International Journal of Advanced Biochemistry Research

ISSN Print: 2617-4693
ISSN Online: 2617-4707
IJABR 2024; 8(10): 172-179
www.biochemjournal.com
Received: 14-07-2024
Accepted: 17-08-2024

CH Prathyusha
M.Sc (Hort.), Department of Fruit Science, Dr. YSR Horticultural University, College of Horticulture, Venkataramannagudem, Andhra Pradesh, India

P Vinaya Kumar Reddy
Associate Professor, Department of Fruit Science, Dr. YSR Horticultural University, College of Horticulture, Venkataramannagudem, Andhra Pradesh, India

K Ravindra Kumar
Senior Scientist, Dr. YSR Horticultural University, Horticultural Research Station, Kovvur, Andhra Pradesh, India

S Narasimha Rao
Associate Professor, Department of Plant Pathology, Dr. YSR Horticultural University, College of Horticulture, Venkataramannagudem, Andhra Pradesh, India

V Sekhar
Assistant Professor, Department of Statistics, Dr. YSR Horticultural University, College of Horticulture, Venkataramannagudem, Andhra Pradesh, India

Corresponding Author:
CH Prathyusha
M.Sc (Hort.), Department of Fruit Science, Dr. YSR Horticultural University, College of Horticulture, Venkataramannagudem, Andhra Pradesh, India

Effect of growing conditions and bioagents on hardening of tissue culture banana (*Musa* spp.)

CH Prathyusha, P Vinaya Kumar Reddy, K Ravindra kumar, S Narasimha Rao and V Sekhar

DOI: https://doi.org/10.33545/26174693.2024.v8.i10c.2436

Abstract
The experiment entitled "Effect of growing conditions and bioagents on hardening of tissue culture banana (*Musa* spp.)" was carried out at the Dr. Y.S.R.H.U-Horticultural Research Station, Kovvur, Andhra Pradesh during the year 2023- 24. The experiment was laid out in a factorial randomized block design (FRBD) with two replications conducted in two periods January and April consists of fifteen treatment combinations in cv. Tella Chakkerakeli having two factors recorded *i.e.*, growing conditions at 3 levels (double shade net, tunnel covered with transparent polythene sheet, in more than 6 months aged banana garden) and 5 levels of potting media enriched with bioagents (Control-red earth + FYM + cocopeat 2:1:1, AMF + *Pseudomonas fluorescens*, PSB + *Pseudomonas fluorescens*, AMF + *Trichoderma* sp., PSB + *Trichoderma* sp.) observations are recorded after 15 days of primary hardening. Both growing conditions and bioagents had significant impact on hardening of tissue culture banana. The treatment combination tunnel covered with transparent polythene sheet + AMF + *Trichoderma* sp. recorded maximum survival percent (98.95 and 88.90%), mortality percent (1.06 and 11.1%), no. of days taken for first leaf emergence (2.95 and 3), plant height (7.70 and 7.45 cm), girth (6.05 and 4.8 mm) and number of leaves per plant (2.4 and 2.6) compared to other treatment combinations.

Keywords: Banana, tissue culture, *ex-vitro* hardening, growing conditions, bioagents

Introduction
Banana (*Musa* spp.) belongs to the family Musaceae. Globally, it stands as the fourth largest fruit crop. Banana being native to Southeast Asia is widely grown in tropical, subtropical and the coastal regions of India. India leads the world in banana cultivation, producing 35.9 million tonnes annually from 9,62,600 hectares, with a remarkable productivity of 35.88 tonnes per hectare (Anon, 2022) [2]. The states of Karnataka, Andhra Pradesh, Maharashtra, Tamil Nadu, and Gujarat dominate banana cultivation in India, contributing significantly to both area under cultivation and total production.

Most edible bananas are triploids (2n= 3x= 33) derived from diploid species that is *Musa acuminata* (Malaysia) and *Musa balbisiana* (India), which are highly productive and vigorous. Due to their parthenocarpy nature and sterile seed, sucker propagation is the only natural means for their perpetuation. The advantages of tissue culture grown banana over conventionally grown banana in yield and quality is well established (Bhojwani and Razdan, 1983) [4] It is globally recognized that, tissue culture technique generates homogeneous population of plantlets endowed with totipotency of elite mother plantlets, which are not only agro-climatically adapted, but also attributed with vibrant growth, free from pest & disease and consequently higher productivity. The success of commercial tissue culture industry solely depends on minimal microbial contamination during the growth and hardening processes of tender plantlets (Patil *et al*., 2010) [14]. Therefore, acclimatization is the most crucial step during micro-propagation as the *in-vitro* raised plantlets upon transfer to *ex-vitro* conditions are exposed to abiotic stress and biotic stress conditions, like soil microflora (Deb and Imchen, 2010) [7]. The composition of the growing medium influences the quality of the plantlets (Wilson *et al*., 2001) [20]. Banana cv. Tella Chakkerakeli (AAA) is choicest table variety in Godavari districts and highly popular in Andhra Pradesh. Therefore the present research work is focused on standardizing the primary hardening protocol for popular banana genotype.

Hence, the present study was carried out on "Effect of growing conditions and bioagents on hardening of tissue culture banana (*Musa* spp.)" to increase acclimatization of plantlets and reduce mortality using different growing conditions and bioagents.

