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Effect of secondary hardening media on the performance of *in-vitro* raised banana plantlets cv. Grand Naine

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ABSTRACT

The present experiment was conducted at the Instructional Farm of Dept. of Pomology and Postharvest Technology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal during the year 2017-2019. The different hardening media used for the present study were Soilrite, Coco peat, Vermiculite, Vermicompost, Perlite and Sand, which were used in different combinations to study the effect of secondary hardening media on field survivability, vegetative growth and yield performances of the in-vitro plantlets in the field condition. The treatments having the secondary hardening media combination of (Sand + Vermiculite + Vermicompost; 1:1:1) gave significant result with respect to the field survivability (93.33%), vegetative growth and development including plant height (210.37 cm), numbers of suckers (5), maximum number of leaves (17) with pseudostem girth (67.13 cm) and also higher yield attributes including maximum hand weight (2.79 kg), fruit diameter (38.33 mm), maximum fingers per bunch (190.00), pulp weight (90.20 g) and a desirable yield of 25.18 kg/plant. Among the quality parameters, T_4: Sand + Vermiculite + Vermicompost (1:1:1) recorded the maximum values for TSS (19.00 %) and ascorbic acid (13.33 mg100g^{-1}) for major biochemical characters related to fruit quality.

Keywords: Banana, fruit quality, Grand Naine, secondary hardening media, *In-vitro* culture, yield

Banana and plantain is the largest fruit crop produced in the world. They are cultivated in 130 countries, mainly in the tropical and subtropical regions of the southern hemisphere (FAO, 2008). Banana is stated to be the fourth most important crop following rice, wheat and maize (Uzaribara *et al*., 2015). Banana is known for its antiquity and is interwoven with Indian heritage and culture. Having greater socio-economic significance and multiple uses, banana is referred as '*Kalpavriksh*' which means plant of virtue (Singh, 2009). India ranks first in area and production of banana in the World. Its annual production is 30.2 MT (2018-19) from an area of 8.47 lakh hectares (ICAR-National Research Centre for Banana 2018-19).West Bengal accounts for 3.7 % of total banana production in the country, which shows that it has a huge potential in banana production. Suckers and rhizomes are well known conventional methods of banana propagation, whereas on a commercial scale banana is usually propagated through tissue culture methods as this provides means to surmount the constrains portrayed by its high levels of sterility, polyploidy and other biotic and abiotic stresses (Uzaribara *et al*., 2015; Hazarika, 2003). With the increasing demand and vast export potential conjoined with the farmer's desire to grow tissue culture propagated banana on a large area has hyped the pressure and demand for the *in-vitro* cultured banana which provides better performances in different agro climatic conditions, and it is thus becoming important for rapid multiplication of quality planting material. The major advantages accrued through tissue culture are being substantial increase in the yield in terms of bunch with higher weight, more fingers and hands and more uniform fruit size and shape, improved quality of fruits and reduced maturity period (Bhojwani 1990; Lalrisanga *et al* 2013). Comparatively, a potentially better tissue culture or micro-propagation also has its own setbacks to overcome, as the in vitro plantlets produced are often observed with either higher mortality (Mathur *et al.*, 2008) or poorer acclimatization (Hazarika, 2003) when transferred from an *in vitro* controlled condition to an *ex vitro i.e*. natural conditions (Uzaribara *et al.*, 2015). Thus, a good quality and balanced growing media serves the purpose to mitigate the constrains with healthy and strong plant foundation and aim at hardening for better survivability in variety of environment conditions, as it provides sufficient anchorage or support to the plant, that serves as reservoir for nutrients and water, allow oxygen diffusion to the roots and permit gaseous exchange between the roots and atmosphere outside the root substrate (Abad *et al.,* 2002) and ultimately supplements to better performances in the field conditions. And also since the tissue culture plants are poorly adapted to the external environmental condition, a good composition of different secondary hardening medium is required to supplement the developing plantlets with all its basic nutrition to develop with stronger roots and a healthy biomass for better anchorage

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and yield in field conditions. The composition of the growing medium influences the quality of the seedlings (Wilson *et al.,* 2001) and its yield performance in the field conditions. Interestingly, regarding the performances of different secondary hardening media on *in-vitro* raised plantlets of banana scanty information are available in the northern parts of West Bengal. Thus, with the above background, the present investigations were undertaken to determine the effect of different composition of secondary hardening on field performances of *in-vitro* raised banana cv. Grand Naine.

