Cultivation and Management Technologies for New Banana Cultivar 'Refen 1' (*Musa* Spp. ABB, Pisang Awak Subgroup)

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Abstract Banana is one of the most important crops in tropical and subtropical regions of China. Fenjiao (*Musa* Spp. ABB, Pisang Awak subgroup) has been grown by small scale famers because of its stable market price and better sugar-acid blend. However, the traditional Fenjiao variety is susceptible to banana Fusarium wilt, has a high plant height (over 5.0 m), and farmers lack large-scale planting techniques. Based on the traditional Fenjiao variety, we selected 'Refen 1' variety through mutagenesis technology, which has low temperature resistance, suitable to marginal soils and relatively low plant height (3.2 -4.0 m). This paper mainly introduces the major techniques of propagation, cultivation, and post-harvest stages of 'Refen 1' banana, including the selection of explant materials, treatment, disinfection, initial culture, nursery hardening of in vitro-produced banana plants, and transplant of tissue culture seedlings. The major points of the cultivation technology include banana plantation selection and preparation, planting methods, irrigation and fertilizer management, pruning and retaining, and prevention and control of plant diseases and insect pests.

Key words 'Refen 1' banana, Pisang Awak (ABB), Banana propagation, Cultivation technology, Fruit harvest and post-harvest storage technology

1 Introduction

South China is one of the areas where banana originated[1] and has been domestically cultivated for thousands of years. Fen Jiao belongs to Pisang Awak subgroup (ABB) and is also known as milk banana in Hainan, China. It has the advantages of good taste and high nutrient content and is thus much favored by consumers[2], and some studies suggested that Pisang Awak banana consumption was associated with strong reduction in risk of colorectal cancer[3] and had antifungal[4] and antibacterial effects[5]. Its main characteristics include ample height (> 4 m), the back of its leaves is covered with wax powder, fruit fingers are plump and short and 11 -15 cm in length, the peel is thin, and its flesh is white, smooth, sweet, and refreshing[6] (Fig. 1). It is more resistant to black Sigatoka disease, banana bunchy top virus, and cucumber mosaic virus than *M.* Cavendishii subgroup (AAA). In addition, it is highly resistant to drought and cold (Fig. 2). It has higher economic value than the ordinary banana[7], but it is vulnerable to Panama disease[8-9]. Since 1992, the suckers of the local Fenjiao variety 'Xinglong Milk Banana' (Pisang Awak subgroup) in Xinglong Town, Hainan Province have been asexually propagated. In 2004, somatic mutants with thick pseudo-stems, high yield, and excellent quality were obtained. Since May 2005, the agronomic traits of this species have been evaluated in the field. Several years of variety comparison and ecological area and production experiments have shown that it has excellent traits, such as high field yield, quality, appearance, high stress resistance, and superior genetic stability. In July 2015, it was designated as 'Refen 1' [10]. To further extend the distribution of this variety, we assessed major technical points ranging from its propagation to post-harvesting.

2 Commercial seedling production techniques

Note: A. Growth of 'Refen 1' field four months after planting; B. Refen 1 fruiting after 13 to 14 months of growth in the field; C. A bunch of ripened 'Refen 1' fruit. (bar = 5 cm).

Fig. 1 A new banana cultivar 'Refen 1'.

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3 Major features of the propagation technology

To generate 'Refen 1' banana seedlings, suckers or tissue culture seedlings may be used as propagation materials. Generally, the newly reclaimed banana plantation should select tissue culture seedlings. These can give high-quality plants free of disease and nematodes, which are mainly used in commercial plantations and lately in smallholder production via mass micropropagation[11]. The major features of the 'Refen 1' tissue culture technology are hereby described.

3.1 Selection of explants For banana propagation, shoot tip cultures have been most widely used[12], Healthy suckers of excellent lines were selected for tissue culture of explants to produce disease-free seedlings. It is better to select the strong and healthy 'Refen 1' buds in disease-free banana plantations after 9:00 AM on days with better weather. Before 9:00 AM or during overcast or rainy days, materials may carry more microorganisms, which are relatively more difficult to disinfect and thus buds are easily infected.

3.2 Treatment of explants The roots and parts of the pseudostems were cut off the suckers, washed with washing powder water, then flushed with tap water three to four times. After flushing, five to six leaf sheaths that were wrapped around each sucker were peeled off. The suckers were initially treated, and then transferred to the clean bench for disinfection.

3.3 Disinfection of explants To disinfect the explants and before placing these on a clean bench, one to two layers of the outermost sheaths were peeled off, and the browning part at the base was cut. Then, the explants were placed in a disinfecting cup and sterilized in 75% alcohol for 30 sec, followed by washing with sterile water three to four times, 6% sodium hypochlorite for 30 min, then sterile water three to four times.