Materials and Methods

The present experiment "Effect of growing conditions and bioagents on hardening of tissue culture banana (*Musa* spp.)" was carried out at Horticultural Research Station, Kovvur, East Godavari district, Andhra Pradesh. The location falls under Agro- climatic zone -10, humid, Southern plateau and hills region (Andhra Pradesh) with an average annual rainfall of 1017.67 mm. The experimental site was located at Horticultural Research Station, Kovvur, East Godavari district, Andhra Pradesh. The experimental site was geographically situated at 17.00^0N latitude, 81.43^0E longitude and altitude of 34 m above mean sea level. The experiment was laid out in factorial RBD with two factors that replicated twice. The first factor has three levels *i.e.* (G_1- double shade net, G_2-tunnel covered with transparent polythene sheet, G_3-in more than 6 months aged banana garden) while the second factor has 5 levels of potting media enriched with bioagents (B_1-Control-red earth + FYM + cocopeat 2:1:1, B_2-AMF + *Pseudomonas fluorescens*, B_3-PSB + *Pseudomonas fluorescens*, B_4-AMF + *Trichoderma* sp., B_5 -PSB + *Trichoderma* sp.). Tissue culture bottles of genotype Tella Chakkerakeli were brought from tissue culture laboratory, HRS, Kovvur under shade net and pre-hardened for 10 days.

Observations recorded

The following observations were recorded for banana cv. Tella Chakkerakeli in the months of January and April respectively, the survival percentage placed under different growing conditions were recorded by dividing the number of plantlets survived at the end of hardening to the total number of plantlets planted initially and multiplied by 100, mortality percentage of plantlets placed under different growing conditions were recorded by dividing the number of plantlets died at the end of primary hardening to the total number of plantlets planted and multiplied by 100, number of days taken for first leaf emergence were recorded from the day of planting, plant height was measured from the base of the stem in the polybag to the angle made between the youngest and 1st open leaf by using measuring scale and average value was expressed in centimetre, girth of the plant was recorded per treatment per replication at 5 cm from the ground level with a Vernier callipers and mean girth was expressed in millimetres and number of leaves per plant are recorded.

Results and Discussion

Survival percentage

The maximum (95.24 and 80.03% in January and April months) survival percentage was recorded at tunnel covered with transparent polythene sheet, minimum (87.73 and 61.93% in January and April months) was observed in, In more than 6 months aged banana garden. Similarly, among bio agents, maximum (96.81 and 76.83% in January and April months) survival percentage was showed at primary hardening in AMF + *Trichoderma* sp. and minimum (85.10 and 61.44% in January and April months) was observed with media containing red earth, FYM and cocopeat (2:1:1) (Control) for both the genotypes.

In Tella Chakkerakeli, significant difference was observed with regard to interaction effect of growth condition and bioagents in January. Among the interactions, maximum survival percent was recorded (98.95 and 88.9% in January and April months) for plantlets under treatment T_9 -G_2B_4 Tunnel covered with transparent polythene sheet + AMF + *Trichoderma* sp*p*) followed by (98.03 and 83.95% in January and April months) treatment T_8 -G_2B_3 Tunnel covered with transparent polythene sheet + (PSB + *Pseudomonas fluorescens*) and lowest (85.20 and 51.67% in January and April months) was recorded in the G_3B_1.

This may be due to the effect of bioagents which promotes root initiation through more nutrient uptake, root cell elongation and initiation. AMF + *Trichoderma* sp*p* being rich source of phosphate fertilizer showed significant increase in survival percentage. The bioagent VAM are reported to substantially enhance the establishment rate of the micro propagated plantlets which might have helped in gaining better acclimatization. *Trichoderma* spp. considered a potent biocontrol agent, that can survive under stressed conditions, antagonistic against phyto-pathogenic microorganisms, induces a defense mechanism in plantlets, and helps in plant growth promotion by secreting several secondary metabolites (Adnani *et al*., 2017; Poveda *et al*., 2020) [1, 15], Singh *et al*. (2011) [16] in micro propagated grape plantlets and Singh *et al*. (2012) [17] in pomegranate plantlets.

Interior of the poly house became warm to warmer and temperature remained at optimum level for the growth and development of seedlings as a result that the air temperature inside the polyhouse gradually increased due to the greenhouse effect and paves way for higher survival percent of seedlings. The results were in line with the findings of Verma *et al*. (2019) [19] in aonla.

Mortality percentage

Among different growing conditions for banana cv. Tella Chakkerakeli, maximum (12.27 and 38.07% in January and April months) mortality percentage was recorded at, In more than 6 months aged banana garden and minimum (4.76 and 19.97% in January and April months) was observed in, tunnel covered with transparent polythene sheet. Similarly, among bio agents, maximum (14.90 and 38.55% in January and April months) mortality percentage was showed at B_1 (red earth, FYM and cocopeat (1:2:1) (Control) primary hardening in and minimum (3.19 and 23.17% in January and April months) was observed with media containing AMF + *Trichoderma* sp. for both the genotypes.

