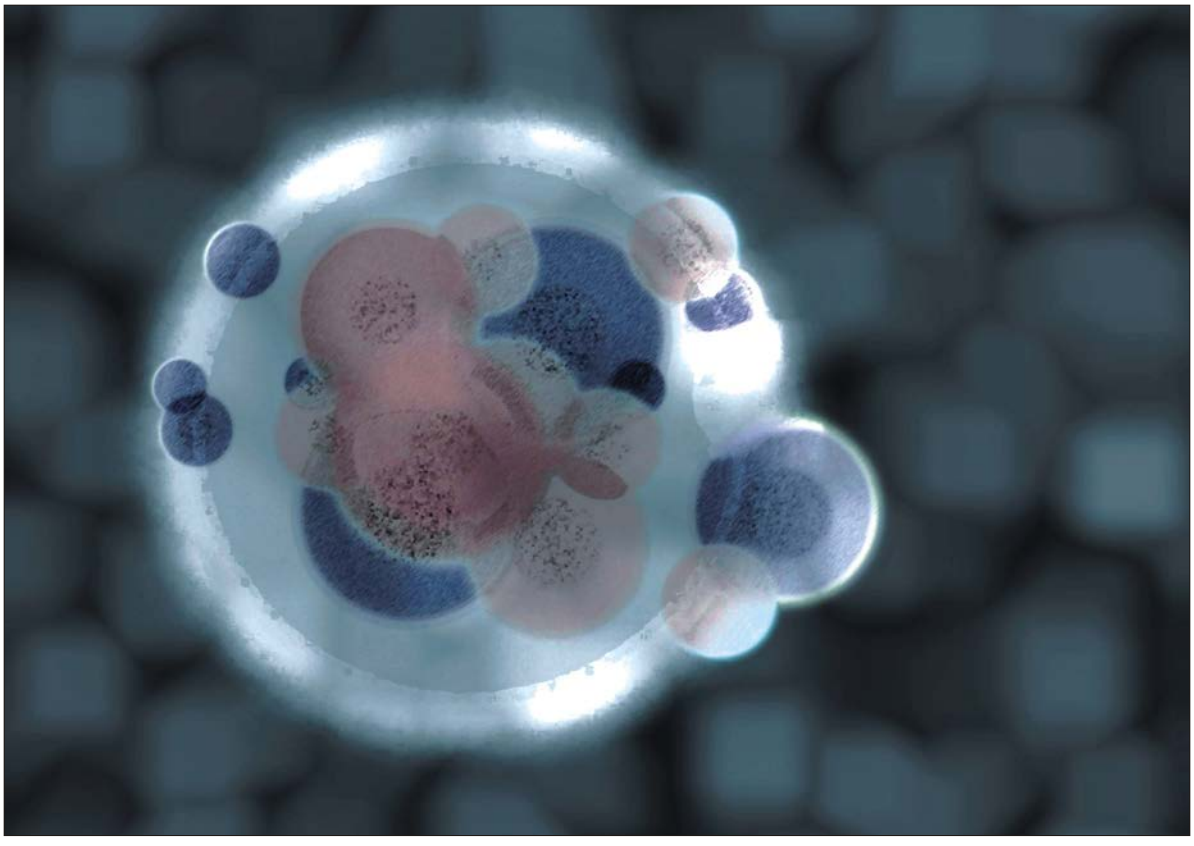

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Coconut Water from Fresh and Dry Fruits as an Alternative to BAP in the *in vitro* Culture of Dwarf Cavendish Banana

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ABSTRACT
The ability of coconut water to support the regeneration of banana plantlets *in vitro* was investigated from March to September 2014 at the Plant Genetic Resource Institute, Bunso, Ghana. The 100 mL L^{-1} coconut water was compared with 4.5 mg L^{-1} BAP. The tissues were cultured for 16 weeks at 26°C, 16 h. photoperiod, 3000 Lux light intensity and relative humidity of 60% with data taken on Fresh weight, Dry weight, Shoot number, Shoot height and other parameters. Explants cultured on 4.5 mg L^{-1} BAP produced 37 shoots/explant while those on 100 mL L^{-1} coconut water recorded 25 shoots/explant. However, for plant height, plant cultured on medium supplemented with coconut water from fresh green fruits had height of 18 cm but those cultured on medium with BAP had a height of 15 cm at 16 weeks. The differences between the performance of plants on BAP and coconut water were however not significant. Coconut water from the fresh green fruits was observed to be a suitable alternative to BAP in the *in vitro* culture of banana plants.

