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EFFECT OF DIFFERENT MEDIA ON SECONDARY HARDENING OF BANANA TISSUE CULTURE PLANTS IN GREENHOUSE CONDITIONS

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ABSTRACT

An investigation on secondary hardening of banana plantlets of the Grand Naine variety was carried out at Hi-tech horticulture park, Junagadh Agricultural University, Junagadh during May 2023. Among the six combinations of growing media, the combination T_5- comprising soil, sand and vermicompost in a 2:1:1 ratio produced maximum plant height (29.39 cm), highest stem diameter (11.57 mm), maximal leaf length (18.87 cm), leaf width (8.53 cm), highest chlorophyll content (49.75) and maximum survival percentage (92.42). Maximal number of leaves (6.30), highest number of primary roots (7.55), maximum root length (25.61 cm) was recorded in T_3 which consist of Soil and Vermicompost in 2:1 ratio. In contrast, the soil only medium (T_1) recorded minimum plant height (18.88 cm), minimal stem diameter (6.40 mm), lowest number of leaves (4.65), number of primary roots (4.75), minimal leaf length (12.56 cm), width (5.10 cm), minimum root length (12.81 cm), lowest chlorophyll content (29.64), minimum survival percentage (62.87). Media combination T_5- (Soil + Sand + Vermicompost- 2:1:1 v/v) gives better performance in Secondary hardening.

Key words : Banana, Tissue culture, Secondary hardening, Media, Vermicompost.

Introduction

Banana is considered as 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 *et al*., 2011). The banana is a fruit that is produced by herbaceous plants belonging to the *Musa* genus. Majority of cultivated bananas are a result of natural hybridization between two diploid species *M. acuminata* and *M. balbisiana* (Simmonds, 1996) 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). Green Banana contains 2-hexanal, ripe banana contains Eugenol and overripe banana contains isopentanol.

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). Micro propagation is a unique process for banana large numbers production. Acclimatization is the most crucial process during banana micro propagation (Ozdemir *et al.*, 2020). Tissue culture, as a propagation method which gives a reliable way to produce planting materials free from disease which can serve as the first line of defence in creating an integrated program for managing banana diseases. The current contaminated germplasm needed to be replaced with healthy and high-yielding seed material due to the high occurence of banana bunchy top and panama wilt diseases in banana fields (Al-Amin *et al*., 2009).

Due to the micro propagation of tissue culture grown

plants under controlled conditions, direct planting in the field is not feasible. Farmers suffer losses as a result of their direct plantation in the natural environment, which encourages high death of plants or low survival rates. That's why they gradually acclimatise in green houses and shade houses before transferring it to the field conditions and before being given to the farmers for plantation work. (Hudson *et al*., 1990). The physiological and anatomical characteristics of micro-propagated plantlets necessitate that they should be gradually acclimatized to the environment of the greenhouse or field (Hazarika, 2003). Development of cuticle, epicuticular waxes, and effective stomatal regulation of transpiration occurs leading to stabilization of water potential of field transferred plantlets (Silva *et al.*, 1999). 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).

Hardening is the process of acclimating plants cultivated in tissue culture. Since the plantlets, which are produced in vitro are not easily acclimated to in vivo condition. Acclimatization also known as hardening is the most important step in the micro-propagation of bananas. (Vasane *et al*., 2006).

Thus, one essential and crucial step in the entire tissue culture technology process is primary and secondary hardening. Improper hardening causes the industry as a whole and as well as individual technologies to fail. An industry's ability to harden is essential for industry's existence (Radheshyam and Subramani, 2008). Moreover, an effective acclimatization technique saved time, labour, and money, including the culture medium used, is really needed (Gantait *et al.*, 2009). The composition of the growing medium affects the quality of the seedlings (Wilson *et al*., 2001) 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. Random plantlets were selected from primary hardened banana plantlets. The experiment followed a Completely Randomized Design (CRD) with four repetitions and six treatments: T_1 (Soil only), T_2 (Soil + Sand- 2:1 v/v), T_3 (Soil + Vermicompost- 2:1 v/v), T_4 (Soil + FYM- 3:1 v/v), T_5 (Soil + Sand + Vermicompost- 2:1:1 v/v) and T_6 (Soil + Sand + Vermicompost + FYM- 2:1:1:1 v/v) for 30 days.

Observation Recorded During Secondary Hardening

Plant height (cm)

The plant height was measured at around 60 days after transplanting. It was measured from ground level to the highest growing tip of the plant from all the five tagged plants of each treatment and repetition. The measurement was done in centimeters with the help of the standard meter scale and average height of plant was worked out and recorded.