In Tella Chakkerakeli, significant difference was observed with regard to interaction effect of growth condition and bioagents in January and April. Among the interactions, maximum mortality percent was recorded (19.60 and 48.34% in January and April months) for G_3B_1 plantlets under treatment followed by (15.20 and 38.80% in January and April months) treatment T_{15} -G_3B_{15} (In more than 6 months aged banana garden + PSB + *Trichoderma* sp*p*) and lowest (1.06 and 11.10% in January and April months) was recorded in the T_9 -G_2B_4 Tunnel covered with transparent polythene sheet + (AMF + *Trichoderma* sp*p*).

However, mortality of plantlets may be due to injuries to the root system during transferring and sudden exposure to the harsh environment. The lowest mortality rate in VAM

treated plantlets confirmed its importance for plant establishment during hardening. This might be due to the maximum mortality of micro-propagated plantlets occurs during the acclimatization process as plantlets undergo several morphological, physiological, and biochemical changes (Pati *et al.* 2013) [13].

No. of days taken for first leaf emergence

Among the different growing conditions for Tella Chakkerakeli, the plantlets placed under polyhouse condition (G_2) significantly induced early emergence of leaves (3.46 days and 3.57 days in January and April months), followed by double shade net (G_1) condition (3.64 and 3.81 days in January and April). The maximum (4.44 and 4.82 days in January and April months) average number of days recorded for no. of days taken for 1st leaf emergence are recorded for (G_1) -In more than 6 months aged banana garden.

Among the different bioagents (AMF, PSB, *Trichoderma*, *Pseudomonas*) in Tella Chakkerakeli, significant differences were observed with respect to the number of days taken for 1st leaf emergence. AMF + *Trichoderma* (B_4) recorded the minimum number of days to initiate leaf emergence, (3.39 and 3.46 days in January and April months) which was significantly different from other treatments, maximum number of days to initiate leaf emergence (4.23 and 4.72 days in January and April months) was recorded in the B_1 (Red earth +FYM + Cocopeat 2:1:1) (Control) in primary hardening.

Interaction between different growing conditions and bioagents was found to be non-significant with respect to no. of days taken for 1st leaf emergence in Tella Chakkerakeli in January and April. The data revealed that minimum no. of days taken for 1st leaf emergence was recorded (2.95 and 3.00 days in) in G_2B_4treatment combination (Tunnel covered with transparent polythene sheet + AMF + *Trichoderma*) and maximum no. of days (4.85 and 5.65 days) in G_3B_1 (In more than 6 months aged banana garden + Red earth +FYM + Cocopeat 2:1:1) treatment combination.The leaf emergence rate serves as a good index of the vegetative growth rate of banana plant (Turner, 1981) [18].

Plant height (cm)

At 2nd week after hardening in Tella Chakkerakeli, the data pertaining to pseudo stem height revealed that at different growing conditions, maximum plant height (6.42 and 6.37 cm in January and April months) was recorded in tunnel covered with transparent polythene sheet and minimum (4.65 and 4.89 cm in January and April months) was recorded at, In more than 6 months aged banana garden. Similarly, among bio agents, maximum (6.25 and 6.54 cm in January and April months) plant height was showed at B_4 (AMF + *Trichoderma* sp.) primary hardening in and minimum (4.58 and 4.39 cm in January and April months) was observed with media containing B_1 (Red earth +FYM + Cocopeat 2:1:1) (Control).

The data revealed that maximum plant height was recorded (7.70 and 7.45 cm) in G_2B_4 treatment combination and minimum plant height was observed in (3.80 and 3.70 cm G_3B_1 treatment combination. Findings corroborates with the results obtained by Vega *et al.* (1995) [8] in pineapple and banana and Mwashasha *et al.* (2011) [11], Patel *et al*. (2015) [12] and Biswalk *et al*. (2016) [5] in banana.

Girth

At 2nd week after hardening in Tella Chakkerakeli, the data pertaining to pseudo stem girth revealed that at different growing conditions, maximum plant girth (5.56 and 3.85 mm in January and April months) was recorded in tunnel covered with transparent polythene sheet and minimum (3.59 and 3.58 mm in January and April months) was recorded at, In more than 6 months aged banana garden.

Similarly, among bio agents, maximum (5.09 and 4.17 mm in January and April months) plant girth was showed at B_4 (AMF + *Trichoderma* sp.) in primary hardening and minimum (4.20 and 3.32 mm in January and April months) was observed with media containing B_1 (Red earth +FYM + Cocopeat 2:1:1) (Control).

The data revealed that maximum plant girth was recorded (6.05 and 4.80 mm) in G_2B_4treatment combination and minimum plant girth was observed in (3.44 and 3.35 cm in January and April months) G_3B_1 treatment combination. Finding corroborates with the results obtained by Biswas *et al.* (2018) [6] in *in vitro* raised banana plantlets, Khatik *et al.* (2019) [9] in strawberry.

Number of leaves per plant

The plantlets placed under polyhouse condition (G_2) showed maximum number of leaves (2.28 and 2.31 in January and April months), which is on par with double shade net (G_1) condition (2.19) in January month. In April month it showed (2.01) significant difference. The minimum (1.73 and 1.73 in January and April months) number of leaves per plant are recorded for (G_3) -In more than 6 months aged banana garden.