Key words: *Cocos nucifera*, BAP, Dwarf Cavendish

INTRODUCTION

Banana is tropical and subtropical tree-like plant that bears fruit and belongs to the family Musaceae. It is regarded as the first fruit crop in the world and is the eighth most important food crop in the world and the fourth most important food crop among the world's least-developed countries, with India being the leading producer in the world with a production of 24,868,390 MT (FAOSTAT., 2012). Banana is considered one of the most important fruits in the world as a staple food as well as, a major export commodity for many tropical and sub-tropical countries. For instance in Uganda banana is an important staple crop and they have the highest per capita consumption of cooking banana in the world (Clarke, 2003).

Bananas and plantains are propagated vegetatively because almost all cultivated banana cultivars are triploid, parthenocarpic and seeds are nearly sterile. The materials used for conventional propagation include corms, large and small suckers and sword suckers (Cronauer and Krikorian, 1984; Arias, 1992). However, conventional planting materials are not the ideal propagule, because they carry weevils, fungal pathogens, nematodes and viruses (Arias, 1992; Sagi *et al*., 1998) and also suffer from slow multiplication, bulkiness and poor phytosanitary quality (Vuylsteke, 1989). Mutation breeding and biotechnological methods can offer useful tools for banana improvement. Therefore, since 1985, shoot tip culture has been increasingly used as an alternative to the generation of conventional plant material (Robinson, 1996). *In vitro* propagation of bananas provide excellent advantages over traditional propagation, these include a high multiplication rate, physiological uniformity, the availability of disease-free material all the year round, rapid dissemination of new plant materials throughout the world, uniformity of shoots, short harvest interval in comparison with conventional plants and faster growth in the early growing stages compared to conventional materials (Vuylsteke, 1989; Daniells and Smith, 1991; Arias, 1992; Suprasanna *et al*., 2008; Patade *et al*., 2008). Recently, *in vitro* propagation has gained great attention because of it numerous benefits. Several researchers have reported the regeneration of *Musa* spp. via micropropagation (Cronauer and Krikorian, 1986; Jarret, 1986;

Diniz *et al*., 1999; Nguyen and Kozai, 2001; Gallez *et al*., 2004; Madhulatha *et al*., 2004; Roels *et al*., 2005).

However, for cost effective and the quest to improve the *in vitro* techniques research efforts have focused on the growth regulating compounds and salt mixes and it seems that there is a good scope toward substituting the expensive chemical nutrient media by low cost natural extracts. Numerous researchers have investigated the effect of using yeast and plant extracts in different *in vitro* culture media. One of the earliest report addressing this point is that of Van Overbeek *et al.* (1941), who succeeded in growing immature Datura embryos by including the liquid endosperm of *Cocos nucifera* (coconut milk) in their culture medium. Thereafter, Nasib *et al.* (2008), Al-Khayri (2010), Beshir *et al.* (2012), Iqbal *et al.* (2013) and De Souza *et al.* (2013) cultured banana and other plants *in vitro* using coconut milk.

Coconut water has been used as a supplement in many laboratories to improve regeneration of plant cells (Maddock *et al*., 1983; Mathias and Simpson, 1986; Al-Khayri *et al*., 1992; Boase *et al*., 1993; Jayasih and Wattimena, 1994; Murthy and Pyati, 2001; Mandal *et al*., 2002; Pyati *et al*., 2002; Krug *et al*., 2005; Yoon *et al*., 2007; Aktar *et al*., 2008; Nasib *et al*., 2008; Baskaran *et al*., 2009; Cheong *et al*., 2009; Pena-Ramirez *et al*., 2010; Bhattacharya *et al*., 2010). Coconut water contains mainly water (94%) and growth promoting substances that can influence *in vitro* cultures including inorganic ions, amino acids, organic acids, vitamins, sugars, sugar alcohols, lipids, nitrogenous compounds and phytohormones (Yong *et al*., 2009).

