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An integrated approach to primary and secondary hardening of banana var. *Grand Naine*

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For improving banana productivity, virus-indexed *Grand Naine* plantlets were used for primary and secondary hardening. Among nine combinations of bio-fertilizers (including VAM) used, GNP_2V_1 medium comprising of peat fortified with nitrogen fixing and phosphate solubilizing microbes (each 1 g/plantlet) and VAM (2 g/plantlet) emerged as the optimal growth medium for primary hardening on the basis of statistically significant 98.9% survival, besides corroborating optimal morphological features (viz. root length, number of primary roots, % VAM colonization, height, girth, number of leaves, leaf area and chlorophyll content) at the end of primary hardening. For secondary hardening, among four combinations of bio-fertilizers and VAM used in conjunction with soil, press mud cake (PMC) and vermi-compost (VC), SNP_2V_1 medium comprising of soil, PMC and VC in a ratio of 1:1:1 (v/v/v) emerged as the optimal growth medium on the basis of statistically significant 97% survival, corroborated by optimal morphological features as above, along with uptake of macro- and micro-nutrients. The positive feedback by several monitoring parameters have left no doubt about the integrated outcome at the end of primary and secondary hardening.

Keywords: banana, bio-fertilizers, primary hardening, secondary hardening, VAM

Introduction

Preliminary results on secondary hardening of plantlets of banana var. *Grand Naine* were reported by us[1]. Subsequently, improved banana production technologies were reported incorporating the application of (i) virus-indexed, tissue culture-derived, primary and secondary hardened plantlets, (ii) organic manure and bio-fertilizers (inclusive of mycorrhizae) to supplement chemical fertilizers for meeting nutrient needs for optimal growth productivity, (iii) drip irrigation to cater the need of moisture and alleviate heat stress during its crop cycle, and (iv) bio-pesticides to control pest (if any)[2]. In view of the above, it became imperative to devise primary and secondary hardening processes in an integrated way for improving qualitative/quantitative productivity of banana. Efforts made in this direction are theme of the present article.

Materials and Methods

Chemical Fertilizers

Water soluble fertilizer (composition: 19:19:19% for N, P_2O_5 and K_2O, respectively) was procured from Riechfield Company, Belgium, and Multiplex, a complex micro-nutrient (composition: Zn 3.0%, Fe 2.5%, Mn 1.0%, Cu 1.0%, Bo 0.5% and Mo 0.1%) from Karnataka Agro Industries, Bangalore.

VAM and Biofertilizers

Vesicular arbuscular mycorrhiza (VAM, *Glomus fasciculatum* 50-100 spores per g) was procured from TERI, New Delhi. *Azotobacter* as a nitrogen fixer (1×10^9/mL) and *Aspergillus* as a phosphate solubilizer (1×10^8/mL) were procured fresh in liquid state from the School of Life Sciences, North Maharashtra University, Jalgaon.

Media Ingredients for Primary Hardening

Besides above ingredients, Peat (N 2.37%, P_2O_5 0.07% and K_2O 1.14%, *p*H 5.5, EC 1.0 mS cm^{-1}) was procured from Pindstrup, Denmark. Various insecticides, fungicides and bactericides used during these trials were procured from the standard companies.

Media Ingredients for Secondary Hardening

River bed soil, press mud cake (PMC), vermi-compost (VC) used in matrix and polybags (20×15 cm^2, black, 150-200 gauge) were procured locally.

Banana Plantlets

For primary hardening trials (27^oC, humidity gradually reducing from 100% to 60% over 45 d,

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12000-15000 Lux), banana plants (var. *Grand Naine*) were procured from the tissue culture laboratory of Jain Irrigation Systems Ltd. (JISL). The same plants were used for secondary hardening in shade house (ambient temperature, 30-40% humidity, 50% light cut off) for 45 d.

Water

Demineralised (DM) water (M/s Millipore India Ltd., Bangalore) was used for analysis. For primary and secondary hardening, water of the following composition was used: [cation ($meqL^{-1}$)] Ca 3.5; Na 3.2 and Mg 2.0; [anions ($meqL^{-1}$)] bicarbonate 7.7; chlorides 2.3 and carbonates 0.2; sodium absorption ratio (SAR) 2.0; residual sodium carbonate (RSC) 2.2; *p*H 7.6; EC 1.4.

