Evaluation and Tissue-Culture Conservation, Multiplication and Distribution of Rare and Carotenoid-**Rich Fe'i Banana Cultivars in Micronesia**

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Abstract

Musa, a plant genus of extraordinary significance to human societies, produces the fourth most important food in the world today, the edible bananas and plantains. Banana is one of the most important staple food crops grown in the Micronesian Region for local consumption as well as for export. Two cultivars of rare F**e'i banana, 'Kulasr' and 'Kulondol'**, have yellow and orange flesh, and are rich in provitamin A and other carotenoids. Both are robust plants bearing erect bunches of brilliant orange-gold to purple fruits on maturity, which are delicious and nutritious. Tissue-culture multiplication of banana provides excellent advantages over traditional multiplication, including a high multiplication rate, physiological uniformity and the availability of disease-free material throughout the year, ensuring safe and rapid distribution of new planting materials. A study was undertaken to develop an efficient, rapid and reproducible regeneration **protocol for 'Kulasr' and 'Kulondol' for mass multiplication and in vitro conservation. Banana plantlets were produced at mas**s scale through tissue culture, acclimatized in the greenhouse, **distributed and established in the field. Ultimately, this exercise helped greatly to save rare Fe'i bananas on Micronesian i**slands. This paper discusses the research and extension work done on evaluation and tissue-culture conservation, multiplication and distribution of these two rare and carotenoid-**rich Fe'i banana cu**ltivars on small islands of Micronesia.

INTRODUCTION

Bananas, a major global food staple, are the fourth most important food in the world, after rice, wheat, and maize. Bananas, consumed cooked or raw, either as green, half-ripe, or ripe banana, are one of the most ignificant sources of calories for the human diet worldwide. Bananas are particularly good source of potassium. The yellow and orange-fleshed bananas are also rich in provitamin A and other carotenoids. Two of the carotenoid-**rich cultivars from Micronesia, 'Kulasr' and 'Kulondol', are Fe'i bananas, referred as** *Musa troglodytarum* (Stover and Simmonds, 1987). These bananas are characterized by an erect bunch and red sap (Daniells, 1995; Sharrock, 2000). The **β-carotene content of 'Kulasr' and 'Kulondol' is 2,230 and 4,960µg 100g**$^{-1}$, respectively (Englberger et al., 2003), whereas, it is only 21µg 100g^{-1} in common Cavendish banana (Holden et al., 1999).

Bananas and plantains are propagated vegetatively because almost all cultivated banana cultivars are triploid, seedless, or seed sterile. The materials used for conventional propagation include corms, large and small suckers, and sword suckers from around the base of a mature plant (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 poor phytosanitary quality, bulkiness, and slow multiplication since only a few suckers are produced each year (Vuylsteke, 1989). Therefore, the propagation process can be speeded up dramatically by using the technique of micropropagation as an alternative to conventional plant propagation (Robinson, 1996). In vitro propagation of banana provides excellent advantages over traditional propagation, including a high multiplication rate, physiological uniformity, the availability of disease-free material throughout the year, safe and 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). Tissue culture also plays a vital role in the distribution and conservation of germplasm, safe exchange of internal planting material and rapid propagation of newly selected hybrid cultivars.

Apart from the influence of genotypes, shoot proliferation rate and elongation are affected by cytokinin types and their concentration. Adenine-based cytokinins are used in several *Musa* species for tissue culture. 6-benzylaminopurine (BAP) is the most commonly preferred cytokinin (Cronauer and Krikorian, 1984; Vuylsteke, 1989). The others are isopentyladenine (Dore Swamy et al., 1983), zeatin (Vuylsteke and De Langhe, 1985) and 6-furfurylaminopurine (Cronauer and Krikorian, 1984). The concentration of exogenous cytokinin appears to be the main factor affecting multiplication. The optimum recommended concentration of BAP is 20µM for banana micropropagation (Vuylsteke, 1989). The auxins are most frequently used to induce root initiation in the banana (Vuylsteke, 1989). Rooting is also achieved on Murashige and Skoog medium (MS) (1962) without any growth regulators (Cronauer and Krikorian, 1984; Jarret et al., 1985).

The important factor affecting the efficiency of micropropagation system is the rate of multiplication. It has been observed that banana multiplication rate is genotype dependent. The present research is focused on in **vitro multiplication, conservation and field evaluation of Fe'i banana cultivars 'Kulasr' and 'Kulondol'** on small islands of Micronesia.