However, according to Jackson *et al.* (2004) the chemical composition of coconut water is affected by several factors. Jackson *et al.* (2004) showed that coconut water of different coconut varieties contain different concentration of compounds and that the chemical contents also varied during the different stages of maturity.

One of the limiting factors to many laboratories in Ghana and other African countries in the *in vitro* culture of plants is the cost of procuring chemicals for the preparation of tissue culture medium. Ghana abounds in coconut which stretches across the whole coastline of the country and in almost all parts of the southern and middle sections of the country. The fruits are sold cheaply at all times. This study was therefore conducted to explore the possibility of using coconut water as a substitute for BAP in the *in vitro* culture of banana.

MATERIALS AND METHODS

The study was carried out at the tissue culture laboratory of the Plant Genetic Resource Institute, at Bunso in Ghana, from March 2014 to September 2014.

Preparation of plant material: The preparation of the plant followed the method of Buah *et al.* (2011). The banana cultivar, Dwarf Cavendish (*Musa accuminata*) was used as the explant source. Sword suckers were taken from a farmer's field at Bunso in the Eastern Region of Ghana. This was done in the morning, with earth chisel to separate the sucker from the parent at the point of attachment. Roots of the suckers were trimmed off to remove debris, soil and other pathogens that might be present of the plant before washing them thoroughly under running tap water. The leave sheaths were carefully removed to reduce the size of the material. As a first step of the sterilization process, the plant materials were sterilized with 70% ethanol for 3 min and washed three times in sterilized distilled water (Buah *et al*., 2011). More leaf sheaths were then removed aseptically in a clean bench until about two leaves covered the shoot meristem. This process was followed by sterilization with 1% Sodium hypochlorite solution containing a drop of polyoxyethylenesorbitan monolaurate (Tween 20) for five minutes with occasional shaking and there after washed three times with sterilized distilled water. Prior to their inoculation on the medium, each shoot tip (about 1 cm) was longitudinally divided into two halves and again sterilized with 1% Sodium hypochlorite (NaClO) as above for 1 min.

Preparation of coconut water: Coconut water from fresh mature and dry fruits was extracted in sterile chamber from the fruits, by drilling holes through the two micropyles according to the method of Nasib *et al.* (2008). The water was then filtered using a 0.45 μm sterile-mesh and heated at 80-100°C for 10 min with continuous stirring to precipitate out the proteins, fats and other materials. The precipitates were separated by filtration and the filtrate is stored at -20°C for future use (George, 1993).

Media preparation and composition: Three types of media were prepared according to whether coconut water from fresh and dry fruits or BAP was used as the hormone source. Basic MS medium (Murashige and Skoog, 1962) was supplemented with either 4.5 mg L^{-1} 6-Benzylaminopurine or 100 mL L^{-1} coconut water from either fresh or dry fruit. The 100 mL L^{-1} coconut water was used based on previous preliminary study. The 30 g L^{-1} of sucrose was used because it had been the optimal sucrose concentration from previous study with Musa species (Buah *et al*., 2011; Shirani *et al*., 2009). The pH of the media was adjusted to 5.8. About 6 g L^{-1} agar was dissolved in each medium on a hot plate magnetic stirrer before autoclaving the media for 15 min at 121°C. The explants were inoculated into the media and kept under a temperature of 26°C, 16 h photoperiod with an intensity of 3000 Lux and a relative humidity of 60%. The initial sub culturing was done 4 weeks after placing the explants in the media and subsequently at two weeks interval. During sub culturing, materials with multiple shoots were separately removed and placed into different vessels. In all, seven subcultures were done during which data were taken.