Drip Irrigation

The drip irrigation system procured from M/s JISL was installed in the shade house.

Experimental Design

Completely randomized design (CRD) was chosen for the primary and secondary hardening trials. Each set of treatment was in triplicate in all the trials; 490 plantlets per replication during primary trials and 832 plantlets per replication during secondary trials

Green House for Primary Hardening

The Gothic arch type green house [constructed with UV stabilized poly-sheet (200 μ), equipped with water-repellant benches, four-way misting system, thermal shade net, thermometer, hygrometer and lux-meter to monitor temperature, humidity and light intensity] was used for primary hardening trials.

In vitro grown plantlets were removed from glass bottles, cleansed to remove agar medium sticking to roots and planted in plastic pro-trays containing coco peat and soilrite. They were delicate because of (i) poorly developed cuticle, (ii) poor stomatal activity, (iii) limited mesophyll, and (iv) plenty of intra-cellular cavities. Hence, they have to undergo several anatomical and physiological changes for their establishment in greenhouse conditions. For this purpose, in this growth phase, they undergo adaptation from heterotrophic to autotrophic condition. This transition is achieved by gradually exposing the plantlets from condition of highest (90%) humidity and diffused light, to decreasing (70%) humidity and increasing light intensity. To achieve this objective, these plantlets were maintained at 70% humidity and 25°C for 40-45 d, after which they were shifted to shade houses for secondary hardening. During this period, their profiles were monitored as summarized in Table 1.

Shade House for Secondary Hardening

The shade house [constructed with 50% light cut-out black shade net, equipped with drip irrigation system, supplying water/fertilizers to every plant with an individual dripper (2 L per h discharge), fertigation equipment (ventury) and micro-sprinklers (40 L discharge per h)] was used for secondary hardening trials.

Table 1—Profiles of survival and morphological features of *Grand Naine* during primary hardening

No.	Treatment	Survival (%)		Root length (cm)		No. of primary roots		Colonization (%)		Height (cm)		Girth (cm)		No. of leaves		Leaf area (cm^2)		Chlorophyll (mg/g)	
		GH1	GH2	GH1	GH2	GH1	GH2	GH1	GH2	GH1	GH2	GH1	GH2	GH1	GH2	GH1	GH2	GH1	GH2
1	GNP_1V_I	97.0	95.2	10.0	10.2	6.2	6.0	67.4	70.6	11.0	10.9	2.3	2.3	6.1	6.1	127.1	124.3	4.2	4.3
2	GNP_1V_2	97.8	95.6	10.5	10.4	6.3	6.3	68.3	72.2	11.1	11.1	2.3	2.1	6.3	6.3	129.4	126.3	4.2	4.3
3	GNP_1V_3	95.8	93.0	9.8	9.8	5.9	5.9	55.5	59.2	10.7	10.5	2.1	2.1	5.9	5.5	119.9	120.0	4.1	4.1
4	GNP_2V_I	98.9	96.0	11.2	11.0	6.8	6.8	76.9	78.4	11.5	11.5	2.7	2.5	6.5	6.4	136.8	137.6	4.3	4.4
5	GNP_2V_2	98.5	96.0	10.8	10.8	6.6	6.6	76.1	76.1	11.2	11.3	2.3	2.3	6.4	6.1	135.4	138.2	4.3	4.5
6	GNP_2V_3	97.4	97.4	10.3	10.3	6.2	6.2	67.9	70.0	11.2	10.7	2.3	2.1	6.3	6.1	128.4	127.6	4.2	4.3
7	GNP_3V_I	98.3	96.1	10.8	11.0	6.8	6.6	73.4	71.0	11.1	11.5	2.4	2.4	6.4	6.3	133.6	136.6	4.3	4.3
8	GNP_3V_2	98.1	95.6	10.8	10.6	6.3	6.3	68.5	69.2	11.1	11.0	2.4	2.2	6.4	6.0	130.0	133.5	4.2	4.2
9	GNP_3V_3	94.3	93.7	9.3	9.6	5.7	5.7	66.8	68.6	10.7	10.7	2.2	2.0	5.6	5.6	114.7	122.1	4.0	4.1
10	GNP_0V_0	95.8	94.2	9.7	9.3	6.1	6.1	0.0	0.0	10.8	10.5	2.0	1.9	6.0	5.8	121.6	121.0	4.1	4.3
11	G_0	93.5	91.0	9.2	9.0	5.7	5.7	0.0	0.0	10.6	10.0	1.8	1.7	4.9	4.8	114.5	115.0	3.7	3.7
	CD at 5%	1.71	1.43	0.37	0.35	0.32	0.25	2.20	1.71	0.16	0.29	0.14	0.20	0.16	0.27	1.96	1.64	0.24	0.22