Similarly, among bio agents, maximum (2.34 and 2.47 in January and April months) no. of leaves was showed at B_4 (AMF + *Trichoderma* sp.) and minimum (1.86 and 1.67 in January and April months) was observed with media containing B_1 (Red earth +FYM + Cocopeat 2:1:1) (Control).

In Tella Chakkerakeli, interaction between different growing conditions and bioagents was recorded. Maximum no. of leaves are observed in G_2B_4 (2.40 and 2.60 in January and April months) treatment, which is found to be on par with all other treatments G_2B_3, G_2B_2, G_2B_5 and G_2B1 in polytunnel (G_2) condition in January and April and minimum plant girth was observed in (1.33 and 1.30) G3B1. These results are in line with Bharati *et al.* (2018) [3] in banana.

Table 1: Effect of different growing conditions and bio agents on survival percentage of banana cv. Tellachakkera Keli after primary hardening stage in tissue culture banana

Growing conditions (G)	January						April					
	Bioagents (B)						Bioagents (B)					
	B_1	B_2	B_3	B_4	B_5	Mean (G)	B_1	B_2	B_3	B_4	B_5	Mean (G)
G_1	87.13	91.93	95.73	97.75	90.50	92.61	60.94	67.60	71.70	73.93	64.70	67.77
G_2	87.78	96.83	98.03	98.95	94.65	95.24	71.73	79.55	83.95	88.90	76.03	80.03
G_3	80.40	87.25	92.48	93.73	84.80	87.73	51.67	63.18	65.93	67.68	61.20	61.93
Mean (B)	85.10	92.00	95.41	96.81	89.98		61.44	70.11	73.86	76.83	67.31	
Factors	SE m±		CD at 5%				SE m±		CD at 5%			
G	0.19		0.59				0.22		0.68			
B	0.25		0.76				0.29		0.88			
G x B	0.43		1.32				0.50		1.53			

G_1-Double shade net
G_2-Tunnel covered with transparent polythene sheet
G_3-In more than 6 months aged banana garden
B_1-Control (Red earth +FYM + Cocopeat 2:1:1
B_2-AMF + *Pseudomonas fluorescens*
B_3-PSB + *Pseudomonas fluorescens*
B_4- AMF + *Trichoderma* sp.
B_5- PSB + *Trichoderma* sp.

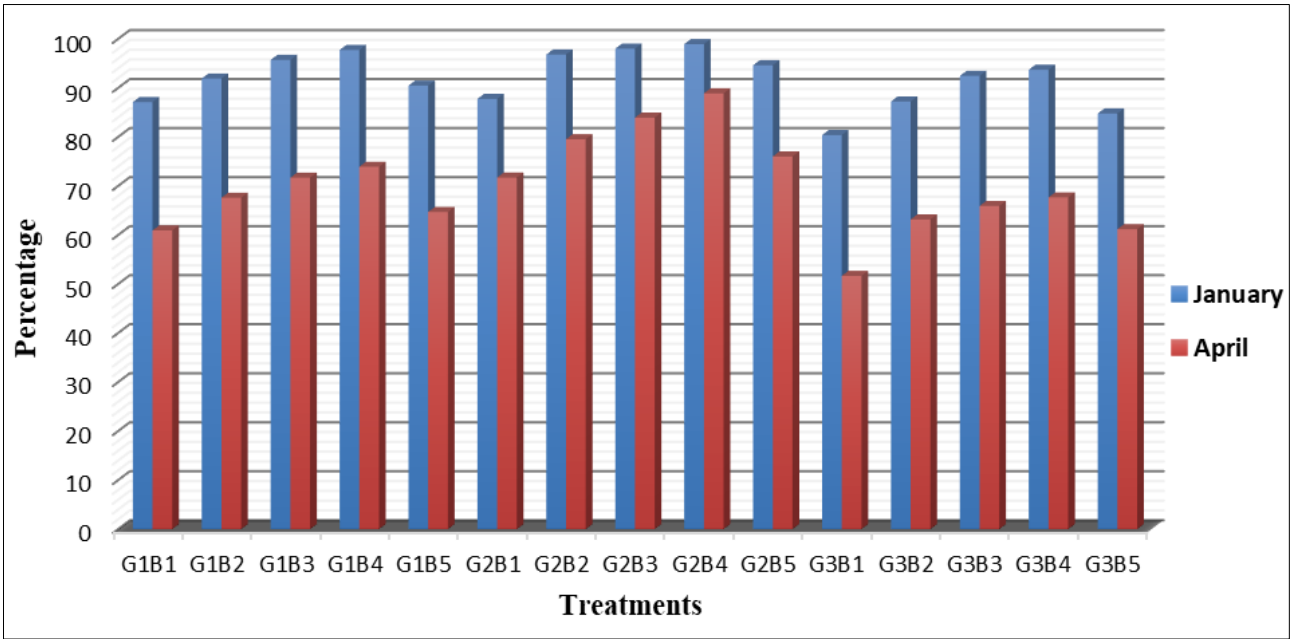

Fig 1: Influence of different growing conditions and bio agents on survival percentage of banana cv. Tella Chakkerakeli after primary hardening stage in tissue culture banana

Table 2: Effect of different growing conditions and bio agents on mortality percentage of banana cv. Tella Chakkerakeli after primary hardening stage in tissue culture banana

