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Effect of biostimulants on growth of Red banana plantlets during the primary hardening phase

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Abstract
An experiment was conducted to evaluate the effect of various biostimulants during the primary hardening phase of tissue culture plantlets of Banana cv. Red Banana at the North Farm, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore in the year 2022-2023. Among the various treatments, the maximum survival percentage (100%) during the primary hardening phase was observed in Soil + Cocopeat + Chitosan (50 ppm), Soil + Cocopeat + Chitosan (100 ppm), Soil + Cocopeat + Moringa leaf extract (3%), Soil + Cocopeat + Moringa leaf extract (4%), Soil+ Cocopeat + Vermiwash (3%), Soil + Cocopeat + Fermented coconut water. Among the various treatments given the maximum plant height (12.65 cm), pseudostem girth (4.12 cm), leaf length (7.51 cm), leaf width (4.27 cm), leaf area (25.70 cm^2) and number of leaves per plant (4.75) was observed in Soil + Cocopeat + Moringa leaf extract (4%). The SPAD value was highest (38.24) in the treatment of Soil + Cocopeat + Chitosan (50 ppm).

Keywords: Red banana, biostimulants, chitosan, hardening, tissue culture

Introduction
Bananas and plantains (*Musa* spp.) are the first crop plants domesticated by humans. Banana is a large monocotyledonous and monocarpic herbaceous perennial plant that belongs to the family of Musaceae and Zingiberales order. Bananas are otherwise called "Apple of Paradise" or "Kalpavriksh" which means the plant of virtue (Singh *et al*., 2009) [1]. Banana is the most important tropical fruit next to mango. India is the largest producer of bananas in the world with an area of 880,000 ha and a production of 32454'000 MT (NHB, 2022) [2]. The Red banana (*Musa acuminata*) is the most relished and highly prized variety of bananas. It is a triploid cultivar (AAA) that is commercially grown in the Kanyakumari and Tirunelveli districts of Tamil Nadu.

Healthy and disease free planting material is an important factor for long term banana production. To meet the ever increasing demand for elite planting material, traditional propagation methods must be supplemented with modern propagation techniques. This increasing demand for high quality banana planting material can only be met if conventional and non-conventional banana propagation methods are used on a commercial scale, with need-based modifications involving modern propagation technologies.

Micropropagation or tissue culture is the only viable option for large scale banana multiplication. This method is free of seasonal bonds and allows for the multiplication of the chosen plants. Other benefits include product uniformity, disease free plants, and the ease of exchanging germplasm and planting material (Murashige *et al*., 1974) [3]. Acclimatization or hardening is a critical step in the micropropagation process. It is a process of adaptation to the natural environment for various plant species that have undergone *in vitro* growth and development. Most *in vitro* species require an acclimatization process to ensure that a sufficient number of plants survive and grow vigorously when transferred to soil. Acclimatization of *in vitro* raised plants to *ex vitro* conditions is critical and a major determinant of these plants subsequent field establishment (Singh *et al*., 2012) [4]. The benefit of any micropropagation system can only be realized if plantlets are successfully transferred from the tissue culture system to the natural environment. When *in vitro* plantlets were successfully transferred to the soil through the acclimatization process, morphological characteristics can be visible (Singh *et al*., 2017) [5]. The selection of suitable media and biostimulants are important during the acclimatization process in crops.

Natural biostimulants are gaining popularity in sustainable agriculture for improving crop productivity and quality (Abdalla, 2013) [6]. Biostimulants are a diverse group of compounds capable of enhancing certain physiological processes that promote crop growth and development and also reduces the use of synthetic fertilizers while maintaining crop productivity. Plant biostimulant is also a substance or microorganism which is applied to plants to improve nutrition efficiency, abiotic stress tolerance, and crop quality traits, regardless of nutrient content. Biochar, chitosan derivatives, humic substances, microbial inoculants, moringa leaf extract, vermiwash, and seaweed extracts are the most widely used natural biostimulants in crop production (Jardin, 2015) [7].

The plantlets when treated with various biostimulants may improve establishment and overall performance. A beneficial organic extract in commercial cultivation would reduce pesticides, saving costs and reducing pollution to the environment. With the above background, the experiment was done to the study the efficiency of various biostimulants on survival percentage and growth parameters of tissue cultured red banana plantlets.

