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Acclimatization of *In Vitro* Propagated Grand Naine Banana Plantlets

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ABSTRACT
The tissue culture plantlet of Grand Naine of Banana is most popular among the farmers; mainly the ready planting materials are generally costly. The study was carried out at Plant Tissue Culture Laboratory; Department of Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola during 2015-2016 with objective to find out suitable potting mixture for hardening. Hardening process is integral and vital activity of banana tissue culture. In my study it is evaluated that the Ex Agar plant can be successfully hardened to produce the healthy planting material in two stages hardening, (Primary & Secondary hardening) by the cost effective process. In that primary hardening media (cocopeat+ perlite) and secondary hardening mixtures containing different potting mixture of garden soil (control), garden soil+ cocopeat (3:1) , garden soil+ farm yard manure (3:1), garden soil+ vermicompost (3:1), garden soil+ cocopeat+ FYM+ vermicompost (2:1:1:1) and garden soil+ sand+ FYM+ cocopeat(2:1:1:1) showed that cocopeat was best medium for primary hardening for plantlets survival (95 %), In secondary hardening plant height 30.86 cm, pseudostem girth 2.15 cm, number of leaves 7.15, leaf area 417.63 cm², root length 28.20 cm, root mass 29.17 gm and 100 % survival was recorded in treatment garden soil+ FYM (3:1) whereas garden soil + vermicompost (3:1) recorded best results for secondary hardening. Cocopeat+ perlite for primary hardening and combination of garden soil+ FYM (3:1) was best followed by garden soil + Vermicompost (3:1) medium for primary and secondary hardening respectively in vitro propagated banana. The cost incurred around half of the cost of ready plant which was directly bought from the tissue culture lab. This process can be easily used by farmers for their need of planting material and also as source of income generation.
Keywords: Banana Plantlets, garden soil, vermicompost, Grand Naine

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INTRODUCTION

Grand Naine (*Musa accuminata*) is one of the most popular cultivars which is known for its unique aroma and fruit quality, because of its qualities and taste it is internationally accepted and most suitable for the export. After ripening, the fruit develop excellent aroma and good bright yellow colour, which attracts people widely. Now days, the major source of planting material is tissue culture companies and is most widely accepted by the farmers because of the superiority of the tissue culture plants over the conventional rhizome sucker plantation [8].

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 [4]. 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 [6]. The physiological and anatomical characteristics of micropropagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field [5]. Development of cuticle, epicuticular waxes, and effective stomatal regulation of transpiration occurs leading to stabilization of water potential of field transferred plantlets [5]. 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 [6-9].

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 [14]. The success in acclimatization of *in vitro* produced banana plantlets largely depends not only on the post transfer growth conditions but also on the pre-transfer culture condition [2]. The present investigation was carried out to study the influence of different hardening and acclimatization treatments on micropropagated banana plantlets for better field survival and cost effective to the farmers.

MATERIALS AND METHODS

The study was carried out at the Department of Botany, Dr. Panjabrao Deshmukh Krishi Vidyapeeth Akola in the year 2015-16. Plantlets of tissue culture banana cv. Grandnaine were collected from tissue culture nursery. 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 for getting maximum growth and survival. Different hardening treatments which include polythene bags kept in shade net for getting maximum growth and survival. Different potting mixtures combinations were tried which include garden soil, FYM, cocopeat, vermicompost and sand. The experiment was carried out using RDB design with four replications. The Data were recorded for different treatment with parameters and analyzed by statistically as per Panse and Sukhatme).

RESULTS AND DISCUSSION

1. Standardization of hardening treatments:

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. In the present study cocopeat showed to be far superior to other potting media in primary hardening the in vitro raised plantlets; so as to make them adapted to the natural 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 the adapted percent plantlets survived when they were kept in shadenet with maintained condition (i.e. relative humidity and light intensity). According to them plantlets develop their stomatal control mechanism during this period.

A) Primary Hardened Plantlets

2. Influence of potting mixture in secondary Hardening:

After primary hardened plantlets were transferred from micropots to polybags. containing substrate made of the mixtures containing garden soil+ FYM (3:1) and garden soil+ vermicompost (3:1) which was superior to all other treatments. Only 94.23 per cent survival was recorded in potting mixture containing garden soil only. The maximum height of plantlet (30.86 cm) was recorded in potting mixture containing garden soil+ FYM which was closely followed by mixture containing garden soil+ vermicompost (3:1) (28.08 cm) as well as garden soil+ FYM+ sand + cocopeat (2:1:1:1). The potting mixture containing garden soil 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.For better hardening in FYM and 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 be responsible for producing sufficient aeration. Hence, mixing of garden soil, and FYM might have helped in giving better grip for the roots, ample aeration and sufficient organic matter. The result obtained better survival and growth of banana plantlets in the potting mixture containing soil: FYM (3:1) represented in table number 1.

