International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(7): 736-740
www.biochemjournal.com
Received: 27-05-2024
Accepted: 30-06-2024

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Effect of different media on primary hardening of banana tissue culture plants in greenhouse conditions

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DOI: https://doi.org/10.33545/26174693.2024.v8.i7i.1585

Abstract
An experiment was conducted on primary hardened banana plantlets of the Grand Naine variety. Among the eight combinations of growing media, the combination T_5- comprising soil, vermiculite, and vermicompost in a 2:1:1 ratio produced the highest number of roots (6.87), leaves (5.27), leaf area (12.71 cm²), and survival percentage (80.25%). It also showed the highest stomata frequency per mm² on both the upper (37.33) and lower (129.67) leaf surfaces. The maximum shoot length (5.01 cm) was observed in T_4, which consisted of soil and vermicompost in a 2:1 ratio. In contrast, the soil only medium (T_1) recorded the shortest shoot length (3.56 cm), fewest roots (4.07), lowest number of leaves (3.93), smallest leaf area (10.27 cm²), highest survival percentage (39.64%), and lowest stomata frequency on both the upper (23.00) and lower (74.00) leaf surfaces. Media combination T_5- (Soil + Vermiculite + Vermicompost- 2:1:1 v/v) gives better performance in primary hardening.

Keywords: Banana, tissue culture, primary hardening, media, vermicompost

Introduction
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 *et al.*, 2011) [14]. Banana evolved in the humid tropical regions of S. E. Asia with India as one of its centers of origin. Modern edible varieties have evolved from the two species – *Musa acuminata* and *Musa balbisiana* and their natural hybrids, cultivars belonging to *M. balbisiana* tend to be starchy bananas, which are also known as plantains and cultivars belonging to *M. acuminata* tend to be sweet dessert, peeled and eaten types of bananas (Zaini *et.al.*, 2022) [18] originally found in the rain forests of S. E. Asia.

The average banana consumption is 12 kg per capita, making it the world's leading food crop after rice, wheat and maize. The world production of bananas has increased steadily over the last 20 years, from approximately 70 million tonnes in 1999 to around 117 million tonnes in 2019 (FAOSTAT., 2020) [7]. Banana is a very popular fruit due to its low price and high nutritive value. It is consumed in fresh or cooked form both as ripe and raw fruit. Green Banana contains 2-hexanal, ripe banana contains Eugenol and overripe banana contains isopentanol.

Banana is generally propagated vegetative through suckers, which grows from lateral buds originating from corms and suckers. Suckers are used for production of individual plants. The process requires a long period of time and rate of multiplication of suckers through conventional vegetative means has been found to show several negative impacts which include transmission of diseases, low production and poor preservation of original plant genetic material (Hussein, 2012) [9]. Tissue culture as a propagation technique provides a robust means to prepare disease-free planting materials that can provide the first line of defence in developing an integrated disease-management program for banana. Due to incidences of banana bunchy top and panama wilt diseases in banana field, it was the need of the time to replace the existing infected germplasm with healthy and high yielding seed material (Al-Amin *et al.*, 2009) [2].

Since the tissue culture grown plants are micro propagated under controlled conditions, they cannot be planted directly in the field. Their direct plantation in nature invites high mortality or low survival rate, causing loss to the farmers. They are, therefore, slowly acclimatized for

the field conditions in green houses and shade houses before being released to the farmers for plantation (Hudson *et al*., 1990) [8]. High mortality is observed upon transfer of micro shoots to ex vitro conditions as the cultured plants have non-functional stomata, weak root system and poorly developed cuticle (Mathur *et al*., 2008) [12].

The process of acclimatization of tissue culture grown plants is called as hardening. Acclimatization or hardening is the most crucial process during banana micro-propagation as the *in-vitro* raised plantlets are not readily adapted for in vivo conditions (Vasane *et al*., 2006) [16].

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) [13]. The composition of the growing medium influences the quality of the seedlings (Wilson *et al.,* 2001) [17] and its yield performance in the field conditions. The finding of this research may help banana growers to bring very good effect on tissue culture banana plants.