G= Green house; GH1= Green house 1; GH2= Green house 2; N= Nitrogen fixer (*Azotobacter*); P= Phosphate solubilizer (*Aspergillus*); NP_0= Without nitrogen fixer and phosphate solubilizing microbes; NP_1= with nitrogen fixer and phosphate solubilizing microbes, each @ 0.5 g/plant; NP_2= with nitrogen fixer and phosphate solubilizing microbes, each @ 1 g/plant; V_o= without VAM; V_1= 2 g VAM/plant; V_2= 4 g VAM/plant; V_3= 6 g VAM/plant, Treatments 1-9 did neither receive chemical fertilizer, nor fungicide. Treatment 10 did not receive any bio-fertilizer and plants were hardened by using only water soluble chemical fertilizers on weekly basis (0.5 g/L) and fungicidal treatments applied @ 1 g/L at alternate wk; Treatment 11 served as a control (without any chemical and bio-fertilizers).

Optimization of Medium

The primary hardened plants (not exposed previously to bio-fertilizer combinations) in the green house were ready in 45 d for transferring to poly-bags filled with different (T1-T5) growth media. For optimizing these media, locally and economically available ingredients were explored, so that an optimized medium appealing to an industrial house emerges for commercial exploitation. They were kept in shade house at 50% light intensity by judicious application of water and soluble fertilizers during the next 45 d for secondary hardening. Their growth was monitored with the same parameters studied during primary hardening. These observations are summarized in Table 2.

Optimization of Biofertilizers

After having decided over the choice of soil, PMC and VC (1:1:1; v/v) to serve as poly-bag medium for secondary hardening, it was necessary to study the effect of various bio-fertilizer combinations, so as to get the plants of optimal health and vigour, which would give optimum survival, growth and yield in the field. For this purpose, primary hardened plants in the green house were used.

Rhizospheric Count

The soil samples were analyzed for total viable count (TVC) by serial dilution and plating technique (30°C for 48 h) on nutrient agar as per Subba Rao[3].

Percent VAM Infection to Roots

Hairy, absorbing root samples were collected at a depth of 25 cm, cleared with 10% KOH solution for 40 min to remove brownish phenolic compounds, stained with trypan blue and per cent mycorrhizal infection determined by root slide technique[4].

Analytical Methods

Nitrogen, phosphorus and potassium content were analyzed as per Tandon[5]. Chlorophyll content of leaf lamina, third from emergence, was determined as per Jayaraman[6]. For reproducibility of the results, all data were analyzed statistically[7] and tested for its significance using ANOVA software (M/S Indostat Services Ltd., Hyderabad).

Results and Discussion

Optimization of Biofertilizers during Primary Hardening

Survival was more than 97% in most of the bio-fertilized treatments where GNP_2V_1 recorded optimal % survival in GH1, at par with GNP_2V_3 in GH2, while G_0 (master control) and GNP_0V_0 (control) performed poorly (Table 1). It is peculiar and rather unexplainable as to why GNP_1V_3 and GNP_3V_3 performed poorly (while GNP_2V_3 performed optimally), in spite of maximum presence of VAM in all of them. This trend was also noted in root length, number of primary roots, % colonization by VAM, height, girth of pseudo-stem, number of leaves, leaf area and chlorophyll content, confirming the optimal performance of GNP_2V_1 above all media.