B) Plantlets established in different potting

3. Initial Field performance of Banana

The observations were recorded of initial growth period for 120 days after planting on growth parameter aspects viz., height of plant, circumference of pseudo stem, number of leaves, leaf area, root mass, root length, The observations were recorded during 15 days interval from September to January in secondary hardening and January to May at its initial field performance. From overall assessment of results obtained it may be concluded that potting mixture of garden soil and FYM (3:1) may prove better survival in hardening and also field condition. The observations were based on the results of experiment conducted for vegetative growth and their survival in main field therefore these results are suggestive. The results were found significant.

The initial field performance of the plantlets the plant height was significantly maximum 185.68 in cm at 120 DAP in T_3 - garden soil+ FYM (3:1) Whereas, minimum plant height was recorded in T_1 - garden soil 176.49 cm. The performance of the plantlets the pseudostem girth was significantly maximum 23.90 cm at 120 DAP in T_3 - garden soil+ FYM (3:1) whereas, minimum plant height was recorded in T_1 - garden soil 17.36 cm. The number of functional leaves was significantly maximum leaves 13.83 at 120 DAP in T_3 - garden soil+ FYM (3:1). The initial leaf area was significantly maximum 238.93 dm^2 in T_3 - garden soil+ FYM (3:1). The number of suckers influenced by different potting mixture was recorded significantly minimum in T_1 - garden soil (control) throughout the growth period (1.50). Whereas, maximum number of suckers were recorded in T_3 - garden soil + FYM (3:1) (2.75).Chlorophyll content in leaf was significantly maximum 1.405 mg/g in T_3 - garden soil+ FYM (3:1). Whereas, minimum chlorophyll content was recorded in treatment T_1 - garden soil 1.035 mg/g. The data pertaining to the influence of different potting mixtures on survival and growth of plantlets are 93.75 per cent survival was obtained in the potting mixture containing garden soil and FYM (3:1) which was superior to all treatment. Only 85.41 per cent survival was recorded in potting mixture containing garden soil in initial field performance. The data represented in Table No. 2.

Table:1

Treatments	Plant height (cm)	Pseudo stem girth (cm)	Number of leaves	Leaf area (cm^2)	Root length (cm)	Root mass (g)	Survival (%)
T_1 - Garden soil	18.05	1.35	6.40	403.58	19.02	22.52	94.23
T_2 - Garden soil+ cocopeat	20.01	1.55	6.45	405.54	20.42	23.14	98.07
T_3 – Garden soil+ FYM	30.86	2.15	7.15	417.63	28.20	29.17	100
T_4 - Garden soil+ vermicompost	28.08	1.85	6.69	413.84	25.83	27.60	100
T_5 -Garden soil+ coco+ FYM+ vermin	21.82	1.60	6.53	409.84	21.79	24.69	98.07
T_6 - Garden soil+ FYM+ sand+ coco	25.28	1.65	6.65	411.98	24.99	25.91	94.22
CD at 5 %	1.812	0.193	0.214	2.305	1.813	1.704	7.335

Table: 2

Treatments	Plant height (cm)	Pseudostem circumference	Number of functional leaves	Leaf area(dm^2)	Number of suckers	Chlorophyll content (mg/g)	Survival (%)
T_1 - Garden soil	176.49	17.36	8.03	229.84	1.50	1.035	85.41
T_2 - Garden soil+ cocopeat	178.97	18.89	8.97	230.71	2.00	1.075	89.58
T_3 – Garden soil+ FYM	185.68	23.90	13.83	238.93	2.75	1.405	93.75
T_4 - Garden soil+ vermicompost	183.45	21.83	11.88	237.92	2.55	1.250	91.66
T_5 -Garden soil+ coco+ FYM+ vermin	180.94	19.88	9.08	232.16	2.00	1.350	83.33
T_6 - Garden soil+ FYM+ sand+ coco	182.05	20.99	10.89	234.39	2.25	1.363	87.49
CD at 5 %	2.132	1.910	1.701	2.200	0.424	0.107	12.469

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