoot length about 8.4%. Similar pattern also been observed for 1% and 2% sucrose when the coconut water concentration increased, the shoot also become longer till 4%. When it reached 6% coconut water, it no longer boost up the shoot elongation. However, this phenomenon is opposite when only 6% coconut water was applied without any sucrose, the elongation of the shoot seems to be continue.

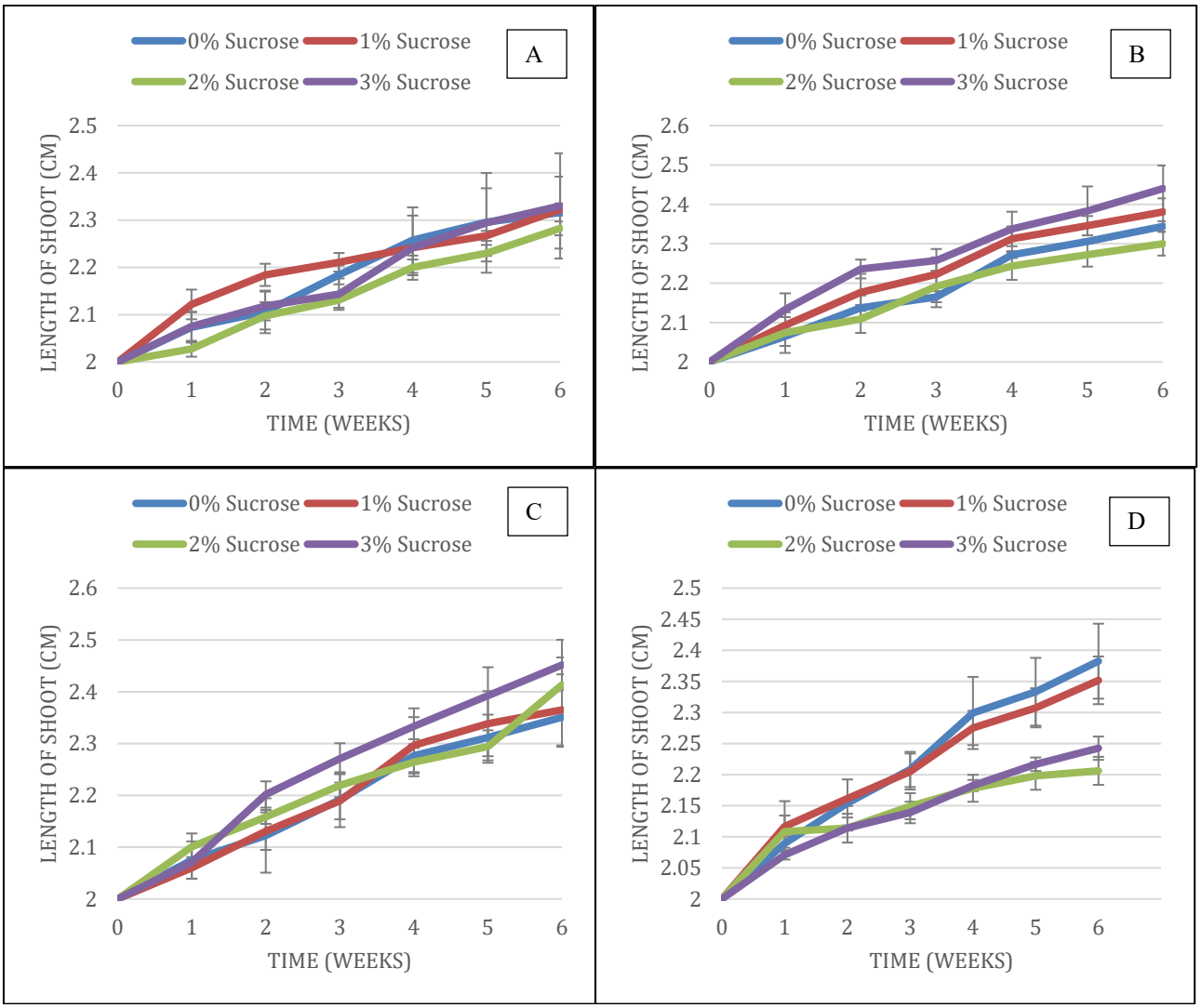


Fig. 1 Effect of different concentration of sucrose on the length of shoot of dragon fruit tree on MS media containing 0.01mg/L NAA, 0.03mg/L BAP and 0% coconut water (A), 2% coconut water (B), 4% coconut water (C), 6 % coconut water after 6 weeks of *in-vitro* culture. The results were expressed as means ± SE (n = 3)