Materials and Methods

Experiment was conducted at Hi-tech Horticulture Park, College of Horticulture, Junagadh Agricultural University, during April-May 2023. Junagadh, located in South Saurashtra Agro-climatic Zone, Micro-propagated banana plantlets (*M. Paradisica* L.) *cv*. Grand naine were sourced from the Department of Genetics and Plant Breeding. Healthy and uniform rooted plantlets with 3 to 4 leaves were selected from *in-vitro* cultures. The experiment followed a Completely Randomized Design (CRD) with three repetitions and eight treatments: T_1 (Soil only), T_2 (Soil + Coco peat- 2:1 v/v), T_3 (Soil + Vermiculite- 2:1 v/v), T_4 (Soil + Vermicompost- 2:1 v/v), T_5 (Soil + Vermiculite + Vermicompost- 2:1:1 v/v), T_6 (Soil + Coco peat + Vermiculite- 2:1:1 v/v), T_7 (Soil + Coco peat + Vermicompost- 2:1:1 v/v), and T_8 (Soil + Coco peat + Vermiculite + Vermicompost- 2:1:1:1 v/v) for 30 days.

Results and Discussion

From Table no.1 Maximum survival percentage (80.25) maximal number of roots (6.87), highest number of leaves (5.27), maximum leaf area (12.71 cm2), highest stomata frequency per mm^2 on upper surface (37.33) and lower surface (129.67) of leaves were observed in Soil + Vermiculite + Vermicompost (2:1:1) (T_5).

Maximum survival percentage, maximal number of roots, highest number of leaves, maximum leaf area, highest stomata frequency per mm^2 on upper surface and lower surface of leaves might be due to Vermiculite which enhances soil aeration, water retention and providing a conducive environment for growth of roots. In addition to changing the anchoring and nutrient content of the medium and enhancing the growing plant's capacity to retain water and get oxygen (Beardsell and Nicholas, 1982) [3], vermiculite would have also increased the plant's ability to grow and develop. Vermiculite corroborated similar results when combined with vermicompost, which offers vital nutrients and enhances soil structure, improving overall plant health and growth (Kuepper *et al*., 2003) [10]. Vermicompost includes micro and macronutrients that are easily absorbed by plants and increases soil microbial activity, both of which are good for the cycling of nutrients (Edwards *et al*., 1988) [6]. It was found to include growth-promoting auxins, cytokinins, and gibberellins (Suhane, 2007) [15]. Synergy of soil, vermiculite and vermicompost provides a steady environment with sufficient nutrients, moisture and aeration leading to optimal plant growth and health (Atiyeh *et. al.,* 2002) [2].

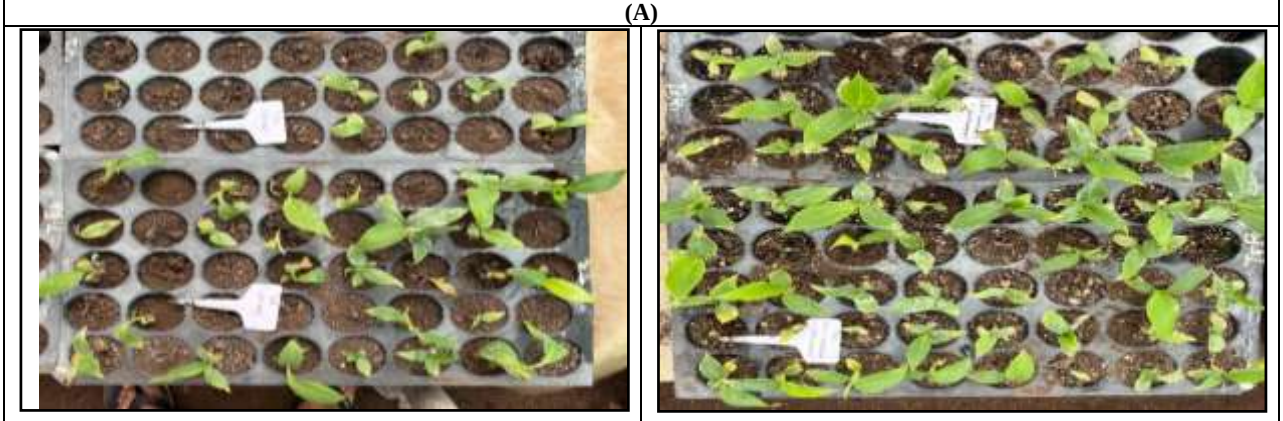
Whereas maximum shoot length (5.01 cm) was observed in Soil + Vermicompost (2:1) (T_4) which can be attributed to Vermicompost which improves the consistent moisture supply, which promotes root respiration, and tight contact between plantlets and media promotes general root growth to improve the distribution and absorption of nutrients (Chatterjee and Choudhary, 2007) [5]. These results also showed that vermicompost has hormonal and biochemical effects in addition to nutritional ones on plant growth and productivity. Vermicompost increases the amount of nutrients that plants take up, possibly by giving them all in a form that is easily absorbed. According to Mahmud *et al.* (2020) [11], applying vermicompost decreased soil acidity and increased the amount of macro- and micronutrients (N, P, K, Mg, Ca, S, Fe, Zn, B, and Al) in the soil and plants.