Stem diameter (mm)

The diameter of plant stem was calculated with the help of Vernier caliper (in mm) from five tagged observational plants from each treatment and repetition was recorded and average was calculated.

Number of leaves

Number of leaves of five tagged observational plants from each treatment and repetitions was recorded at the end of the secondary hardening or 60 days from transplanting and average was calculated and expressed in numbers.

Number of primary roots

Number of roots emerged from corms of five tagged observational plants from each treatment and repetitions was recorded at 60 days from transplanting and average was calculated and expressed in numbers.

Leaf length and leaf width (cm)

Leaf length and leaf width of five tagged observational plants from each treatments and repetitions were determined by measuring of the second leaf of the plant (Razani *et al*., 2020) by using the standard meter scale and average length and width of the leaf was worked out and recorded.

Root length (cm)

Root length was measured from the base of corm to the tip of root from five tagged observational plants of each treatment and repetitions was recorded at 60 days from transplanting and average was calculated and expressed in centimeters.

Chlorophyll content (SPAD)

Chlorophyll content was measured with the help of SPAD 502 chlorophyll meter at 60 days from five tagged observational plants of each treatment and repetitions of

youngest fully expanded leaf of selected plants in SPAD unit.

Statistical analysis

Statistical analysis of the individual data of various characters studied in the experiments was carried out as per Completely Randomized Design (CRD) through computer. Analysis of variance was worked out using standard statistical procedures as described by Panse and Sukhatme (1985). Standard error of mean (S. Em ±), Critical Difference (C.D.) at 5 probability and coefficient of variance (C.V.%) was worked out for the interpretation of the results. Statistical analysis was carried out in the computer cell, Department of Agricultural Statistics, College of Agriculture, J.A.U., Junagadh.

Results and Discussion

From Table no.1 Maximum survival rate (92.42) maximal number of primary roots (6.87), maximum leaf length (18.87 cm), highest leaf width (8.53 cm), highest chlorophyll content (49.75), maximum plant height (29.39 cm), and maximal stem diameter (11.57 mm) were observed in Soil + Sand + Vermicompost (2:1:1) (T_5) whereas longest root length (25.61 cm), and highest number of leaves (7.55) were recorded in Soil + Vermicompost (2:1) (T_3).

Maximum survival rate, maximal number of primary roots, maximum leaf length, highest leaf width, highest chlorophyll content, maximum plant height, and maximal stem diameter might be due to 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 (Chamling and Bhowmik, 2021). Sand may be responsible for producing sufficient aeration and 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 (Ahmed *et al.*, 2014). Addition of vermicompost to growth environments improved banana leaves content of chlorophyll, nitrogen, phosphorus and potassium. Similarly, chlorophyll a which got increased in plants grown in sugarcane compost at all concentrations used compared to control plants (Najarian and Souri, 2020). 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).

Whereas, maximum number of leaves (7.55) and highest root length (25.61 cm) were observed in Soil + Vermicompost (2:1) (T_3), which can be attributed to Vermicompost promotes direct contact between plant roots and the growing medium, improves a consistent moisture supply, and supports root respiration and growth, leading to better nutrient absorption and distribution in plants (Chatterjee and Choudhary, 2007). Vermicompost contain fair amount of macro and micro nutrients and different growth promoters like cytokinin, gibbrelin and auxins (Hassan *et al.*, 2022). 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), applying vermicompost decreased soil acidity

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

Treatments	Survival rate (%)	Number of primary roots	Root length (cm)	Number of leaves	Leaf length (cm)	Leaf width (cm)	Chloro-phyll content (SPAD)	Plant height (cm)	Stem diameter (mm)
T_1	62.87	4.75	12.81	4.65	12.56	5.10	29.64	18.88	6.40
T_2	81.05	5.65	14.76	5.45	14.60	6.61	35.24	24.64	7.99
T_3	87.87	7.55	25.61	6.30	17.68	7.43	45.63	27.61	9.19
T_4	74.24	6.10	20.07	5.60	16.74	7.14	30.78	25.28	8.61
T_5	92.42	6.80	25.19	6.20	18.87	8.53	49.75	29.39	11.57
T_6	81.05	6.55	20.35	5.75	15.80	7.39	34.38	24.29	9.26
S. Em. ±	2.531	0.180	0.566	0.162	0.466	0.207	0.606	0.619	0.175
C. D. at 5%	7.52	0.53	1.68	0.48	1.39	0.62	1.80	1.84	0.52
CV %	6.33	5.76	5.72	5.73	5.82	5.90	3.22	4.93	3.96