MATERIALS AND METHODS

Healthy and young suckers of 'Kulasr' and 'Kulondol' (*Musa troglodytarum* cultivars) were collected from mother plants to generate explants. Explants were thoroughly washed with tap water and were surface sterilized by immersion in 6% sodium hypochlorite solution with 5 drops of Tween 20 (Sigma-Aldrich, USA) for 30 min followed by a treatment with 0.1% (w/v) mercuric chloride solution for 5 min. Explants were trimmed and shoot meristems were used to initiate cultures.

MS medium solidified with 0.8% agar containing different combinations of growth regulators and 3% sucrose was used throughout the study. The pH was adjusted 5.8 before autoclaving at 121°C and 1.05kg cm^{-2} for 15 min. Cultures were initiated on MS medium containing 100mg L^{-1} ascorbic acid (AA), 1g L^{-1} activated charcoal (AC) and augmented with 1µM IAA and 10µM BAP, and were given a passage after 4 weeks. The established cultures were transferred on MS medium augmented with 5µM BAP. For further growth and subsequent multiplication, passages were given every 8 weeks. Every 8 weeks, multiplication rate and shoot length were recorded, and each experiment was replicated three times. A 12-h photoperiod with room temperature of 24-26°C, light intensity of 40µmol m^{-2} s^{-1} (cool white fluorescent), and 55% relative humidity were maintained for multiplication.

Fully elongated shoots with approximately 5-7cm lengths were separated from the clumps and used for rooting experiments. MS medium containing 1g L^{-1} AC and augmented with 5µM IAA was used for root induction. After 4 weeks of incubation, percentage of rooting, number of roots per shoot and root length were recorded and each experiment was replicated three times.

To standardize the acclimatization procedure, well-rooted plantlets with approximately 8-10cm shoot lengths, thoroughly washed in tap water, were transplanted into 72-well plastic trays containing sterilized potting mix. The trays were placed in a greenhouse. They were watered every day with tap water and once per week with one-fourth strength MS mineral salts. Survival of plants was recorded after 4 weeks. After 4 weeks of ex vitro growth in the greenhouse, plants were fully acclimatized and transplanted into a screened nursery in the poly bags. The plants were kept in the screened nursery for 8-12 weeks until they reached a height of 60cm. At this stage, the plants were distributed to growers to plant in the field for evaluation.

RESULTS AND DISCUSSION

To initiate cultures of Fe'i banana cultivars 'Kulasr' and 'Kulondol', shoot meristems were used as explants, and were inoculated on MS + 1µM IAA + 10µM BAP + 100mg L^{-1} AA + 1g L^{-1} AC. Base of the explants embedded in the medium became slightly brown due to leaching of phenolics and, therefore, a passage was given after 4 weeks. After 8 weeks of passage small, creamy nodular structures (Fig. 2) appeared on the surface of the explants. These nodular structures were transferred on MS + 5µM BAP, which resulted in continuous proliferation of 8±4 green shoot buds (Fig. 3) per explant within 8 weeks of transfer. For further growth and subsequent multiplication, passages were given every 8 weeks. During third passage, 20±6 multiple shoots per explant were recorded. After 5 subsequent passages, 70-92 shoots per explant with 5-7cm lengths (Fig. 4) were recorded. Collected data proved that the cultures that showed high multiplication rate in first two passages, continued to do so in further passages, therefore, discarding the cultures with low multiplication rate after first two passages could be appropriate to save resources.

Shoots of 5-7cm lengths were transferred on MS medium augmented with 5µM IAA, and 100% rooting was recorded in 1 week. After 4 weeks, 8±6 roots per shoot with 10-18cm lengths were observed.

Well-rooted plantlets (Fig. 5) with approximately 8-10 cm lengths were transplanted into 72-well plastic trays. The trays were kept in the greenhouse for initial 4 weeks. After that, the plants were transplanted in the poly bags and were exposed directly to nursery conditions in a screened nursery. After 4 weeks in the nursery, 89% survival rate was observed. Fully acclimatized plants of minimum 60cm lengths (Fig. 6) were distributed (Fig. 7) to growers to plant in the field for evaluation. Field evaluation data proved that tissue culture multiplied (Fig. 1) plants showed vigorous growth, early maturity, uniformity, more disease-freeness and better yield (Fig. 8-9) when compared with plants produced through conventional methods. There was no deterioration observed in the quality of plants conserved in vitro up to three years.

(Fig. 1-5) Tissue culture multiplication; nodular structures; green shoot buds; multiple shoots; well-rooted plantlets

(Fig. 6-**9) Acclimatized plants in the screened nursery; distribution, 'Kulasr' fruits; 'Kulondol' fruits**

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