MATERIALS AND METHODS

The present experiment was conducted at the instructional farm of Pomology and Post-harvest Technology, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal during the year 2017-2019. This area lies under the *Terai* agro-ecological zone of West Bengal. The banana cultivar selected for the designated experiment was Grand Naine. Though a common tissue culture cultivar it is also renowned for its well adaptability, brilliant performance, for its aroma and fruit qualities (Singh *et al.,* 2011). The tissue cultured plantlets of banana cultivar Grand Naine were collected from the commercial laboratories and initially kept in Biotechnology Laboratory of Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal, India for the primary hardening process. After the primary hardening, the same plants were subjected to different combinations of secondary hardening media for secondary hardening process which was maintained in shade house. The different hardening media used in the present study were Soilrite, Coco peat, Vermiculite, Vermicompost, Perlite and Sand. After 30 days of secondary hardening process the plantlets were transferred to the field conditions. The experiment was laid out in Randomised Block Design (RBD) comprising of 7 (seven) treatments and 3 (three) replications. The treatments included different combinations of the secondary hardening media in different ratio *viz.*, T_1: Soilrite (control), T_2: Coco peat + Vermicompost (1:1), T_3: Vermiculite + Coco peat + Vermicompost (1:1:1), T_4: Sand + Vermiculite + Vermicompost (1:1:1), T_5: Soil+ Sand + Vermicompost (1:1:1), T_6: Coco peat + Vermiculite (1:1), T_7:Soilrite + Coco peat + Vermiculite + Perlite + Vermicompost (1:1:1:1:1).

The percentage of survivability of plantlets at field condition was calculated using formula:

$$Mean\ percent\ survival\ of\ explant = \frac{Total\ number\ of\ plants\ survived}{Total\ number\ of\ plant} \times 100$$

The vegetative characters such as the plant height, pseudo stem girth, and the number of leaves were observed for ten plants in each replication for each treatment and then their average was finally recorded. Plant height was measured using a measuring tape from collar region to the base of a newly emerged leaf, and was expressed in centimetres (cm). Pseudo stem girth was measured above 5cm from the ground level with a measuring tape and was expressed in centimetres (cm). Number of leaves and number of suckers per plant were counted manually and the average values were recorded. Number of days to first flowering stage as well as the number of days taken from flowering to fruit set were recorded to check the effect of treatments.

The yield and its attributes were recorded immediately after the harvest by adopting the standard procedures. Bunch weight of 3 random plants for each treatment was taken for each replication and the mean was recorded. The peduncle weight was taken by measuring 20cm above the first hand and was expressed in kilograms (kg) for 3 random plants and the mean was recorded. The peduncle length for each of these plants was measured manually using a centimetre scale for calculating the mean value. The fruit diameter was measured at the middle portion of the fingers by using a vernier calliper. Pulp and peel weight were measured separately after removing the peel and expressed in grams (g).

Excellent fingers were allowed for natural and uniform ripening and these fruits were used for determining different quality parameters. The total soluble solids in a sample were recorded by using a Handheld Refractometer. The methods described by Ranganna (1978) were adopted for estimation of Titratable Acidity, Ascorbic Acid, Total Sugar, Reducing Sugar and Non-reducing Sugar. Titratable acidity was determined by titrating the fruit sample against 0.1 N NaOH solutions using phenolphthalein as an indicator (light pink end point) and expressed as percentage in terms of malic acid. Ascorbic acid content was estimated by using 2, 6-dichlorophenol indophenol visual titration method. Total sugar was determined by Lane and Eynon's titration method. A measured amount (20 ml) of the extract was taken in a 100 ml volumetric flask to which 1.0 ml concentrated HCl was added and kept for hydrolyzation overnight at room temperature. Next day, the solution was neutralized with saturated NaOH solution followed by a drop of phenolphthalein, finally the volume will be made upto the mark with distilled water. This solution was then titrated against Fehling's

A and B solutions using methylene blue as indicator. Reducing sugar was also estimated by Lane and Eynon's titration method. Non reducing sugar was calculated by subtracting the value of reducing sugar from that of total sugar.