Growing conditions (G)	January						April					
	Bioagents (B)						Bioagents (B)					
	B_1	B_2	B_3	B_4	B_5	Mean (G)	B_1	B_2	B_3	B_4	B_5	Mean (G)
G_1	12.88	8.08	4.28	2.25	9.50	7.40	39.06	32.40	28.30	26.08	35.30	32.23
G_2	12.23	3.18	1.98	1.06	5.35	4.76	28.26	20.45	16.05	11.10	23.98	19.97
G_3	19.60	12.75	7.53	6.28	15.20	12.27	48.34	36.83	34.08	32.33	38.80	38.07
Mean B	14.90	8.00	4.59	3.19	10.02		38.55	29.89	26.14	23.17	32.69	
Factors	SE m±		CD at 5%				SE m±		CD at 5%			
G	0.19		0.59				0.22		0.68			
B	0.25		0.76				0.29		0.88			
G x B	0.43		1.32				0.50		1.53			

Table 3: Effect of different growing conditions and bio agents on no. of days taken for first leaf emergence (days) of banana cv. Tella Chakkerakeli after primary hardening stage in tissue culture banana

Growing conditions (G)	January						April					
	Bioagents (B)						Bioagents (B)					
	B_1	B_2	B_3	B_4	B_5	Mean (G)	B_1	B_2	B_3	B_4	B_5	Mean (G)
G_1	4.06	3.74	3.46	3.16	3.79	3.64	4.35	3.80	3.70	3.30	3.90	3.81
G_2	3.79	3.47	3.36	2.95	3.72	3.46	4.16	3.59	3.40	3.00	3.72	3.57
G_3	4.85	4.45	4.27	4.06	4.60	4.44	5.65	4.75	4.50	4.07	5.15	4.82
Mean B	4.23	3.89	3.70	3.39	4.03		4.72	4.05	3.86	3.46	4.25	
Factors	SE m±		CD at 5%				SE m±		CD at 5%			
G	0.02		0.21				0.03		0.09			
B	0.03		0.27				0.03		00.11			
Gx B	0.56		NS				0.06		NS			

G_1-Double shade net
G_2-Tunnel covered with transparent polythene sheet
G_3-In more than 6 months aged banana garden
B_1-Control (Red earth +FYM + Cocopeat 2:1:1
B_2-AMF + *Pseudomonas fluorescens*
B_3-PSB + *Pseudomonas fluorescens*
B_4- AMF + *Trichoderma* sp.
B_5- PSB + *Trichoderma* sp.

Table 4: Effect of different growing conditions and bio agents on plant height (cm) of banana cv. Tellachakkera Keli after primary hardening stage in tissue culture banana

Growing conditions (G)	January						April					
	Bioagents (B)						Bioagents (B)					
	B_1	B_2	B_3	B_4	B_5	Mean (G)	B_1	B_2	B_3	B_4	B_5	Mean (G)
G_1	4.33	5.45	5.83	5.90	5.20	5.34	4.30	5.35	5.60	6.48	5.08	5.36
G_2	5.60	6.35	7.15	7.70	5.30	6.42	5.16	6.45	7.20	7.45	5.60	6.37
G_3	3.80	4.65	5.05	5.15	4.60	4.65	3.70	5.00	5.25	5.70	4.80	4.89
Mean (B)	4.58	5.48	6.01	6.25	5.03		4.39	5.60	6.02	6.54	5.16	
Factors	SE m±		CD at 5%				SE m±		CD at 5%			
G	0.02		0.08				0.06		0.20			
B	0.03		0.10				0.08		0.26			
G x B	0.06		0.18				0.14		0.45			

G_1-Double shade net
G_2-Tunnel covered with transparent polythene sheet
G_3-In more than 6 months aged banana garden
B_1-Control (Red earth +FYM + Cocopeat 2:1:1
B_2-AMF + *Pseudomonas fluorescens*
B_3-PSB + *Pseudomonas fluorescens*
B_4- AMF + *Trichoderma* sp.
B_5- PSB + Trichoderma sp.