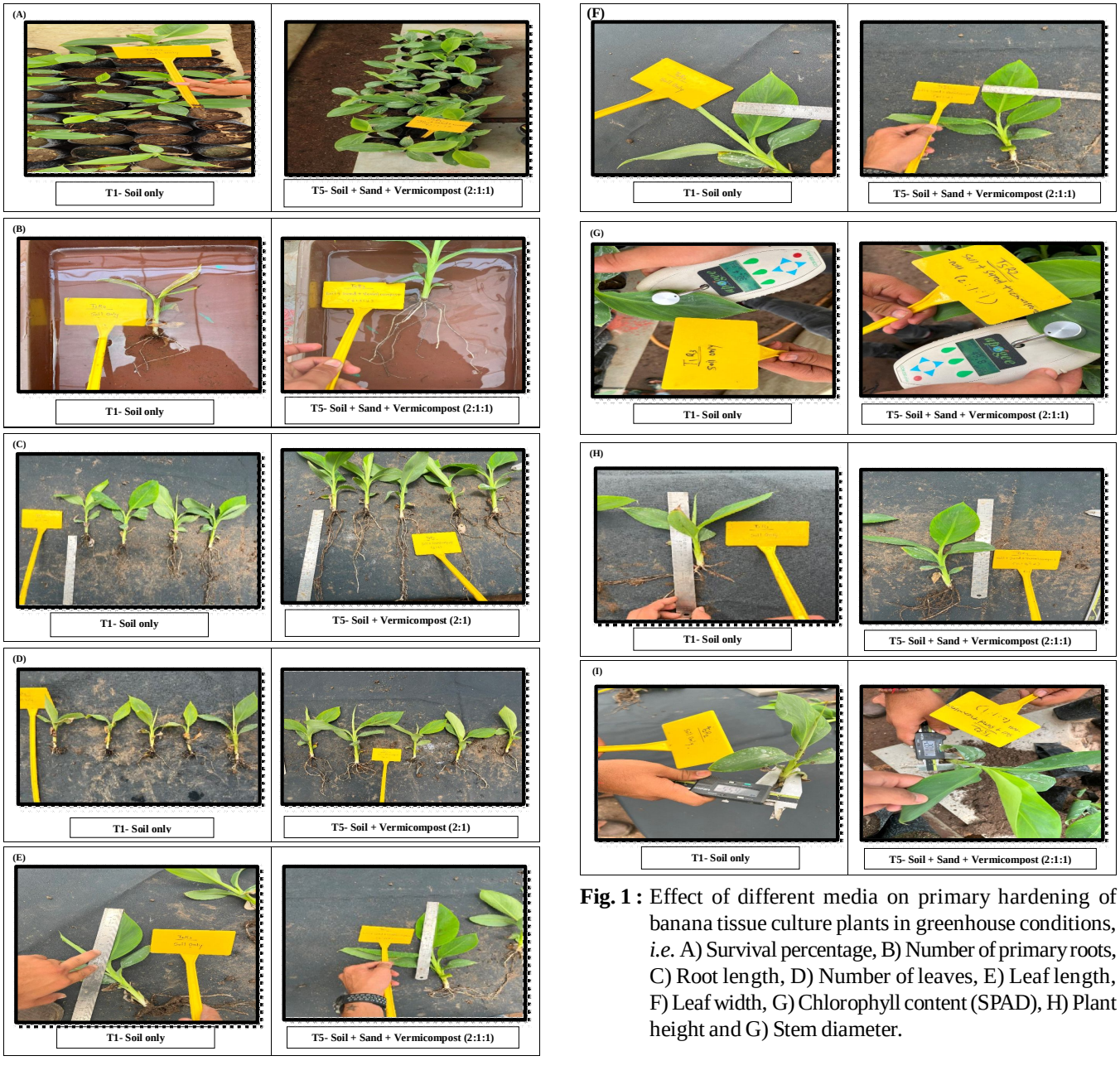

Fig. 1 : Effect of different media on primary hardening of banana tissue culture plants in greenhouse conditions, *i.e.* A) Survival percentage, B) Number of primary roots, C) Root length, D) Number of leaves, E) Leaf length, F) Leaf width, G) Chlorophyll content (SPAD), H) Plant height and G) Stem diameter.

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 plant height (18.88 cm), minimal stem diameter (6.40 mm), lowest number of leaves (4.65), number of primary roots (4.75), minimal leaf length (12.56 cm), width (5.10 cm), minimum root length (12.81 cm), lowest chlorophyll content (29.64), minimum survival percentage (62.87) recorded in Soil only (T_1) these findings might be due to Soil by itself lacking sufficient nutrients and correct structure which results in inadequate plant growth and development. Soil might have lower nutrient accessibility, less effective soil aeration and reduced water retention (Brady *et al.*, 1984).

Conclusion

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

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