3.4 Establishment of regeneration system of Refen 1 The washed explants were blotted dry with filter paper, any excess leaf sheaths were cut, and then inoculated in the initial culture medium. To induce adventitious bud formation in 'Refen 1', the culture medium was supplemented with 6-BA (6-Benzylaminopurine) (4 mg/L). At the proliferation culture stage, the MS medium was supplemented with 6-BA (2.5 mg/L) and AD (3.0 mg/L). After several subcultures, tube seedlings and proliferated seedlings were observed. At the rooting stage, the MS medium was supplemented with BA (2.0 mg/L) and NAA (0.2 mg/L) to regenerate complete plantlets, with a rooting rate reaching 98%.

3.5 Nursery hardening of tissue culture seedlings The tissue culture seedlings obtained from the laboratory were acclimatized to shady conditions. Rooted 'Refen 1' tissue culture seedlings were transferred to the greenhouse and allowed to grow and acclimatize for 10 – 15 d. Then, the tissue culture seedlings were soaked in 0.1% carbendazim for 30 sec to wash off the culture medium, then transferred to soilless nutrient medium (10:1 coconut husk:organic fertilizer, nutrient cup size: 10 cm × 10 cm), sprayed with root water, and then covered with arched film to maintain high humidity. Upon emergence of new leaves, the film covering the seedlings were removed, and the seedlings were sprayed with 0.5% potassium dihydrogen phosphate. The humidity of the nutrient medium was checked twice a day, and water was added according to the humidity. Liquid compound fertilizer (N: P: K = 15: 15: 15, 1% – 2% concentration) was applied once every 5 – 10 d, and the seedlings were regularly inspected for leaf spots and nematodes. After 45 – 60 d of environmental acclimatization, the seedlings had gradually adapted to the field environment. When the seedlings had developed six to eight functional leaves, reached a plant height of 30 – 50 cm, and exhibited ample growth, these were transplanted to the field (Fig. 3).

4 Cultivation and management techniques

The main points of cultivation and management of 'Refen 1' included plantation selection and preparation planting methods, interim management, water and fertilizer management, deflowering and bagging, and prevention and control of plant diseases and insect pests.

4.1 Banana plantation selection and preparation 'Refen 1' banana can be planted during any season in Hainan, China. and its growth period is 12 – 14 months. To avoid the typhoon season, it is advisable to plant 'Refen 1' banana in March. Due to its ample height and developed root system, the soil at the planting site of 'Refen 1' should be > 60 cm in depth, within a pH range of 5.5 – 7.0, and far from the source of Panama disease. The optimal soil is loose, fertile, and sandy, with excellent drainage. Before transplantation, soil ploughing to a depth of > 30 cm should be conducted to form wide ridges and deep gullies. Debris and malignant weeds in the field should be removed and the soil dried in the sun for two weeks. Fully fermented farm manure with calcium superphosphate should be used as base manure, applying about 5 kg of organic fertilizer per hole, 0.5 kg of calcium, magnesium, and phosphate fertilizer, and the topsoil should be filled with organic fertilizer. Using fields that have not been planted with banana, melon, tobacco, and solanaceous crops can reduce the occurrence of banana diseases[13]. The field that has been planted with bananas should be rotated with other crops, such as Chinese Chive to reduce the incidence of banana panama disease[14].

4.2 Planting method Since 'Refen 1' banana plants are stout, these are generally planted in a single row. If the banana plantation has excellent drainage, these can be planted in a triangular form (with one ridge and two rows). The row spacing of the flat field plants is 2.5 m × 2.5 m, and about 1 500 plants may be planted in one hectare field; the row spacing of slope plants is 3.0 m × 2.5 m, and about 1 350 plants may be planted in one hectare field. During planting in the field, adequate water must be sprayed onto the roots. Bananas are vulnerable to underground pests such as nematode worms[15]. to prevent damage by underground pests, when planting banana tissue-culture seedlings, avermectin can be used for treatment. The method of using avermectin is to dilute 1 000 times with 1.8% avermectin emulsifiable concentrate. If mix the rot straws in the soil for planting to the field and make film covering treatment, the effect will be better[16].

Note: A: Comparison of soluble sugar and starch content between two varieties, namely, Refen 1 (RF) and Baxi (BX). Ps, pseudostems; B: Ra, rachis. Cold resistance of Refen 1; C: Baxi in Nanning, Guangxi, China in 2014.