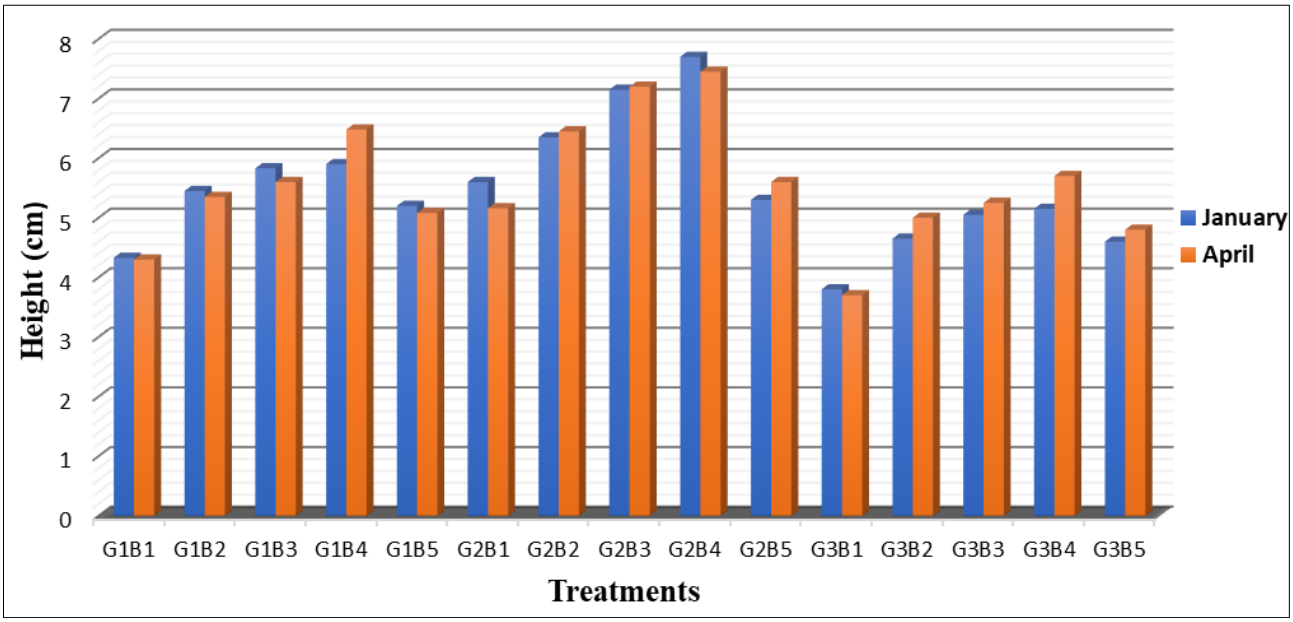

Fig 2: Influence of different growing conditions and bio agents on plant height (cm) of Tella Chakkerakeli after primary hardening stage in tissue culture banana

Table 5: Effect of different growing conditions and bio agents on girth (mm) of banana cv. Tella Chakkerakeli after primary hardening stage in tissue culture banana

Growing conditions (G)	January						April					
	Bioagents (B)						Bioagents (B)					
	B_1	B_2	B_3	B_4	B_5	Mean (G)	B_1	B_2	B_3	B_4	B_5	Mean (G)
G_1	4.22	4.45	5.25	5.45	4.85	4.84	3.36	3.62	3.69	3.76	3.51	3.59
G_2	4.95	5.63	5.70	6.05	5.45	5.56	3.26	3.53	4.19	4.80	3.48	3.85
G_3	3.44	3.55	3.68	3.76	3.53	3.59	3.35	3.62	3.56	3.97	3.41	3.58
Mean (B)	4.20	4.54	4.88	5.09	4.61		3.32	3.59	3.81	4.17	3.47	
Factors	SE m±		CD at 5%				SE m±		CD at 5%			
G	0.04		0.13				0.01		0.05			
B	0.05		0.17				0.02		0.06			
G x B	0.09		0.29				0.03		0.12			

G_1-Double shade net
G_2-Tunnel covered with transparent polythene sheet
G_3-In more than 6 months aged banana garden
B_1-Control (Red earth +FYM + Cocopeat 2:1:1
B_2-AMF + *Pseudomonas fluorescens*
B_3-PSB + *Pseudomonas fluorescens*
B_4- AMF + *Trichoderma* sp.
B_5- PSB + *Trichoderma* sp.

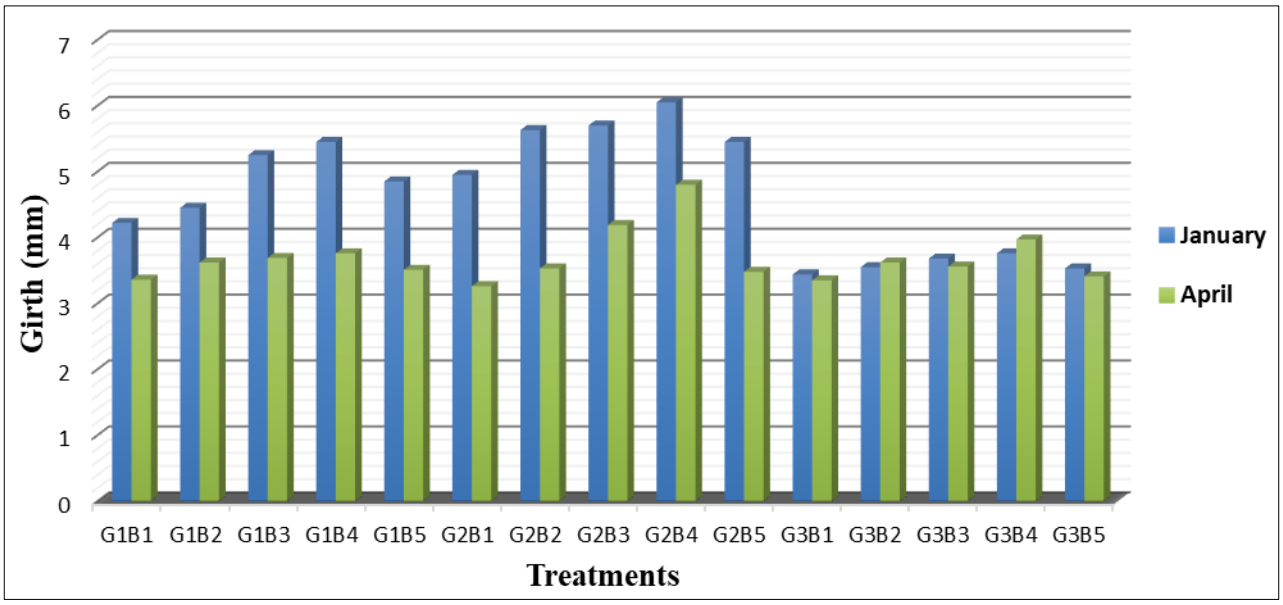

Fig 3: Influence of different growing conditions and bio agents on girth (mm) of banana cv. Tella Chakkerakeli after primary hardening stage in tissue culture banana