All the observations were subjected to the statistical analysis of variance for RBD. The statistical analysis of the data on the mean values of individual characters was analysed using OPSTAT software. Significance and non-significance of the variance due to different treatments was determined by calculating the respective 'F' values according to the method described by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

Field survivability and vegetative growth of banana

Field survivability is the most significant parameter of the secondary hardening which directly indicates the contribution of the secondary media mixture on the plant life span. In the present investigation, it was found that the field survivability percentage ranged between 76.67 % - 93.33 %. And out of all the treatments, T_5 (93.33 %) recorded the highest field survivability whereas T_2 (86.67%), T_3 (90.00%), T_4 (90.00%), T_6 (83.33%), and T_7 (86.67%) were found to be statistically par with the treatment T_5. Minimum field survivability was recorded in treatment T_1 (76.67%). Similarly T_1 was similar with T_6.

All the parameters except for the days to first flowering were found to be significantly different for all the treatments. It was observed that the treatment T_4 performed better than other treatments for maximum of the characters (Table 1). The plantlets obtained from treatment T_4 were found to be significantly superior for characters like plant height (210.37 cm), pseudo stem girth (67.13 cm), number of suckers (5), maximum number of leaves (17) while significantly inferior performance was recorded in T_1. The plantlets from treatment T_4 were the first to flower (311 DAT) as compared to the other treatments and recorded the shortest time period required for fruiting (41 DAF) whereas, T_1 showed delayed flowering and fruiting. It was observed that a good height with a thick pesudostem girth provides strength at the time of fruiting to support and balance out the weight of the bunch (Vasane and Kothari , 2008) and keeps it away from the ground. The maximum number of leaves per plant supports the physiological and biochemical process like photosynthesis, respiration etc (Gonzalez *et al*., 2012). A good number of leaves also provides with the domestic needs of the famers like feeds for cattle, traditional plates and baskets etc. The shortest period of flowering and fruit helps in escaping the various biotic and abiotic stresses (Anonymous, 2006).

Yield attributing characters of banana

From Table 2, it is clearly observed that the yield/ plant ranged from 15.68 kg – 25.18 kg. Among all the treatments the plantlets obtained from treatment T_4 (25.18 kg plant^{-1}) was found to be significantly superior followed by T_3 (21.71 kg yield plant^{-1}), T_5 (21.68 kg yield plant^{-1}). T_2 recorded highest hands per bunch (13.33) which was at par with T_4 (12.00) and T_6 (11.67), whereas lowest was recorded in T_1 (11). T_4 recorded highest fingers per bunch (190.00) which was at par with T_5 (180.00), T_7 (174.00), T_3 (172.00) and T_6 (167.00)

Table 1: Effect of secondary hardening media on field survivability and vegetative growth of *in-vitro* raised banana

Treatments	Field survival percentage (%)	Plant height (cm)	Pseudostem girth (cm)	Days to first flowering	Days from flowering to fruiting	Number of suckers	Number of leaves
T_1	76.67	180.51	52.34	330	53	2	12
T_2	86.67	191.61	58.76	314	45	4	13
T_3	90	198.77	63.93	318	51	5	14
T_4	90	210.37	67.13	311	41	5	17
T_5	93.33	190.49	56.56	314	48	3	13
T_6	83.33	186.4	54.96	326	52	4	13
T_7	86.67	194.64	60.35	316	49	4	13
S.Em. (±)	**2.43**	**0.39**	**0.4**	**4.35**	**1.27**	**0.23**	**0.27**
CD. 5%	**7.58**	**1.22**	**1.25**	**N.S**	**3.97**	**0.71**	**0.84**