RESULTS

Number of root: The mean number of roots obtained with the three treatments (fresh coconut, dry coconut and BAP) is presented in Fig. 1. Root growth was significantly affected by coconut water. Fresh coconut water performed slightly better than BAP. Cultures augmented with fresh coconut and those with BAP commenced root formation earlier than cultures with water from dry coconut fruits (by observation). All explants cultured started rooting after the fifth week in culture. Fresh coconut and BAP recorded early rooting at 6th week. However, those cultured onto media with water from dry coconut took longer time (8 weeks) to root. The maximum number of roots was obtained with medium containing fresh coconut water at 16 weeks was 6.5 roots/plant while those on medium supplemented with BAP had 5 roots/plant (Fig. 1).

The number of leaves increased over the weeks. At the fourth week, all the different treatments recorded 2 leaves/plant. The number of leaves per plant for each week for all the treatment was not significantly different from each other especially between plants cultured on BAP and those on coconut water from fresh green fruits. However, at 16th week dry coconut was significantly different from fresh coconut and BAP (Fig. 2). Dry coconut recorded the least number of leaves almost every week and at 16 weeks while plants cultured on coconut water from dry fruits had 5 leaves/plant, those cultured on medium with coconut water from fresh green fruits had 9 leaves/plant.

It was also noted that plant height was highly influenced by the three treatments. However, the medium with dry coconut showed much lesser growth as compared to the cultures grown with the fresh coconut and BAP (Fig. 3). Water from fresh coconut preformed slightly better than BAP. At 16 weeks, plants cultured on medium supplemented with water from dry coconut fruit had significantly lower shoot height values of 10 cm compared with those cultured on water from fresh fruits and BAP which were 17 and 16 cm, respectively. The various treatments had influence on the fresh weight. No significant difference was observed with the treatments used till week 10 (Fig. 4). On the whole, BAP had slightly

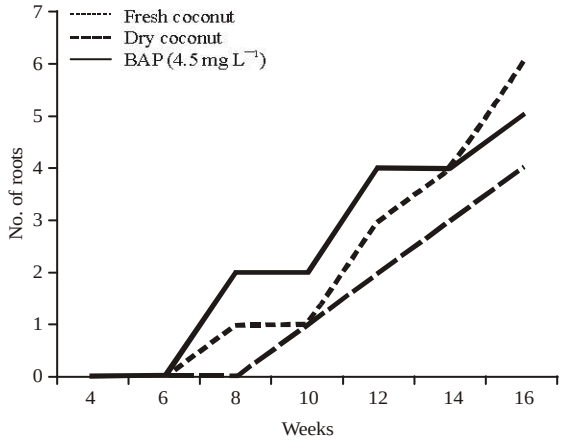

Fig. 1: Number of roots per plantlet of banana after 16 weeks of culture on BAP and coconut water

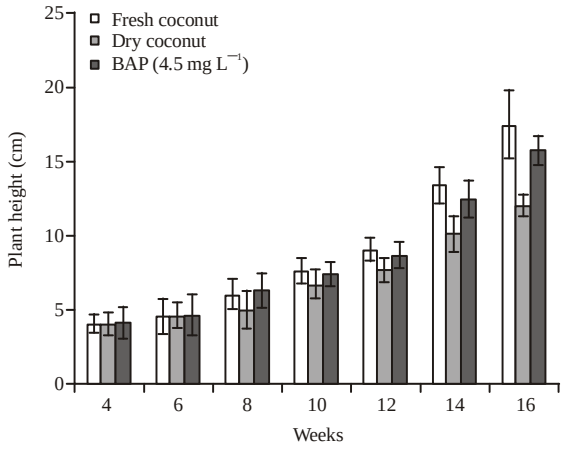

Fig. 3: Mean plant height of banana plantlets cultured for 16 weeks on BAP and coconut water

