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ACCLIMATIZATION OF *IN VITRO* PROPAGATED RED BANANA *(MUSA ACUMINATA)* PLANTLETS

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ABSTRACT
The present study was carried out at Plant Tissue Culture Laboratory, Division of Horticulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendr, Bangalore during 2011-2012. Primary and secondary hardenings are integral and vital activities of the process of tissue culture banana. Propagation Studies on primary hardening media (cocopeat, vermicompost, sand and vermiculite) and secondary hardening mixtures [(red soil + sand + cocopeat (1:1:1 v/v), red soil + sand + FYM (1:1:1; v/v) and red soil + sand + vermicompost (1:1:1; v/v)] of *in vitro* propagated Red bananan plantlets showed cocopeat was the best medium for primary hardening in terms of per centage survival of plantlets (95.00 %), plantlet height (5.58 cm), number of leaves (3.20), plantlet diameter (4.59 mm), number of primary roots per plantlet (5.20), length of primary roots (5.18 cm) and number of secondary roots per plantlet (25.50), whereas red soil + sand + cocopeat (1:1:1; v/v) recorded best results in terms of plantlet height (20.50 cm), plantlet diameter (11.60cm) length of leaves (15.43 cm), width of leaves (6.47 cm), number of primary roots per plantlet (12.30) and number of secondary roots per plantlet (331.20) followed by red soil + sand + FYM (1:1:1; v/v) mixture were good for secondary hardening. Finally concluded that combination of red soil + sand + cocopeat (1:1:1 v/v) was best medium for primary and secondary hardening of *in vitro* propagated Red bananan.

INTRODUCTION

Bananas and plantains are giant perennial herbs and provide an essential food source for more than 400 million people throughout the developing countries of the tropics and the subtropics. It is the most important and most widely grown fruit crop in the world. It ranks as the fourth major crop after rice, wheat and maize and is considered as a poor man's apple in tropical and subtropical countries. Generally, banana cultivars are good sources of carbohydrates, proteins, vitamins and minerals. Red banana is a cultivar with geographical indication in Kamalapura, Karnataka, India. As the banana cultivars are having high degree of sterility and polyploidy the conventional breeding methods are difficult in banana improvement. Many pests and diseases are also threatening the good production of banana cultivars. In order to augment conventional breeding and to avoid constraints imposed by pests and pathogens, transgenic and *in vitro* approaches are being considered (Jain and Swennen, 2004). Therefore plant tissue culture or micropropagation of banana has been extensively used for rapid production of high quality, disease free and uniform planting material irrespective of the season and weather. However a large scale application of this technology is hindered by high mortality experienced by micropropagated plantlets when transferred to *ex vitro* conditions. During *in vitro* conditions, plantlets grow under special conditions in relatively air-tight vessels *i.e.,* air humidity is higher and irradiance is lower than in conventional culture. Microshoots, upon transfer to *ex vitro* conditions are exposed to abiotic stress (altered temperature, light intensity and humidity conditions) and biotic stress conditions *i.e.*, soil microflora (Deb and Imchen 2010). High mortality is observed upon transfer of microshoots to *ex vitro* conditions as the cultured plants have non functional stomata, weak root system and poorly developed cuticle (Mathur *et al.,* 2008).The physiological and anatomical characteristics of micropropagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field (Hazarika, 2003). Development of cuticle, epicuticular waxes, and effective stomatal regulation of transpiration occurs leading to stabilization of water potential of field transferred plantlets. (Silova *et al.,* 1999). Therefore Primary and Secondary hardening is an integral and vital activity of the whole process of tissue culture technology. Improper hardening leads to the failure of whole technology and the industry itself. Success in hardening is a must for an industry for its survival (Radheshyam and Subramani, 2008). Despite this important status it remains underexploited and it is hard to find any research being done to enhance the supply of quality banana planting material to the farmers. After studies on shoot proliferation and rooting *in vitro* were carried out, the present study was undertaken to identify the optimum media for acclimatization of *in vitro* propagated Red banana plantlets under *ex vitro* conditions.