While minimum shoot length (3.56 cm), minimal number of roots (4.07), lowest number of leaves (3.93), minimum leaf area (10.27 cm^2), minimum survival percentage (39.64) lowest stomata frequency per mm^2 on upper surface (23.00) and lower surface (74.00) of leaves recorded in Soil only (T_1) these findings might be due to Soil alone which may lack adequate nutrients and proper soil structure which leads to poor plant growth and development. Soil alone may possess less nutrient availability, weaker soil aeration and water retention would be poorer. These findings were supported by Brady *et al.* (1984) [3].

Table 1: Effect of different media on primary hardening of banana tissue culture plants in greenhouse conditions

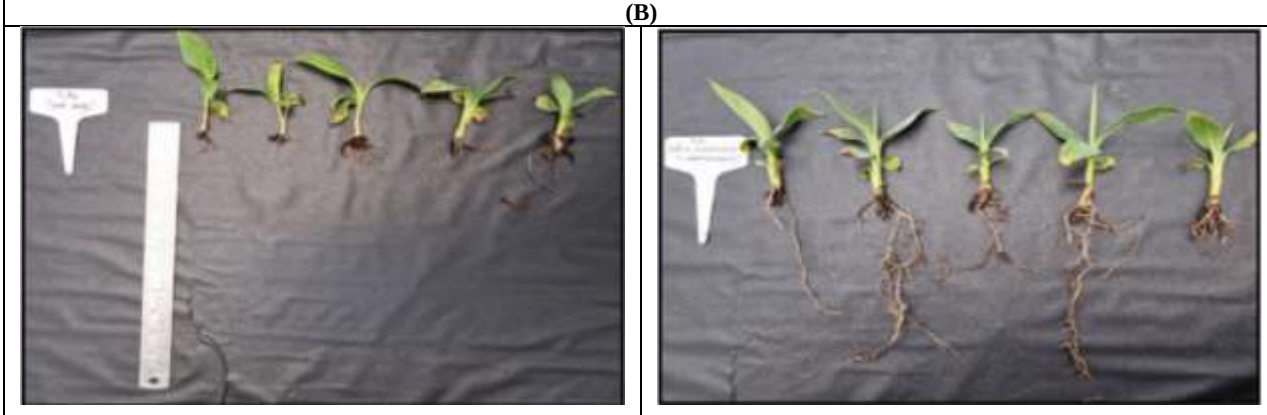
Treatments	Survival rate (%)	Number of roots	Number of leaves	Leaf area (cm^2)	Stomata frequency on upper surface (mm^{-2})	Stomata frequency on lower surface (mm^{-2})	Shoot length (cm)
T_1	39.64 (38.99)	4.07	3.93	10.27	23.00	74.00	3.56
T_2	48.07 (43.88)	4.40	4.00	10.41	27.00	83.33	3.96
T_3	51.42 (45.82)	6.07	4.33	10.44	23.33	74.67	3.96
T_4	59.18 (50.38)	5.07	4.53	11.33	26.00	78.33	5.01
T_5	80.25 (63.93)	6.87	5.27	12.71	37.33	129.67	4.99
T_6	53.35 (46.93)	5.13	4.33	10.66	36.00	127.33	4.14
T_7	53.49 (47.01)	4.80	4.20	12.27	28.33	82.67	4.04
T_8	57.20 (49.14)	6.60	4.13	11.83	32.00	97.00	3.59
S. Em.±	3.096	0.108	0.104	0.283	1.181	3.070	0.119
C. D. at 5%	8.81	0.31	0.30	0.80	3.36	8.74	0.34
CV %	13.33	4.80	5.73	5.99	9.65	7.83	6.79