Materials and Methods

The present investigation was taken up at the North Farm, School of Agricultural Sciences, Karunya Institute of Technology and Sciences, Coimbatore of Tamil Nadu. The experiment was laid out in a Completely Randomized Design with eleven treatments and three replications. The following treatments T_1: Soil + Cocopeat (Control), T_2: Soil + Cocopeat + Chitosan (50 ppm), T_3: Soil + Cocopeat + Chitosan (100 ppm), T_4: Soil + Cocopeat + Moringa leaf extract (3%), T_5: Soil + Cocopeat + Moringa leaf extract (4%), T_6: Soil + Cocopeat + Vermiwash (3%), T_7: Soil + Cocopeat + Vermiwash (4%), T_8: Soil + Cocopeat + Cow urine (10 times diluted), T_9: Soil + Cocopeat + Cow urine (5 times diluted), T_{10}: Soil + Cocopeat + Fermented coconut water and T_{11}: Soil + Cocopeat + Humic acid were given to the red banana plantlets at fortnight intervals.

Results and Discussion

The salient findings of the study are presented in Table 1 and discussed below.

Survival Percentage: The survival percentage of red banana plantlets during the primary hardening phase was recorded maximum of 100% in T_2: Soil + Cocopeat + Chitosan (50 ppm), T_3: Soil + Cocopeat + Chitosan (100 ppm), T_4: Soil + Cocopeat + Moringa leaf extract (3%), T_5: Soil + Cocopeat + Moringa leaf extract (4%), T_6: Soil+ Cocopeat + Vermiwash (3%) and T10: Soil + Cocopeat + Fermented coconut water whereas it was minimum in the control (66.67%). The higher survival percentage observed in the red banana plantlets treated with moringa leaf extract may be due to the presence of antioxidants in it. The above result is in accordance with the findings of Guler *et al*., (2021) [8] and it is further supported by the research findings of Veraplakorn and Kudan (2021) [9] using chitosan, Molnar *et al*., (2011) [10] reported that the liquid endosperm in coconut water contains a variety of amino acids, organic acids, nucleic acids, vitamins, sugars, sugar alcohols, plant hormones (auxins and cytokinins), minerals that are entirely responsible for the growth promoting activity in plants.

Plant height (cm)

Plant height recorded in the tissue cultured red banana plantlets during the primary hardening phase was maximum (12.65 cm) in the treatment of T_5: Soil + Cocopeat + Moringa leaf extract (4%) followed by T_9: Soil + Cocopeat + Cow urine (5 times diluted) (12.05 cm) whereas it was minimum in the control (9.32 cm). The increased plant height may be due to the presence of zeatin in moringa leaf extract (Culver *et al*., 2012) [11]. The above result is also supported by the findings of Sardar *et al*., (2021) [12] who opined that Moringa Leaf Extract (MLE) is effective in improving vegetative growth, yield, and quality of numerous crops around the world. It is also quite inexpensive compared to synthetic growth regulators. According to Bashir *et al*., (2014) [13], Moringa leaf extract increased the average plant height, number of leaves, branches, and yield parameters. The increased plant height observed in plantlets receiving the treatment of T_9: Soil + Cocopeat + Cow urine (5 times diluted) may be due to the presence of macro and micro nutrients present in it (Ambiga and Balakrishnan, 2015) [14].

Pseudostem girth (cm)

Pseudostem girth was recorded maximum (4.12 cm) in the treatment of T_5: Soil + Cocopeat + Moringa leaf extract (4%) followed by T_2: Soil + Cocopeat + Chitosan (50 ppm) (3.85 cm) whereas it was recorded minimum in the control (2.40 cm). The increased pseudostem girth may be due to the presence of cytokinin present in moringa leaf extract which promoted the cell division and cell elongation (Yusuff *et al*., 2020) [15]. The increase in pseudostem girth due to chitosan is supported by the findings of El-Miniawy *et al*., (2013) [16] who observed that chitosan spray on strawberries after transplantation increased the vegetative growth and yield.

Leaf length (cm)

Leaf length was recorded maximum (7.51 cm) in the treatment of T_5: Soil + Cocopeat + Moringa leaf extract (4%) followed by T_9: Soil + Cocopeat + Cow urine (5 times diluted) (7.42 cm). The control plants which were not treated by any biostimulants recorded the least length of 5.03 cm. The above result is in accordance with the findings of Ahmed *et al*., (2020) [17] and Jandaik *et al*., (2015) [18]. Hasegawa *et al*., (2000) [19] opined that by activating enzymes, osmoregulation, and photosynthesis, the presence of calcium and potassium in Moringa Leaf Extract can potentially promote plant growth and development.