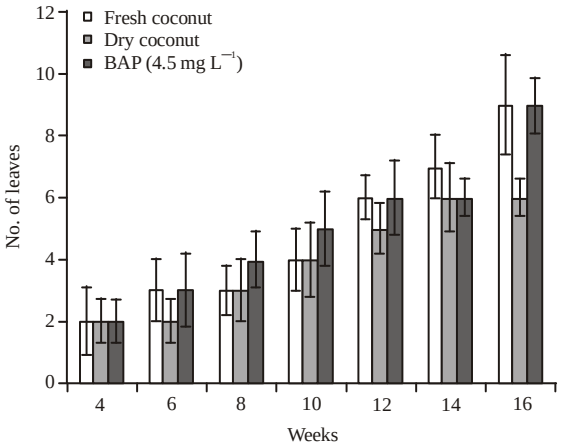

Fig. 2: Number of leaves per plantlet of banana cultured for 16 weeks on BAP and coconut water

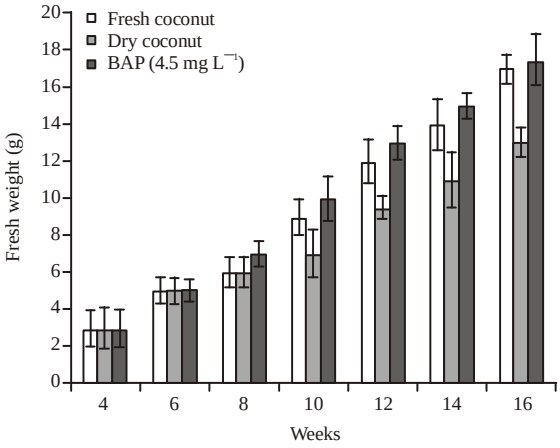

Fig. 4: Mean fresh weight of banana plantlets cultured for 16 weeks on BAP and coconut water

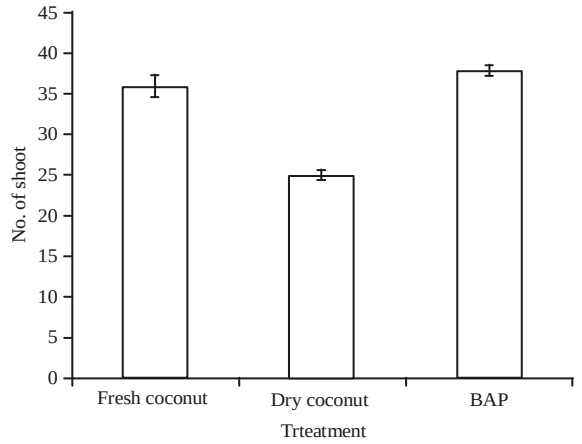

Fig. 5: Mean number of shoots per banana plantlet cultured for 16 weeks on BAP and coconut water

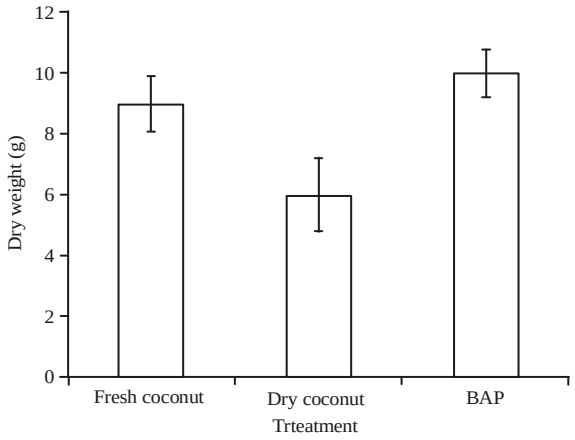

Fig. 6: Men dry weight (g) per banana plantlet cultured for 16 weeks on BAP and coconut water

superior influence on fresh weight than the coconut water. This was clear from the 10th to the 16th week. At 16 weeks plants cultured on BAP had a fresh weight of 16 g plant^{-1} while those on coconut water from green fruits had a fresh weight of 15 g plant^{-1}. The difference between BAP and coconut water from fresh green fruits on one hand and those cultured on coconut water from dry fruits were significant (Fig. 4).