MATERIALS AND METHODS

Primary hardening

The present study was carried out at Plant Tissue Culture

Laboratory, Division of Horticulture, University of Agricultural Sciences, Gandhi Krishi Vignana Kendr, Bangalore during 2011-2012. *In vitro* rooted plantlets were removed from the culture bottles and washed with water. They were given a quick dip in 0.1% Carbendazim solution and transferred to individual micropots in a protray containing media (cocopeat, vermicompost, sand and vermiculite) and placed in polytunnel located in shade house for five weeks. Inside the shade house the temperature ranged between 25 to 27ºC and the relative humidity was maintained between 80 to 90 per cent inside the polytunnels (Jarret, 1986 and Wong, 1986). The experiment was arranged in completely randomized design (CRD) with ten replications; each replicate consisted of one micropot. At the end of culture cycle the effect of medium on establishment of plantlets. Data on growth parameters *viz.,* per centage survival, mean plant height (cm), mean plantlet diameter (mm), mean number of leaves, mean number of primary and secondary roots per plantlet, mean length of primary root (cm) were recorded and the data were analysis statistically.

Secondary hardening

After primary hardened plantlets were transferred from micropots to polybags containing substrate made of the mixtures [(red soil + sand + coco peat (1:1:1 v/v), red soil + sand + FYM (1:1:1 v/v), red soil + sand + vermicompost (1:1:1 v/v)] treated with fungicide solution (0.1 per cent Carbendazim). The plantlets were maintained for six weeks inside a green house where the temperature ranged between 25 to 30 °C and relative humidity between 60 to 70 per cent (Jarret, 1986 and Wong, 1986). The experiment was arranged in completely randomized design (CRD) with ten replications; each replicate consisted of one polybag containing one plantlet. At the end of culture cycle the effect of medium on establishment of plantlet. Data on growth parameters *viz.,* Per centage survival, mean plant height (cm), mean plantlet diameter (mm), mean number of leaves, mean length of leaves, mean width of leaves, mean number of primary and secondary roots per plantlet, mean length of primary root (cm) were recorded and the data were analysis statistically.

RESULTS AND DISCUSSION

Primary hardening is an integral and vital activity of the whole process of tissue culture technology. Improper hardening leads to the failure of whole technology and the industry itself. Success in hardening is a must for an industry for its survival (Radheshyam and Subramani, 2008). In the present study cocopeat showed to be far superior to other potting media in

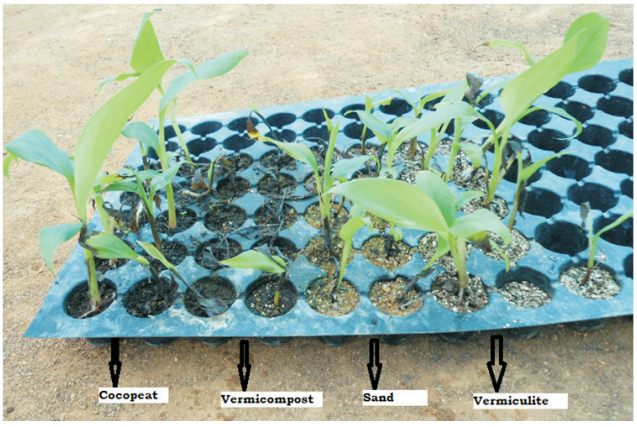

Plate 1: Effect of potting media on primary hardened plantlets

Plate 2: Secondary hardened plantlets a) Red soil + Sand + Cocopeat(1:1:1), b)Red soil + Sand + FYM (1:1:1:1), C) Red soil + Sand + Vermicompost (1:1:1)