Optimization of Growth Medium for Secondary Hardening

It is clear from Table 2 that % survival of plantlets was maximum and statistically significant in T2 and T3 media, whereas medium T1 (control) had minimum % survival, at par with T4 and T5 media in both the shade houses. From these observations, it is clear that soil alone (T1) is not good for secondary hardening; it needs both PMC as well as VC (as in T2 and T3). Absence of either VC (as in T4) or PMC (as in T5) created problems for their survival. This is not at all surprising as both PMC[8] and VC[9] are the established inputs, which impart protection from transplantation shock, pests/diseases and promote plant growth.

Table 2—Profiles of survival and morphological features of *Grand Naine* during secondary hardening

No.	Medium/ Trial site	Survival (%)		Root length (cm)		No. of primary roots		Root mass (g)		Height (cm)		Girth (cm)		No of leaves		Leaf length (cm)		Leaf width (cm)		Chlorophyll (mg/g)	
		SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2
1	T1	89.1	79.0	12.4	12.8	9.1	8.8	10.3	11.0	12.6	13.5	3.1	2.2	5.5	5.2	16.1	17.2	6.4	6.5	4.9	4.7
2	T2	95.3	96.2	27.4	27.6	11.6	11.2	30.1	31.1	21.5	23.3	4.7	4.9	6.6	6.5	23.1	22.6	11.5	11.9	5.6	5.5
3	T3	95.3	95.0	26.4	26.1	11.0	10.8	24.4	26.8	20.1	23.3	4.4	4.6	6.4	6.5	22.6	22.1	11.2	11.6	5.5	5.6
4	T4	90.1	90.1	15.4	15.2	9.4	9.3	18.4	20.0	14.6	14.9	3.4	4.0	5.8	6.0	20.0	20.2	8.1	8.6	5.1	5.1
5	T5	89.9	90.1	16.6	18.6	10.1	1.1	21.0	22.4	16.3	17.0	3.6	4.1	6.0	6.2	20.0	20.2	8.4	9.0	5.1	5.2
	CD at 5%	2.08	1.54	1.07	0.96	0.44	0.58	1.15	1.39	1.63	1.14	0.19	0.13	0.58	0.44	0.96	0.71	0.38	0.39	0.19	0.17

SH1= Shade house 1; SH2= Shade house 2; T1= Soil alone; T2= Soil:PMC:VC, 1:1:1 (v/v); T3= Soil:PMC:VC, 2:1:1 (v/v); T4= Soil:PMC:VC, 3:1:0 (v/v); T5= Soil:PMC:VC, 3:0:1 (v/v).

The same trend (i.e. T2 and T3 superior media over T1, T4 and T5) was witnessed in root length, number of primary roots, root mass, height, girth of pseudo-stem, number of leaves, leaf length and width, and chlorophyll. Cumulatively, these observations unambiguously pointed out that PMC and VC together (as in T2 and T3) gave better performance in terms of plant growth.

Significance of PMC and VC as Medium Ingredients

Press mud cake (PMC), a by product of sugar industry, appears to be a promising indigenous ingredient in the medium devised for secondary hardening as PMC is being used as a (i) source of plant nutrients to raise seedlings[10], (ii) stabilizer and shelf-life enhancer of several bio-fertilizers[8], (iii) promoter of nitrogen and phosphorus uptake, besides improving organic carbon and nutrient status of the soil[11], (iv) enhancer of water holding capacity of soil[9], (v) balancing air-water relationship in the root zone, leading to a well developed root system, (vi) partial substitute to chemical fertilizers[12], and (vii) source of plant growth regulators (PGRs)[8].

For soil, VC (i) serves as a source of organic carbon, (ii) promotes colonization by varieties of useful microbes, (iii) improves medium texture by virtue of its soft and fluffy nature, as also aeration of soil, (iv) enhances its water holding capacity, sustained release of moisture to plants and water drainage, and (v) increases its humus content, as a ready source of food and protection to plants. For plants, it (i) imparts higher rate of germination and root ramification, (ii) soft texture conducive to root ramification, (iii) various nutrients (macro and micro) to the plants, leading to their healthy growth[9], and (iv) increases green cover and biomass[13].