Table 6: Effect of different growing conditions and bio agents on no. of leaves per plant of banana cv. Tella Chakkerakeli after primary hardening stage in tissue culture banana

Growing conditions (G)	January						April					
	Bioagents (B)						Bioagents (B)					
	B_1	B_2	B_3	B_4	B_5	Mean (G)	B_1	B_2	B_3	B_4	B_5	Mean (G)
G_1	2.00	2.20	2.25	2.43	2.05	2.19	1.50	2.00	2.20	2.35	2.00	2.01
G_2	2.25	2.20	2.35	2.40	2.20	2.28	2.20	2.25	2.35	2.60	2.15	2.31
G_3	1.33	1.90	1.83	2.20	1.40	1.73	1.30	1.60	2.10	2.45	1.18	1.73
Mean (B)	1.86	2.10	2.14	2.34	1.88		1.67	1.95	2.22	2.47	1.78	
Factors	SE m±		CD at 5%				SE m±		CD at 5%			
G	0.03		0.11				0.04		0.12			
B	0.05		0.15				0.05		0.15			
G x B	0.08		0.26				0.08		0.27			

G_1-Double shade net
G_2-Tunnel covered with transparent polythene sheet
G_3-In more than 6 months aged banana garden
B_1-Control (Red earth +FYM + Cocopeat 2:1:1
B_2-AMF + *Pseudomonas fluorescens*
B_3-PSB + *Pseudomonas fluorescens*
B_4- AMF + *Trichoderma* sp.
B_5- PSB + *Trichoderma* sp.

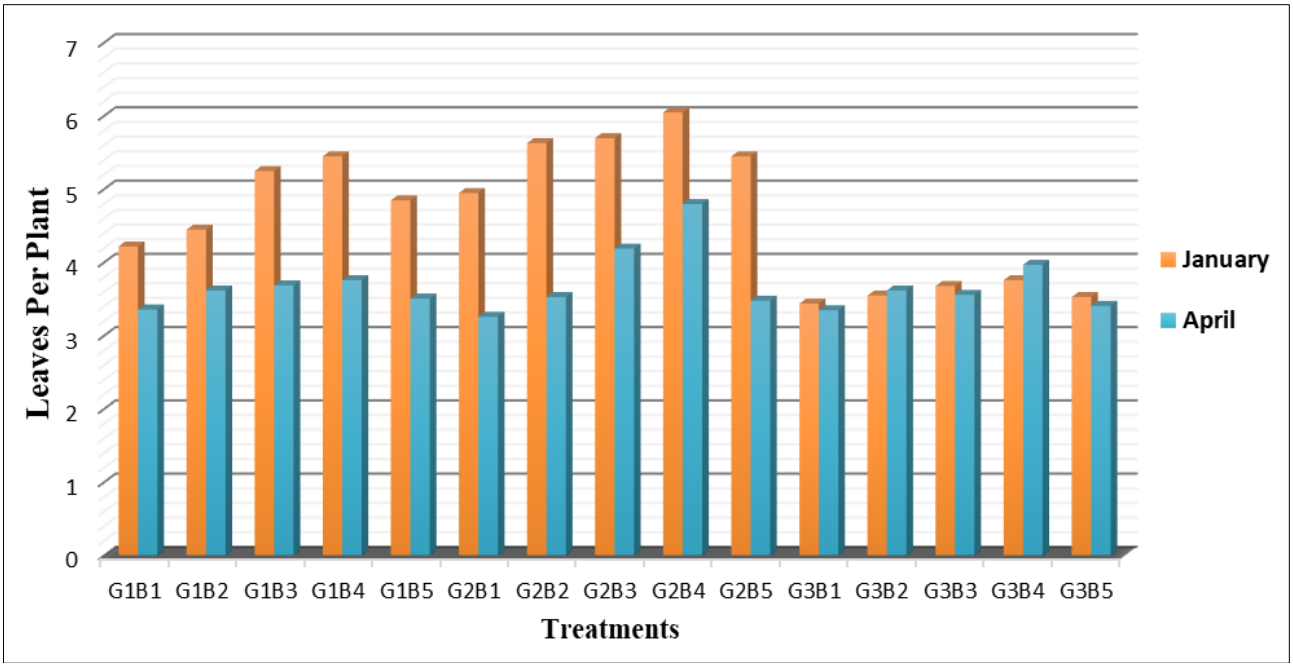

Fig 4: Influence of different growing conditions and bio agents on no. of leaves per plant of banana cv. Tella Chakkerakeli after primary hardening stage in tissue culture banana

Conclusion

The present investigation on the "Effect of growing conditions and bioagents on hardening of tissue culture banana (*Musa* spp.)" revealed that by use of tunnel covered with transparent polythene sheet + AMF + *Trichoderma* sp. showed superior growth performance in terms of survival percent, mortality percent, no. of days taken for first leaf emergence, plant height, girth and number of leaves per plant compared to other treatment combinations. The major problem in extending the cultivation of banana cv. Tella Chakkerakeli is non-availability of disease-free genuine planting material, which can only be ensured by *in vitro* propagation. Mortality of *in-vitro* raised plantlets in large scale at field level performance limits the production. This limitation can only be overcome by proper hardening of tender plantlets using different bio-hardening agents like arbuscular mycorrhiza fungi.

