

STUDIES ON HARDENING AND ACCLIMITIZATION OF MICROPROPAGATED PLANTLETS OF BANANA CV. GRAND NAINE

SHAHNAWAZ AHMED^{1*}, AKASH SHARMA¹, BHARAT BHUSHAN¹, V. K. WALI¹, P. BAKSHI¹ AND A. K. SINGH²

¹Division of Fruit Science, SKUAST-J, FoA, Chatha, Jammu - 180 009

²School of Biotechnology, SKUAST-J, Chatha, Jammu-180009

e-mail: sham_shana@yahoo.com

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*Corresponding
author

ABSTRACT

The experimental plant material of cv. Grand Naine was raised through tissue culture on MS medium using suckers as explants. The MS medium supplemented with BAP 4.00 mg/L + IAA 2.00 mg/L was used for shoot multiplication. The rooting was obtained on MS (half strength) medium fortified with IBA 1.00 mg/L and activated charcoal 200 mg/L. The *in vitro* rooted plantlets were hardened and acclimatized by using different treatments. Plants transplanted at the age of 4 weeks after root initiation gave maximum survival (100.00 %) during transplanting. These plants were hardened in glass beaker and polythene bags singly or in cluster. The maximum survival during hardening (100.00 %) was observed by covering the plantlets with glass beaker individually and kept in culture room. Out of various potting mixture tried, the potting mixture containing soil: sand and FYM (2:1:1 v/v/v) gave maximum height and survival of plantlets. The results showed that out of different potting mixtures used for hardening soil: sand and FYM (2:1:1 v/v/v) showed cent percent survival.

INTRODUCTION

Micropropagation has proved to be an alternative tool for rapid mass multiplication, disease free production and year round availability of banana planting material. With the increasing demand and vast export potential coupled with the farmer's desire to grow *in-vitro* propagated banana on a large area, it is becoming increasingly important for rapid multiplication of quality planting material (Ray *et al.*, 2006). Propagation of banana through *in-vitro* technique has been reported by several workers using different explants sources and methods (Jalil *et al.*, 2006; Resmi and Nair, 2007; Shirani *et al.*, 2009).

Tejavathi and Indira 2012 found that the regenerated plants of *Drymaria Cordata* were washed in sterile water and transferred to plastic cups containing sterilized mixture of cocopeat: soilrite and perlite (1:1:1). Plantlets were nourished with ½ strength MS liquid medium. The cups were covered with plastic cover and kept in the growth room maintained at 25 ± 2°C under cool fluorescent light (25 μmolm⁻²s⁻¹) with 16h photoperiod. After three weeks, the hardened plants were established in garden soil and maintained in the green house for acclimatization. The plants showed 90% survival rate.

In micropropagation, it is desirable to produce plantlets that can grow better after transplanting into the soil. So, acclimatization is the most crucial process during banana micropropagation as the *in vitro* raised plantlets are not readily adapted for *in vivo* conditions (Vasane *et al.*, 2006). The success in acclimatization of *in vitro* produced for banana plantlets largely depends not only on the post-transfer growth conditions but also on the pre-transfer culture conditions

(Allam *et al.*, 2000). Successful acclimatization and hardening of *in vitro* produced banana plantlets have been reported by several workers (Kaushal *et al.*, 2004). Since, the tissue cultured plants are very poorly adapted to external environmental conditions. The present investigation was carried out to study the influence of different hardening and acclimatization treatments on micropropagated banana plantlets for better field survival.

MATERIALS AND METHODS

The explants were established *in vitro* using Murashige and Skoog (1962) medium supplemented with BAP 4.00 mg/L with IAA 2.00 mg/L. These were further sub-cultured on MS medium containing BAP 4.00 mg/L + IAA 2.00 mg/L for shoot multiplication. Rooting was obtained on MS (half strength) medium fortified with IBA 1.00 mg/L and activated charcoal 200 mg/L. The *in vitro* produced plantlets were subjected to different hardening treatments which include covering the plantlets with glass beaker / polythene bags individually or in groups, kept in AC room or in open for getting maximum growth and survival. Different potting mixtures were also tried which include FYM, soil, sand and vermiculite. Data recorded for different parameters were subjected to completely randomized design (CRD) Panse and Sukhatme (2000). Statistical analysis based on mean values per treatment was made using analysis of variance technique of CRD.