Leaf width (cm)

Leaf width was recorded maximum (4.27 cm) in the treatment of T_5: Soil + Cocopeat + Moringa leaf extract (4%) followed by T_6: Soil + Cocopeat + Vermiwash (3%) (4.15 cm). The control plants which were not treated by any biostimulants recorded the least length of 2.13 cm. The results are in accordance with the findings of Foidle *et al*. (2001) [20]. According to Subasashri (2003) [21], vermiwash is an excellent liquid manure that greatly impacts crop growth and productivity when applied as a foliar spray.

Leaf area (cm^2)

In the primary hardening phase of red banana plantlets, leaf area was found to be maximum (25.70 cm^2) in the treatment of T_5: Soil + Cocopeat + Moringa leaf extract (4%) followed by T_9: Soil + Cocopeat + Cow urine (5 times diluted) (24.05

cm^2). The control plants which were not treated by any biostimulants recorded a minimum leaf area of 8.60 cm^2. The results are in accordance with the findings of Rehman and Basra, (2010) [22] who stated that the foliar application of moringa leaf extract promotes earlier cytokinin synthesis, prevents premature leaf senescence, and results in greater leaf area with higher photosynthetic pigments.

Number of leaves per plant

The present study revealed that application of T_5: Soil + Cocopeat + Moringa leaf extract (4%) increased the leaf production (4.75) in red banana plantlets. The above result is on par with T_4: Soil + Cocopeat + Moringa leaf extract (3%), T_8: Soil + Cocopeat + Cow urine (10 times diluted) and T_6: Soil + Cocopeat + Vermiwash (3%). The above result is in accordance with the findings of Nasir *et al*., (2016) [23], Sahu *et al*., (2022) [24].

SPAD value

The chlorophyll content was measured by SPAD chlorophyll meter (model 502; Minolta Corp., Ramsey, N.J) and presented in Table 1. The SPAD value was maximum (38.24) in the plants which received the treatment of T_2: Soil + Cocopeat + Chitosan (50 ppm) followed by T_3: Soil + Cocopeat + Chitosan (100 ppm) (37.32) and it was minimum in control plantlets (29.20). The result is in accordance with the findings of Gornik *et al*., (2008) [25] where dipping stem cuttings of grapevines in chitosan improved the subsequent rooting and increased the chlorophyll content. The above results are also supported by Van *et al*., (2013) [26] who reported that foliar spray of chitosan enhanced leaf chlorophyll content, net photosynthesis rate, and nutrient uptake.

Table 1: Effect of biostimulants on growth of Red banana plantlets during the primary hardening phase

Treatments	Survival Percentage (%)	Plant height (cm)	Pseudostem girth (cm)	Leaf length (cm)	Leaf width (cm)	Leaf area (cm^2)	Number of leaves per plant	SPAD value
T_1	66.33	9.32	2.40	5.03	2.13	8.61	3.22	29.27
T_2	100.00	11.50	3.85	6.89	3.43	18.96	4.21	38.24
T_3	100.00	11.77	3.27	6.37	3.97	20.24	4.38	38.10
T_4	100.00	11.80	3.67	6.93	4.03	22.36	4.69	35.16
T_5	100.00	12.65	4.12	7.51	4.27	25.70	4.75	36.60
T_6	100.00	10.43	3.61	6.12	4.15	20.34	4.58	33.03
T_7	95.00	11.27	3.49	6.85	3.80	20.86	3.52	36.06
T_8	95.00	10.86	3.79	6.32	3.74	18.93	4.62	37.32
T_9	95.00	12.05	3.69	7.42	4.05	24.05	3.61	34.82
T_{10}	100.00	11.33	3.12	6.24	2.58	12.94	3.68	35.45
T_{11}	90.00	10.23	3.32	5.93	3.26	15.49	3.82	32.26
SE.d	4.71	0.17	0.16	0.18	0.11	0.79	0.13	0.51
CD (0.05)	9.82	0.37	0.32	0.38	0.23	1.64	0.28	1.07

Conclusion

From the present study it was concluded that Soil + Cocopeat + Moringa Leaf Extract (4%) (T_5) treatment recorded the maximum survivability, plant height, pseudostem girth, maximum leaf length, leaf width, leaf area and number of leaves per plant in red banana plantlets during the primary hardening phase. It was also found that red banana plantlets which received the treatment of Soil + Cocopeat + Chitosan (50 ppm) (T_2) and Soil + Cocopeat + Chitosan (100 ppm) (T_3) recorded the maximum SPAD values. Hence the effect of various biostimulants on the growth of red banana plantlets during the primary hardening phase were studied.

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