When the sucrose and coconut water concentration is low (Figure 1A and b), the shoot elongation also shorter. The concentration of sucrose that showed the shorter shoot length is in both 0% to 2% coconut water with 0 to 2% sucrose. When 0% coconut water and 2% coconut water were added to 2% sucrose, it only elogated about 14% and 15%, respectively.

Coconut water has been used in tissue culture medium for growth promotion although it is not considered as an essential nutrient of tissue culture medium. The addition of coconut water with a suitable amount of sucrose increased the elongation of the shoot. This finging corresponds to the study

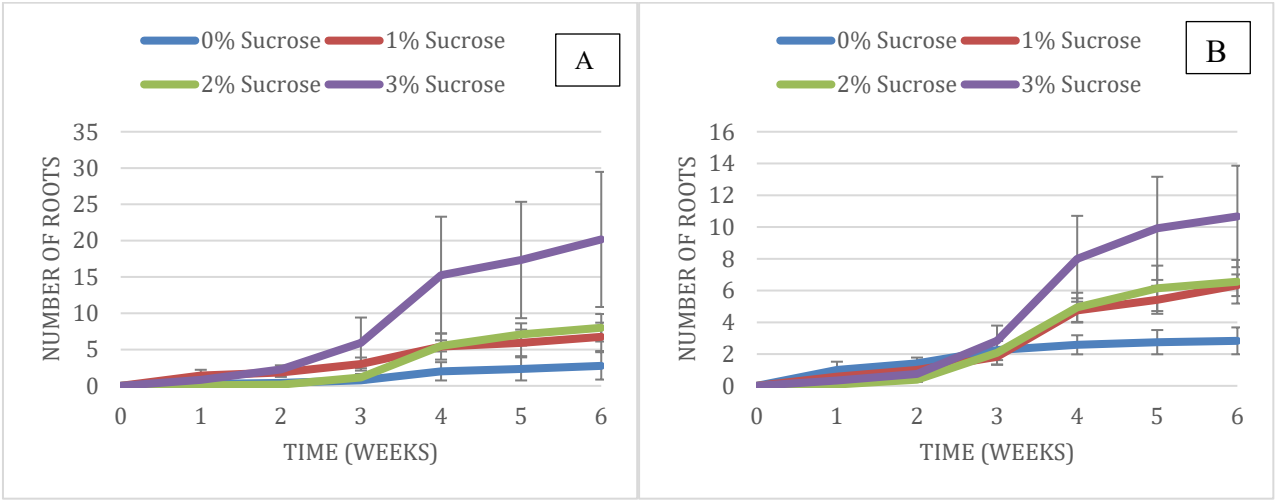
of [12] and [13]. They stated that coconut water could funtion as plant growth regulators because it contains zeatin which is cytokinin. It can help in different growth mode of plant including seed germination, tissue differentiation, cell division and for stimulating the growth of shoots. It also contain inositol, reduced nitrogen compounds and amino acid which stimulates the shoots elongation.

Effect of coconut water and sucrose on root induction. In terms of growth in number of roots, 3% sucrose had shown to has the highest number of roots in MS media that contained all the different concentration of coconut water (0%, 2% and 6% coconut water) except 4% coconut water. In Fig 2, it is obeserved that the number of roots induced were increasing as the concentration of coconut water at 3% sucrose, where the number of roots in these MS media were 10.67 ± 3.20 (2% coconut water), 11.67 ± 3.32 (4% coconut water) and 20.42 ± 2.34 (6% coconut water). However, at 3% surose, the number of roots been induced was comparable between 0% and 6% coconut water where the difference was less more than 1%.