** T_1: Soilrite, T_2: Coco peat + Vermicompost (1:1), T_3: Vermiculite + Coco peat + Vermicompost (1:1:1), T_4: Sand + Vermiculite + Vermicompost (1:1:1), T_5: Soil + Sand + Vermicompost (1:1:1), T_6: Coco peat + Vermiculite (1:1), T_7: Soilrite + Coco peat + Vermiculite + Perlite + Vermicompost (1:1:1:1:1)*

Table 2: Effect of secondary hardening media on yield and yield attributing characters of *in-vitro* raised banana

Treatments	Yield/plant (kg)	Hands/ bunch	Fingers/ bunch	Peduncle length (cm)	Peduncle weight (kg)	Hand weight (kg)	Fruit weight (g)	Fruit length (cm)	Fruit diameter (mm)	Pulp weight (g)	Peel weight (g)
T_1	15.68	11	160.00	81.00	1.34	1.67	123.33	12.76	33.67	75.07	48.44
T_2	19.21	13.33	164.00	87.33	1.71	1.88	130.00	13.23	36.61	86.23	42.75
T_3	21.71	11.33	172.00	86.67	1.67	1.98	132.35	14.35	34.11	85.67	46.17
T_4	25.18	12.00	190.00	80.00	1.37	2.79	129.00	14.02	38.33	90.20	39.98
T_5	21.68	11.33	180.00	92.33	1.88	2.33	130.00	16.45	35.45	78.17	43.50
T_6	16.84	11.67	167.00	82.67	1.43	1.93	126.67	14.85	36.25	77.93	40.50
T_7	16.48	11.33	174.00	78.67	1.11	2.01	128.67	13.73	37.19	84.33	30.67
S.Em. (±)	**0.29**	**0.60**	**8.01**	**2.61**	**0.19**	**0.14**	**1.68**	**0.16**	**0.55**	**2.85**	**4.059**
C.D. at 5%	**0.68**	**1.86**	**24.68**	**8.12**	**N.S.**	**0.45**	**N.S.**	**0.49**	**1.73**	**8.79**	**N.S.**

** T_1: Soilrite, T_2: Coco peat + Vermicompost (1:1), T_3: Vermiculite + Coco peat + Vermicompost (1:1:1), T_4: Sand + Vermiculite + Vermicompost (1:1:1), T_5: Soil + Sand + Vermicompost (1:1:1), T_6: Coco peat + Vermiculite (1:1), T_7: Soilrite + Coco peat + Vermiculite + Perlite + Vermicompost (1:1:1:1:1)*

whereas lowest was recorded with T_1 (160.00). It was observed that T_4 (2.79 kg) was significantly superior for hand weight and fruit diameter (38.33 mm) which was at par with T_7(37.19 mm) in respect to fruit diameter. The maximum pulp weight was recorded with T_4 (90.20 g) which was at par with T_2 (86.23 g), T_3 (85.67 g) and T_7 (84.33 g). For the characters, peduncle length (92.23 cm) and fruit length (16.45 cm), T_5 was found to be superior among the treatments but found to be at par with T_2 (87.33 cm) and T_3 (86.67 cm) in terms of peduncle length. The characters which had higher values for significant characters contributing to the yield consisted of sand and Vermicompost as a common medium in their potting composition, which played a vital role in the performance and deliverances of the plantlets in the external environmental conditions. Vermicompost provides the plant with all the necessary nutrition, protection and root ramification and when combined with sand, it can be prevented from becoming clayey and hard also leading to the improvement in its texture with proper drainage system facilitating in better root system for better grip, necessary for a good foundation and increase in nutrient absorption and better survival with yield in the field conditions. The works of Dewir *et al.*(2005) and Vasane and Kothari (2006) supported the findings of the present investigation. Vermicompost also provides close contact between plantlets and media as well as enhancement in the steady moisture supply which facilitates root respiration and encourages overall root growth for better nutrient allocation and absorption (Chatterjee and Choudhary, 2007). These findings also suggested that the effect of Vermicompost on plant growth and plant productivity were not only nutritional but also hormonal and biochemical.