Shoot number and dry weight were determined at 16 weeks after culture. BAP recorded the highest number of shoot, 37 shoots per explant followed by fresh coconut with 25 shoots per explant but no significant differences existed between them. Dry coconut recorded the least values which was significantly different from values obtained from both fresh coconut and BAP (Fig. 5). The dry weight at the end of week 16 followed the same pattern (Fig. 6). Plants that were cultured on medium supplemented with 100 mL L^{-1} coconut water from fresh green fruits had a dry weight of 8.5 g but those cultured on medium with BAP and coconut water from dry fruits had dry weight values of 9 and 6 g per plant, respectively.

DISCUSSION

This study deals with the micropropagation of banana using MS medium supplemented with either coconut water or BAP (fresh coconut, dry coconut and BAP). Addition of growth hormones proved to be beneficial. From the results, it was evident that coconut water from the fresh fruit, supported the growth of banana *in vitro* almost the same as the medium with 4.5 mg L^{-1} BAP.

Nasib *et al.* (2008) have indicated that the addition of coconut water to medium resulted in 95% increase in overall Phosphorus content and this has also been collaborated by Mezzetti *et al.* (1991). This effect of coconut water ultimately resulted in the doubling of sub-culturing time from 4-8 weeks. Many researchers like Vigliar *et al.* (2006) and Krishnankutty (1987), have reported that coconut water contain minerals, vitamins and sugars as well as Cytokinins and Zeatin.

The difference in plant growth between plantlets that were cultured on BAP and those on coconut water was not wide. Plants on coconut water performed satisfactorily compared with those on the 4.5 mg L^{-1} BAP. This confirms that coconut water contain plant growth hormones which can equally support the growth of plants *in vitro*. Since coconut water contains endogenous cytokinins, it is expected that adding it to the medium should have the same or similar effect on growth or morphogenesis.

Kuraishi and Okumura (1961), observed cytokinin activity in fresh coconut water while George *et al.* (2008) and Peixe *et al.* (2007) have confirmed high levels of cytokinin and zeatin in fresh coconut water.

The ability of coconut water to support plant growth *in vitro* is due to its ability to stimulate cell division and morphogenesis. Moreover, the pH of coconut water is within the range of 4.6-5.9 which falls within the pH, 5.8 of MS medium. Not only does coconut water support plant growth *in vitro* but George (1993) has attributed the robustness and high survival rate of plants cultured on coconut water to the high carbohydrate content which could be used to meet the respiratory demands while surviving the physiological shocks of *ex-vitro* procedures.

The number of shoots, shoot height, fresh weight and dry weight of plants cultured on medium supplemented with 100 mL L^{-1} coconut water from fresh fruits were very close to those cultured on medium with 4.5 mg L^{-1} BAP, compared to those on medium supplemented with 100 mL L^{-1} coconut water from dry fruits.

This is however, contrary to what De Souza *et al.* (2013) reported that coconut water only had positive effect on number of leaves on *in vitro* plants, without affecting shoot length and the other parameters. Baque *et al.* (2011) also observed that high concentrations of coconut water decreased all the growth and morphological features as well as induced abnormal plant growth while 50 mL L^{-1} coconut water gave the best results. This contradiction could be due to the fact that they worked on different a plant apart from banana, giving the indication that the response of coconut water *in vitro* could be species

dependent because 100 mL L^{-1} coconut water has also given best results when Villa *et al.* (2010) cultured olive plants *in vitro*.

Low levels of cytokinins and other minerals in the coconut water from dry fruits compared to those in the fresh fruits could be the reason for the low performance of plantlets cultured on medium with coconut water from dry fruits. This has been confirmed by Vigliar *et al.* (2006) and Krishnankutty (1987) who carried out analysis on coconut water from fresh and dry fruits and observed that minerals and hormones in the dry coconut water were lower than those from the fresh fruits. Physiologically, this may be due to the fact that in mature/dried coconut most nutrients in water might have been remobilized into hardened endosperm/copra.

CONCLUSION

This study has shown that the coconut water can still be used in regeneration of plant cell. However, the results of our study imply that the fresh coconut water is better than dry coconut water in culturing banana.

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