RESULTS AND DISCUSSION

Standardization of hardening treatments: Hardening the *in vitro* raised plantlets; so as to make them adapted to the natural

Table 1: Effect of hardening treatments on survival and growth of *in vitro* produced banana plantlets

Treatments	Survival (%)	Height (cm)	No. of leaves per plantlet
Covering the plantlets with glass beaker individually and kept in culture room	100.00 (90.00)	12.00	6.33
Covering the plantlets with polythene bag individually and kept in culture room	66.66 (55.13)	10.40	5.66
Covering the plantlets with polythene sheet in groups and kept in culture room	83.33 (66.14)	11.00	6.00
Covering the plantlets individually with glass beaker in open	51.00 (45.55)	10.00	5.33
Covering the plantlets individually with polythene bag in open	50.00 (45.26)	9.00	5.00
Keeping the plantlets in open without cover	0.00(0.00)	0.00	0.00
CD (0.05)	2.70	0.54	0.35

Table 2: Influence of potting mixture on survival and growth of banana plantlets

Treatments	Survival (%)	Height (cm)	No. of leaves per plantlet
Soil : Sand (2 : 1 v/v)	63.66 (52.93)	10.00	4.33
Soil : Sand : FYM (1 : 1 : 1 v/v/v)	98.66 (83.70)	15.70	8.66
Soil : FYM (2 : 1 v/v)	96.66 (79.79)	15.00	7.66
Soil : Sand : Vermiculite (1 : 1 : 1 v/v/v)	90.00 (71.55)	12.80	6.33
Soil : Vermiculite (1 : 2 v/v)	79.66 (63.21)	12.00	5.33
CD (0.05)	4.52	0.47	0,52

environment is a critical process due to their anatomical and physiological peculiarities. Excessive water loss from plantlets was prevented by giving various treatments. These treatments were found to influence greatly the survival and growth of plantlets. Out of different treatments (Table 1) adapted cent per cent plantlets survived when they were kept covered with glass beaker individually and kept in culture room (Plate 1A). Similar results have been obtained in rose (Skirvin and Chu, 1979) and acid lime (Manhas, 1999) when young rooted plantlets from culture tube were potted and covered with glass beaker under continuous light. The method of covering the new transferred plantlets with glass beaker followed by misting in green house for initial period and subsequently removing the cover is a gradual process, was successfully adapted by number of workers for hardening the plantlets (Jasrai *et al.*, 1999) and (Vasane and Kothari, 2008). According to them plantlets develop their stomatal control mechanism during this period. Covering the plantlets with polythene sheet in groups and keeping them in culture room was also found to be suitable, recording 83.33 per cent survival. On the other hand, all the plantlets died when kept in laboratory at room temperature without cover. The treatment of covering plantlets individually with glass beaker and keeping them in culture room recorded cent percent survival percentage and maximum height (12.00 cm). This was closely followed by covering

plantlets with polythene sheet in groups and keeping them in culture room (11.00 cm). The data on number of leaves/plantlet did not show much difference between different treatments.

Influence of potting mixture

The data pertaining to the influence of different potting mixtures on survival and growth of plantlets are presented in Table 2 (Plate 11). 98.66 per cent survival was obtained in the potting mixture containing soil, sand and FYM (2: 1: 1 v/v/v) which was superior to all other treatment. Only 63.33 per cent survival was recorded in potting mixture containing soil: sand (2: 1 v/v). The maximum height of plantlet (15.70 cm) was recorded in potting mixture containing soil, sand and FYM which was closely followed by mixture containing soil : FYM (2: 1 v/v) (15.00 cm) as well as soil : sand: vermiculite (12.80 cm). The potting mixture containing soil and sand (2: 1 v/v) was significantly inferior to other potting mixtures. Physical, chemical and biological properties of potting mixture are important for the establishment of *in vitro* produced plantlets. (Kansara *et al.*, 2013) worked on Castor and found the reason for better hardening in vermicompost may be due to presence of rich organic matter source providing strength and essential nutrients for survival to the *in vitro* raised plants. Better performance of FYM may be attributed to its ability to improve biological properties of the soil. On the other hand, sand may

**Figure 1a-b: Acclimatization of banana plantlets (a) Covering the plantlets with glass beakers; (b) Covering the plantlets with polythene sheets**

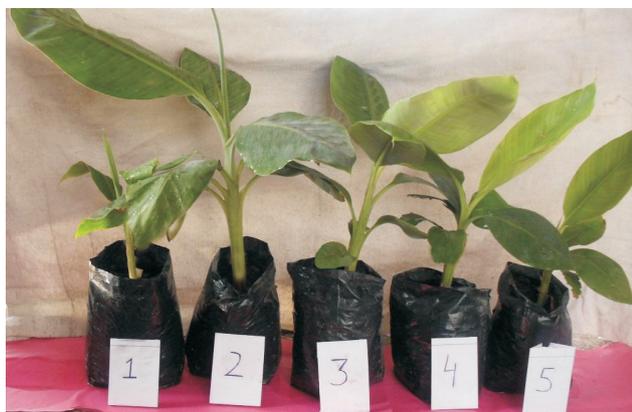


Figure 2: Plantlets established in different potting mixtures

be responsible for producing sufficient aeration. Hence, mixing soil, sand and FYM might have helped in giving better grip for the roots, ample aeration and sufficient organic matter. Reports of (Rahman *et al.*, 2005) and (Ali *et al.*, 2011) support the result as they obtained better survival and growth of banana plantlets in the potting mixture containing soil: sand: FYM (2:1:1 v/v/v).

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