Fig. 2 Comparison of cold hardiness traits in banana

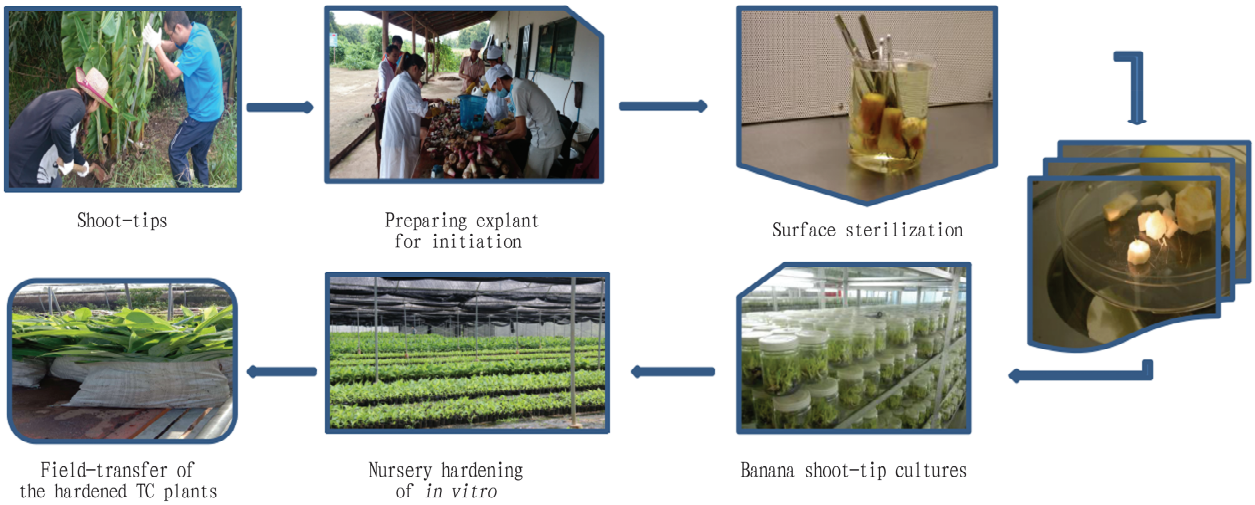

Fig. 3 Flow diagram of micropropagation of 'Refen 1' banana plantlets

4.3 Field weeds management Within two weeks after planting, weeds may grow in the banana orchards. It is essential to manually remove weeds, cover the seedlings with a dark film or directly apply herbicides and only spray during windless days. Mulch not only has characteristics of the common mulch but also has the advantage of having high weeding and degradable ratios as well as safe usage in banana production[17-19]. The chemical herbicides dalapon, diuron, paraquat, and ametryne have been approved for weed control in banana orchards. Paraquat is a general contact herbicide that has been used to knock down or to disrupt weed growth. Dalapon is taken up by plants either via leaf or root absorption. Dalapon is used in severe weed growth. Ametryne and diuron are recommended to control young seeding weeds and grass. After planting for three months, buds emerge; it is necessary to cut off any unnecessary buds to reduce non-essential nutrient consumption.

4.4 Fertilizer and water management 'Refen 1' bananas are tall plants with highly developed root systems. Its yield is high; therefore, its nutrient demand is also high. Studies have shown that under moderate soil fertility conditions, 'Refen 1' has lower nutrient utilization efficiency than Baxi (*M.* Cavendish AAA) banana, more nutrient input, and relatively lower nutrient utilization efficiency[20-21]. The growing period of 'Refen 1' is relatively long. To accelerate the growth of 'Refen 1,' scientific water and fertilizer management must be conducted[22]. Before planting, high-nitrogen high-potassium compound fertilizers (20-5-20) or general-purpose compound fertilizers as base fertilizer should be applied into the planting hole, at a rate of 600 kg to 750 kg ha. Within one month after planting, 225 kg to 300 kg per ha of high-nitrogen and high-potassium compound fertilizer (20-5-20) should be added into a circular shallow ditch. Two months after planting, the circular shallow ditch should be opened again, to which 375 to 400 kg ha of high-nitrogen and high-potassium compound fertilizer (20-5-20) is applied. During the middle and late growth stages, when the 'Refen 1' has grown to 23 to 24 pieces and 27 to 28 leaves, the fertilizer should be applied twice, which includes 675 kg to 750 kg of high nitrogen and high potassium compound fertilizer (20-5-20). Then, three holes should be made within 40 cm to 60 cm from the drip line of the banana leaf edge, and fertilizers are added into these holes, which are then covered with soil and spray water is applied. At the budding stage, 750 kg to 900 kg of high nitrogen and high potassium compound fertilizer (20-5-20) should be applied using the same method as that described during the middle stage of growth. Furthermore, based on the actual growth conditions of 'Refen 1' banana, the top dressing outside the roots should be prepared based on the types of fertiliz-

ers and growth stage of 'Refen 1' banana. For example, at the young seedling stage (7 – 12 pieces of leaves), 0.4% – 0.7 % of urea fertilizer should be applied by spraying; at the young fruit stage (22 leaves to fruit 40% maturity), 0.3% – 0.4% potassium dihydrogen phosphate should be applied by spraying.