(A)



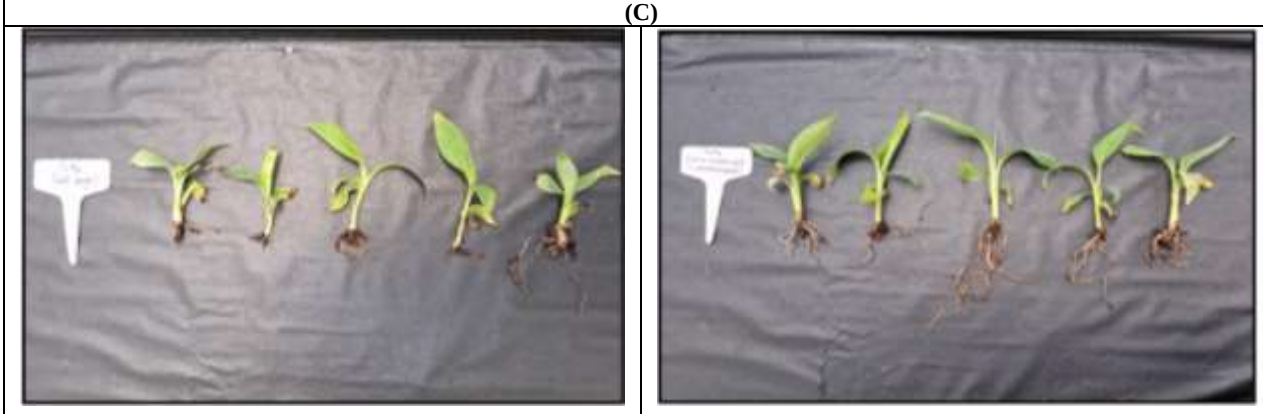
T_1- Soil only | T_5- Soil + Vermiculite + Vermicompost (2:1:1)

(B)



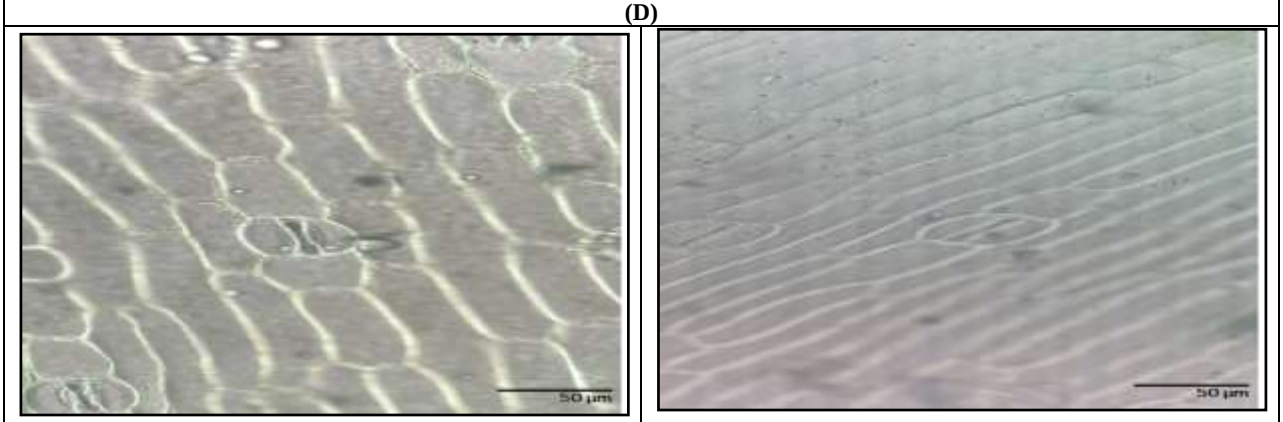
T_1- Soil only | T_5- Soil + Vermiculite + Vermicompost (2:1:1)

(C)



T_1- Soil only | T_5- Soil + Vermiculite + Vermicompost (2:1:1)

(D)



T_1- Soil only | T_5- Soil + Vermiculite + Vermicompost (2:1:1)

(E)