Besides, when there were without any addition of sucrose in the media, it had shown to has the least number of roots in all 4 different concentrations of coconut water containing MS media, where the number of roots was 2.75 ± 1.89 for 0% coconut water, 2.83 ± 0.85 for 2% coconut water, 2.25 ± 0.38 for 4% coconut water and 4.42 ± 0.42 for 6% coconut water.

It is believes that sucrose is an essential carbon source and to support the induction of roots and the growth in *H. polyrhizus*. However, coconut water does not show a significance effect on root induction. This result is accordance with [14]. They found that coconut water supplemeted media gave the least percentage of elongated rooted shoots.

These result suggestd that the effect of coconut water was more pronounced in the elongation of shoots while sucrose influence more in rooting phase. Sucrose can easily move across the cell membran through plasmademata. Sucrose concentration of 2% and 3% are the most commonly carbohydrate source in plant tissue culture [15]. Auxin plays an important role in root induction. Sucrose induces auxin levels in a phytochrome-interacting factors dependent way. Sucrose also induce auxin transport and signal transduction. Sucrose is more efficiently affect in root induction as compare to glucose and fructose alone [16].



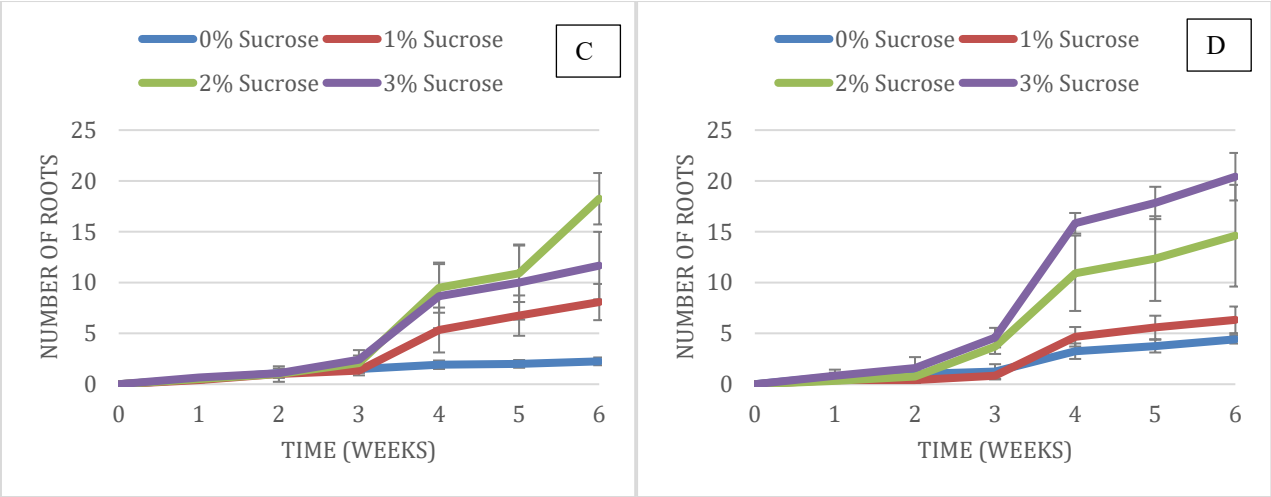


Fig. 2 Effect of different concentration of sucrose on the number of roots of dragon fruit tree on MS media containing 0.01mg/L NAA, 0.03mg/L BAP and 0% coconut water (A), 2% coconut water (B), 4% coconut water (C), 6% coconut water (D), after 6 weeks of *in-vitro* culture. The results were expressed as means ± SE (n = 3)

Conclusion

Based on the findings of present study, it may be concluded that the technique micropropagation of dragon fruit tree can be improved by the addition of 4% waste mature coconut water and 3% sucrose in culture medium has able to increase the elongation of the shoots. It was found that just addition of 3% sucrose was capable to regenerate high number of roots as it was comparable with addition of 3% sucrose and 6% waste mature coconut water into the culture medium.

Acknoledgement

The authors would like to thank the ERGS grant and University Malaysia Pahang RDU160327 for sponsoring this research.

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