Table 1: Effect of media on primary hardening of *in vitro* propagated plantlets

Treatments	Percent survival(%)	Plantlet Height (cm)	Plantlet Diameter (mm)	No of leaves	No of Prim roots/plantlet	Length of prim roots (cm)	No of sec. roots/ plantlet
Cocopeat	95.00	5.58	4.59	3.20	5.20	5.18	25.50
Vermicompost	25.00	3.26	2.90	2.00	1.80	1.52	2.80
Sand	65.00	3.92	3.67	2.20	2.20	1.78	3.30
Vermiculite	80.00	4.55	3.85	2.70	4.10	3.21	9.10
SE m ±	2.73	0.19	0.15	0.16	0.19	0.15	0.36
CD at 1%	7.44	0.52	0.42	0.46	0.52	0.43	0.98

Table 2: Effect of media on secondary hardening of *in vitro* propagated plantlets

Treatments	Percent survival(%)	Plantlet Height(cm)	Plantlet Diameter (mm)	No of leaves	Length of leaves (cm)	Width of leaves (cm)	No of primary roots/ plantlet	Length of primary roots (cm)	No of secondary roots/ plantlet
Red soil + Sand Coco peat (1:1:1)	100(89.47)	20.50	11.60	5.80	15.43	6.47	12.30	15.04	331.20
Red soil + Sand + FYM (1:1:1)	100(89.47)	16.45	10.39	5.80	13.78	5.39	10.70	16.67	259.00
Red soil + Sand + Vermicompost (1:1:1)	100(89.47)	12.70	9.19	5.30	11.52	4.45	8.80	13.50	191.90
SE m ±	NS	0.40	0.33	0.14	0.41	0.22	0.43	0.48	9.59
CD at 1%	-	1.11	0.94	0.39	1.14	0.62	1.21	1.35	26.58

terms of per centage survival of plantlets (95.00 %), plantlet height (5.58 cm), number of leaves (3.20), plantlet diameter (4.59 mm), number of primary roots per plantlet (5.20), length of primary roots (5.18 cm) and number of secondary roots per plantlet (25.50), whereas vermicompost was unsatisfactory (Table1, plate 1). This may be due to better aeration, water holding and nutrient supplying capacity of cocopeat as compared to vermiculite, sand and vermicompost. The poor result obtained with vermicompost may be explained by its structure which became muddy and compact (Dewir *et al.*, 2005). Some of the findings of earlier workers indicated a survival percentage ranging from 80-100 when banana rooted plantlets were transferred to ex vitro hardening media under Green House or Shade House conditions (Palai and Das, 2002; Molla *et al.*, 2004 and Acharjee *et al.*, 2004).

The potting mixture consisting of red soil + sand + cocopeat (1:1:1 v/v) recorded the best results in terms of plantlet height (20.50 cm), plantlet diameter (11.60cm) length of leaves (15.43 cm), width of leaves (6.47 cm), number of primary roots per plantlet (12.30) and number of secondary roots per plantlet (331.20). It was closely followed by red soil + sand + FYM (1:1:1 v/v). This recorded the longest primary roots and was on par with red soil + sand + cocopeat (1:1:1 v/v) in terms of number of leaves. The potting mixture consisting of red soil + sand + vermicompost (1:1:1 v/v) was found inferior to other mixtures apart from per centage survival of plantlets which like other mixtures recorded percent survival (Table 2, plate 2). Probably cocopeat and FYM might have helped in improving physical and chemical properties of the growing media, consequently resulted in better growth of banana plantlets (Hazarika, 2003 and Anbazhagan *et al.*, 2014). The results of the present study, therefore reveal that both red soil + sand + FYM (1:1:1 v/v) and red soil + sand + FYM (1:1:1 v/v) could be used for secondary hardening of *in vitro* propagated Red banana plantlets. Sharma *et al.* (1997) also reported 97% survival of *in vitro* developed Dwarf Cavendish plantlets in sand culture while only 2% loss was observed during the acclimatization of Nanicao and Grande Naine in polythene bags containing equal proportions of organic manure : soil : sand (De-Oliveira and De-Oliveira, 1997 and Robert *et al.*, 2013). These results also agree with the findings of Molla *et al.* (2004) who used soil, sand and cowdung for hardening of *in vitro* banana.

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