Fruits quality of banana

It was evident from the Table 3 that treatment T_4 (19.00 °Brix) was found to be significantly superior for total soluble solids among all the treatments. Similar results were found by Sangeeta *et al.* (2017) where the treatment combination with vermicompost was found to improve the fruit quality parameters. High total soluble solids or titratable acidity is desirable as it helps in determining the fruit flavour with improved sweet taste (Godoy *et al.,* 2016*;* Palijama *et al.,* 2017). Total sugar percentage ranged from 14.02 – 15.37 per cent, the treatments were found to be at par with not much variation among them. The highest reducing sugar level was recorded in T_4 (7.98%) which was found to be at par with T_5 (7.25%) and lowest being observed in T_1

Table 3: Effect of secondary hardening media on fruit quality of *in-vitro* raised banana

Treatments	TSS (Brix)	Total sugar (%)	Reducing sugar (%)	Non-Reducing sugar (%)	Titratable acidity (%)	Ascorbic acid ($mg100g^{-1}$)
T_1	17.67	14.02	6.70	7.32	0.37	10.67
T_2	18.07	14.29	7.11	7.18	0.33	11.20
T_3	18.40	14.85	6.96	7.89	0.35	12.80
T_4	19.00	15.37	7.98	7.38	0.29	13.33
T_5	18.13	14.43	7.25	7.18	0.33	12.27
T_6	18.00	14.29	7.08	7.21	0.34	11.206
T_7	18.07	14.71	6.96	7.76	0.36	11.73
S.Em. (±)	**0.15**	**0.34**	**0.26**	0.38	**0.01**	**0.82**
C.D. at 5%	**0.47**	**1.04**	**0.81**	**N.S.**	**0.04**	**2.54**

** T_1: Soilrite, T_2: Coco peat + Vermicompost (1:1), T_3: Vermiculite + Coco peat + Vermicompost (1:1:1), T_4: Sand + Vermiculite + Vermicompost (1:1:1), T_5: Soil + Sand + Vermicompost (1:1:1), T_6: Coco peat + Vermiculite (1:1), T_7: Soilrite + Coco peat + Vermiculite + Perlite + Vermicompost (1:1:1:1:1)*

(6.70%). The sugar levels are evidently important as these during processing are responsible for non-enzymatic darkening reactions (Oetterer *et al.,* 2006). The titratable acidity ranged between 0.29-0.37 percent, where minimum being observed in T_4 (0.29%) that was found to be at par with T_2 (0.33%), T_5 (0.33%) and the maximum was observed in treatment T_1 (0.37%). Ascorbic acid is a significant characteristic for quality of banana that improved sweet taste and increased ascorbic acid content is consumer preferable and is highly desirable for their antioxidant characteristic. The ascorbic acid content ranged from 10.67 $mg100g^{-1}$ – 13.33 $mg100g^{-1}$ with the maximum being observed in T_4 (13.33 $mg100g^{-1}$) which was at par with T_2 (11.20 $mg100g^{-1}$), T_3 (12.80 $mg100g^{-1}$), T_5 (12.27 $mg100g^{-1}$), T_6 (11.20 $mg100g^{-1}$) and T_7 (11.73$mg100g^{-1}$) and lowest being observed in treatment T_1 (10.67 $mg100g^{-1}$). The role of secondary hardening media in developing the better fruit quality parameters like TSS, total sugar and ascorbic acid may be due to the translocation and breakdown of starch, protein, carbohydrate and other organic acids in fruit also, it is known that nitrogen from vermicompost and other organic amendments assimilate and enhanced the rate of photosynthesis in the leaf and also effects the translocation of photosynthetic product from the leaves to the fruits which helps in improving the quality. The findings were in accordance with the results of Vanilarasu and Balakrishna Murthy (2014), Dey *et al.*(2005).

Therefore, the treatments T_4: Sand + Vermiculite + Vermicompost (1:1:1) and T_5: Soil+ Sand + Vermicompost (1:1:1) can be recommended as secondary hardening media composition for improving the yield and quality of banana plantlets as these combination has high survivability rate and facilitates excellent vegetative and reproductive growth and development.

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