Banana Tissue Culture
by
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Introduction

Banana is an economically important fruit crop that is cultivated in many tropical and sub-tropical countries. It is a perennial herbaceous monocot, which belongs to the *musa* genus of the *musaceae* family. In Malaysia, it is the second most widely cultivated fruit crop with a total production of more than 50,000 metric tones. Banana can be consumed both as staple food as well as an export commodity. Pisang Berangan, Pisang Otel, Pisang Cavendish and Pisang Mas are the preferable dessert cultivars, while the cooking cultivars are Pisang Tanduk and Pisang Nangka. About 50% of the banana plantation in Malaysia is cultivated with Pisang Berangan and Cavendish varieties mainly for export.

Conventionally, bananas are grown from their vegetative suckers. This is because almost all of the banana cultivars are triploid, seedless or seed sterile. However, conventional planting is not the ideal method because planting materials may carry fungal pathogen, viruses, weevils and nematodes. Diseases like panama wilt (*Fusarium oxysporium*), head rot (*Erwinia carotovora*) and banana Bunchy Top virus (BTTV) are often found in banana farms propagated using disease-contaminated suckers. Consequently, another alternative method of propagation is adopted through *in vitro* clonal propagation or tissue culture.

Tissue culture is a propagation technique used to clone a single cell or plant materials in culture medium under strict hygienic conditions. In the case of banana, corms, suckers and sword suckers are used as starting materials. The tissue culture method consists of four steps namely, initiation, multiplication, rooting and acclimatization. This method is commonly used in rapid production of superior banana cultivars especially for the export banana industry. It has been successfully practiced by country like India, Philippine, China and Taiwan. However, because of the high cost of production, it is normally practiced by companies which have their own laboratories set up.

Tissue culture propagation method has many advantages over conventional vegetative methods. One of them is the production of disease-free planting material from certified clean sources. Besides this, it offers a rapid mass propagation of superior planting materials. This is important when a new banana variety is released and need to be multiplied in short duration. Propagation via suckers takes a longer time and can produce only 2-3 suckers per mother plant. Tissue-cultured banana plantlets have higher survival rate in the field and give a significant increase in yield and fruit quality as compare to that of sucker plantlets. Moreover, they are easy to transport and require minimum space for multiplication of large number of planting material.

Tissue-culture technique

Tissue-culture of banana using shoot tip and male floral apices had been practised. There were also reports of somatic embryogenesis and culture in liquid medium e.g. temporarily immersion system (TIS). However, the ideal material for tissue culture of banana was the suckers. Suckers of elite banana were collected from the disease-free mother plant. The suckers (Fig. 2) were peeled off and cut into 3cm x 4cm (basal diameter x length). They were surface sterilized in 70% ethanol followed by three rinses in sterile distilled water. They were trimmed in the laminar flow cabinet to the size of 1.0-2.0 mm in length to obtain the meristem tissue (Fig. 3) and leaving a meristematic dome with one or two leaf initials. Conventional propagation was to quarter a sucker into four. Meristem culture had higher mortality rate and a very slow initiation growth. The prepared meristem tissues were culture on Murashige and Skoog (MS) medium supplemented with different concentrations of cytokinin, 6-benzylamino purine (BAP) ranged from 0-2.0 mg/L. There were 7-9 shoots (Fig. 4) developed from a culture in medium supplemented with 2.0 mg/L BAP but only 1-3 shoots developed from a culture in regulator-free medium. Cultures were incubated at 26 ± 2^{0}C under 16h photoperiod. The cultures were routinely transferred into new medium at monthly interval. The darken tissue at the bottom of the

cultures were removed and the number of shoots produced were counted prior to sub-culturing them.

After three months, clump of shoots were dissected into individual plant and transferred to a regulator-free culture medium, which do not promote further shoot proliferation but to stimulate root formation. Rooting regulator was not necessary but neutralized activated charcoal was added to induce root formation (Fig. 5). When the plantlets were ready to be planted out, they were transferred into polybag (Fig. 6) with sterile potting mixture (soil:sand:peat in 3:2:1 ratio). These plants were acclimatized in green house for about two months (Fig. 7). The planted banana was ready for harvest within one year of planting.

In conclusion, it must be noted that there is possibility for viruses to be transmitted through tissue-cultured plantlets. Thus, virus indexing (ELISA) of source materials used should be carried out to prevent the transmission of viral diseases to plantlets. The formation of multiple shoots is promoted by cytokinin. Consequently, one of the most important factors affecting the efficiency of the rate of multiplication was the concentrations of the cytokinin used. For banana, the recommended concentration is 2.0 mg/L. To avoid mutation of plantlets, only use the same tissue materials from less than 10 cycles of proliferation after which, new clean materials of the said cultivar have to be acquired.

The above mentioned tissue-culture protocol for banana is being practised at ARC, Semongok to supply elite banana materials for research purposes.

Fig. 1
Banana fruiting bunch

Fig. 2
Banana suckers were peeled off and cut into 3cm x 4cm (basal diameter x length)

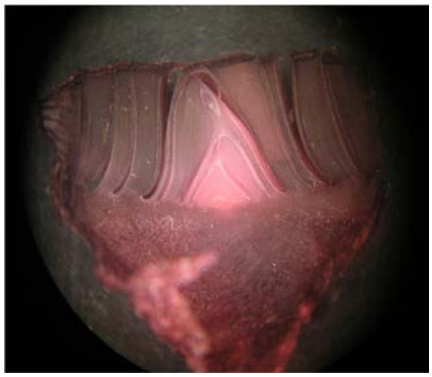

Fig. 3
The meristem tissue of the banana

Fig. 4
Shoot multiplication

Fig. 5
Root formation

Fig. 6
Individual plantlet transplanted into polybag

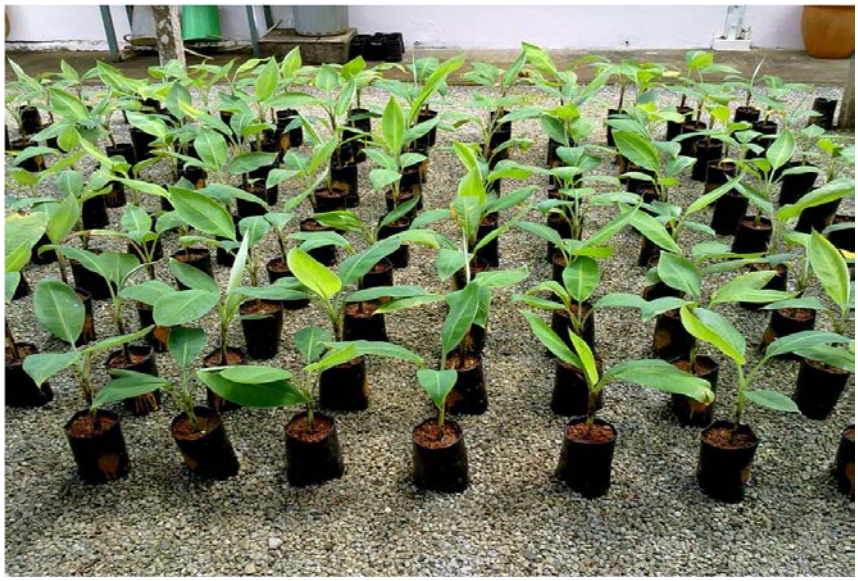

Fig. 7
Micropropagated banana planlets in the planthouse

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