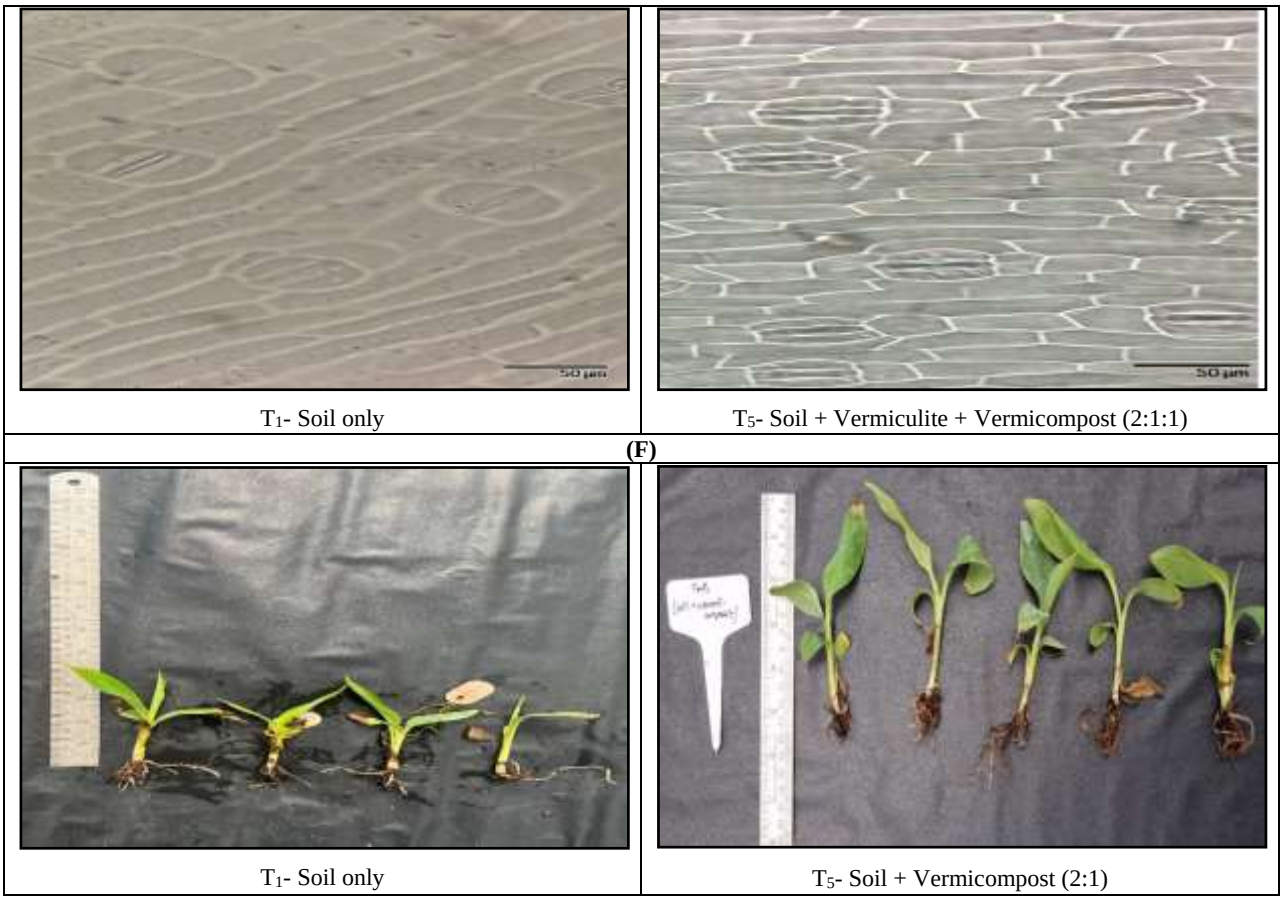


Fig 1: Effect of different media on primary hardening of banana tissue culture plants in greenhouse conditions, i.e A) Survival percentage, B) Number of roots, C) Number of leaves, D) Stomata frequency per mm^2 on upper surface of leaves, E) Stomata frequency per mm^2 on lower surface of leaves and F) Shoot length

Conclusion

The present investigation concluded that treatment Soil + Vermiculite + Vermicompost (2:1:1) is ideal for primary hardening which can enhance the quality of banana plantlets, offering high survivability and excellent growth.

Acknowledgement

I am highly thankful to my guide through my masters Dr. K. M. Karetha. I owe an immense debt of gratitude to my dear parents, Shri. Bhupatbhai and Smt. Laxmiben. Expressing gratitude towards friends.

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