4.5 Pruning After planting in the field and growing to a certain height, 'Refen 1' banana plants start to develop suckers. Generally, a banana mat produces significantly more suckers than needed for fruit production. The problem is that with too many suckers growing at the same time, small bunches are produced and productivity decreases. Consequently, pruning or removing surplus suckers is necessary when growing bananas commercially. To concentrate all of the nutrients for the growth and development of the mother plants, it is necessary to perform several cycles of sucker removal before harvesting. Sucker removal in 'Refen 1' banana should not involve the traditional banana spade to avoid underground injury and infection of Panamanian disease. It is recommended to cut the buds from the ground, then use a sharp iron set to insert into the banana heart, rotate with force to damage the growth point, and then drip 2 – 4 drops of kerosene to prevent the growth of buds. 'Refen 1' banana suckers should be selected within one month after budding based on the principle of healthy, uniform growth, suitable plant spacing, but not in the same direction of bunched fruit.

4.6 Prevention and control of plant diseases and pests Musa crops are often afflicted by various devastating diseases[23]. It is recommended to adopt agricultural and physical control measures as well as advocate biological control. Diseases of 'Refen 1' banana mainly include Panama disease, helminthosporium leaf spot, cordana leaf spot, leaf edge wilt, freckle, and anthrax[24]. Prevention and control measures should mainly concentrate on Panamanian disease. There are currently no specific pesticides that should be used in the prevention and control of 'Refen 1' diseases. Thus, prevention and control measures mainly involve the selection of disease-free seedlings, rational irrigation, and scientific soil management. During planting, it is necessary to consider prevention and control measures against nematode[25-26]; at the middle stage of growth, effects should concentrate on preventing infection with Spodoptera litura Fabricius, Rhyncophorus ferrugineuss; at the late stage, it is essential to prevent the occurrence of thrips, suggesting that the thrips may disperse from the sucker to the mother plant and then to the bunch[27].

Leaf skipper, *Phyllotreta striolata* (striped flea beetle), aphid, *S. litura* Fabricius, *Stephanitis typicus*, and *Tetranychus piercei* can be treated with dilution 1,000 times of 52.25% Nurelle or 800 times of 48% Lorsban. *Rhyncophorus ferrugineuss* can be treated with 15% Lorsban at a concentration range of 6 000 – 7 500 g/ha, mixed with sand at a ratio of 1∶40, and sprayed onto leaf tops. It is necessary to use safety equipment, such as eye shades and gauze masks, while spraying.

'Refen 1' banana is not resistant to Panama disease. Thus, to reduce the harm and spread of Panama disease, sprinkler irrigation or drip irrigation should be used[28], not flood irrigation and vegetated ground cover management can reduce the severity and incidence of Fusarium wilt in Pisang awak banana[29]. Once the banana virus disease and wilted plants are observed, the infected plant is injected with glyphosate, removed, buried *in situ*, and disinfected with lime.

5 Fruit care technology

Three weeks after budding of 'Refen 1' banana, it is necessary to make corrections. When the female flowers blossom to the failure of fruiting of the last 2 – 3 hands of flowers, it is necessary to cut the buds, keeping about nine hands, and keep the cut area about 13 cm from the last hand[30]. If the first hand is far from the second hand, the first hand can be cut off to facilitate uniform growth of each hand. If planting in areas where frost may occur, it is necessary to bag the fruit to reduce the risk of frost damage. Bagging can be conducted 7 – 10 d after cutting off the buds. First, newspaper and PE plastic film bags are employed to wrap the bunches, with the upper part tightly attached to the base of the bunches, and the lower part remains permeable to prevent infection of pathogens, such as *Cladosporium cucumerinum* with rainwater. 'Refen 1' banana rapidly matures using these conditions. When the fruits are plump, these should be treated as soon as possible. The fruits can be harvested when these reach more than 85% maturity. Bananas should be harvested once the dew has dried out during cloudy days or mornings of fine days. At 15 d before harvesting, irrigation should not be performed, and any accumulated water in ridges and ditches of the banana garden should be drained. During harvesting and transport, the banana bunches should be handled with care, should not come in contact with the ground, and avoid scratches or mechanical damage to extend its storage period[31]. It is also necessary to use custom-made pressure- and humidity-resistant cartons. One to two layers of plastic film are placed into these cartons, followed by the disinfected and dried fruit in plastic bags. The bags should be tightly closed to prevent the escape of moisture and inhibit fruit respiration, reduce contact with the carton wall, and ensure excellent external features of the bananas.

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