References

1. Adnani N, Rajski SR, Bugni TS. Symbiosis-inspired approaches to antibiotic discovery. Natural Product Reports. 2017;34(7):784-814.
2. Anonymous. Crop wise area and production of Horticultural Crops for three years. National Horticultural Board, Ministry of Agriculture and Farmers Welfare, Government of India, New Delhi. 2022. Available at: www.nhb.gov.in.
3. Bharati K, Prasad M, Mir H, Pal AK. In vitro regeneration and acclimatisation of banana cv. Malbhog. Current Journal of Applied Science and Technology. 2018;31(4):1-6.
4. Bhojwani SS, Razdan MK. Plant tissue culture: theory and practice. Amsterdam: Elsevier; 1983.
5. Biswal M, Chhuria S, Mandal P, Thriveni V. Impact of integrated nutrient management on soil fertility status of tissue culture banana cv. Grand Naine. International Journal of Agricultural Science and Research. 2016;6(3).
6. Biswas S, Shivaprakash MK, Maina CC. Biosynthesis of phytohormones by potassium solubilising bacteria isolated from banana rhizosphere. International Journal of Agriculture, Environment and Biotechnology. 2018;11(3):497-501.
7. Deb CR, Imchen T. An efficient in vitro hardening technique of tissue culture-raised plantlets. Biotechnology. 2010;9(1):79-83.
8. Jaizme-Vega MC, Azcón R. Responses of some tropical and subtropical cultures to endomycorrhizal fungi. Mycorrhiza. 1995;5:213-17.
9. Khatik PC, Saharan V, Kaushik RA. In vitro propagation of strawberry via leaf. International Journal of Current Advanced Research. 2019;8(4):18387-18393.
10. Kumari A, Sairam RK, Singh SK, Krishna GK. Early growth response: an indicator of subsequent growth and yield of wheat genotypes grown under simulated water stress condition. Indian Journal of Plant Physiology. 2014;19:94-100.
11. Mwashasha RM, Kahangi EM, Jefwa JM, Vanlauwe B. Mycorrhiza and tissue culture banana production. Acta Horticulturae. 2011;911:365-373.
12. Patel SR, Narwade AV, Khatri RT, Singh M, Pracihan S, Jadav KS, Zinzala VN. Acclimatization of banana tissue plantlets (*Musa paradisiaca*) of various genotypes in polybags using different potting mixtures. International Journal of Tropical Agriculture. 2015;33(4):3701-3704.
13. Pati R, Mishra M, Chandra R, Muthukumar M. Histological and biochemical changes in *Aegle marmelos* Corr. before and after acclimatization. Tree Genetics and Molecular Breeding. 2013;3(1).
14. Patil AB, Vasane SR, Moharir KK, Kothari RM. Contamination control and disease management of banana 'Grand Naine' plantlets. Acta Horticulturae. 2010;865:247-253.
15. Poveda J, Abril-Urias P, Escobar C. Biological control of plant-parasitic nematodes by filamentous fungi

inducers of resistance: *Trichoderma*, mycorrhizal and endophytic fungi. Frontiers in Microbiology. 2020;11:992.
16. Singh NV, Singh SK, Singh AK. Standardization of embryo rescue technique and bio-hardening of grape hybrids (*Vitis vinifera* L.) using arbuscular mycorrhizal fungi (AMF) under sub-tropical conditions. Vitis. 2011;50(3):115-118.
17. Singh NV, Singh SK, Singh AK, Meshram DT, Suroshe SS, Mishra DC. Arbuscular mycorrhizal fungi (AMF) induced hardening of micropropagated pomegranate (*Punica granatum* L.) plantlets. Scientia Horticulturae. 2012;136:122-127.
18. Turner DW. Crop physiology of bananas-quo vadis. Madras Agricultural Journal. 1981;68:73-84.
19. Verma R, Pandey CS, Pandey SK, Kumudani S. Influence of pre-sowing seed treatment and growing conditions on growth performance of Indian gooseberry seedlings (*Emblica officinalis* Gaertn.). International Journal of Current Microbiology and Applied Sciences. 2019;8(3):1936-1948.
20. Wilson SB, Stoffella PJ, Graetz DA. Use of compost as a media amendment for containerized production of two subtropical perennials. Journal of Environmental Horticulture. 2001;19(1):37-42.
21. Yasin M, Munir I, Faisal M. Can *Bacillus* spp. enhance K+ uptake in crop species? In: Potassium solubilizing microorganisms for sustainable agriculture; c2016. p. 163-170.