Thus, potting medium of choice was T2 on the basis of biometric data. This outcome may seem to differ from the observations of Sathiamoorthy *et al*[14], who suggested various combinations: (i) peat soil:FYM (12:1; v/v), (ii) sand:top soil:vermi-compost (3:1:1; v/v/v), and (iii) sand:FYM:vermi-compost:red soil (2:1:1:1; v/v/v/v), along with neem seed cake (50 g/kg of matrix). Similarly, Chandre Gowda *et al*[15] suggested the use of soil, compost, coir pith and sand in equal proportions for secondary hardening. Behind all these observations, the common denominator is to avail locally available matrix such as peat, FYM, vermi-compost, coir pith, etc., besides sand to provide porous texture to the medium.

Profiles of Survival and Morphological Features as Functions of Biofertilizers

It is clear from the data in Table 3 that treatment SNP_2V_1 offered maximum (98.5%) survival, followed by SNP_2V_2 and SNP_3V_1; while SNP_3V_3, SNP_0V_0 (control) and S_0 (master control) showed 10-14% mortality in both the shade houses. Almost similar

Table 3—Profiles of survival and morphological features of *Grand Naine* during secondary hardening as functions of bio-fertilizers

No.	Treatment	Survival (%)		Root length (cm)		No. of primary roots		Colonization (%)		Height (cm)		Girth (cm)		No. of leaves		Leaf length (cm)		Leaf width (cm)		Chloro-phyll (mg/g)	
		SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH2
1	SNP_1V_I	94.2	89.0	26.2	27.0	11.2	11.1	72.4	72.0	18.9	18.0	4.9	5.1	7.5	8.0	22.8	22.9	11.0	11.1	5.3	5.4
2	SNP_1V_2	91.0	92.0	24.7	24.2	10.1	10.0	69.0	69.6	18.4	18.4	4.5	4.6	7.4	7.8	22.1	22.2	10.1	10.2	5.1	5.2
3	SNP_1V_3	89.0	89.3	21.5	23.0	9.9	9.5	63.4	62.2	15.3	15.5	4.2	4.2	6.6	6.4	20.2	20.3	9.2	9.4	4.9	4.9
4	SNP_2V_I	98.5	96.2	29.0	28.2	11.6	11.9	81.3	82.4	23.6	23.2	5.2	5.5	8.4	8.5	25.1	25.3	12.6	12.6	5.6	5.5
5	SNP_2V_2	96.3	96.1	27.5	27.2	11.3	11.5	80.4	81.2	22.9	22.1	5.2	5.6	8.2	8.1	24.6	24.8	12.2	12.3	5.6	5.6
6	SNP_2V_3	91.0	93.6	24.4	26.6	10.0	10.3	68.4	69.8	18.4	18.0	4.5	4.3	7.2	7.5	21.7	21.8	10.1	10.2	5.1	5.1
7	SNP_3V_I	96.0	94.2	27.7	27.3	11.0	11.6	73.0	75.6	21.3	21.9	5.0	5.4	8.0	8.2	24.2	24.2	11.1	11.6	5.5	5.5
8	SNP_3V_2	92.0	92.1	25.4	25.4	10.1	10.9	69.7	69.2	18.4	18.0	4.5	4.5	7.4	7.8	22.1	22.2	10.2	10.3	5.1	5.0
9	SNP_3V_3	90.0	86.0	20.4	21.2	9.6	10.1	62.1	63.2	15.7	16.0	4.2	4.3	6.6	6.2	20.2	20.3	9.4	9.5	5.0	4.9
10	SNP_0V_0	90.0	88.0	24.3	24.1	9.9	9.9	0.0	0.0	18.3	18.3	4.3	4.2	7.2	7.4	21.8	22.0	10.1	10.2	5.0	5.0
11	S_0	89.0	86.0	16.6	16.3	9.2	9.3	0.0	0.0	12.0	12.5	3.1	3.2	5.4	5.5	16.0	16.2	6.4	6.7	4.9	4.5
	CD at 5%	2.19	2.87	1.06	0.62	0.55	0.76	2.04	1.76	0.68	0.58	0.20	0.26	0.20	0.41	0.59	0.50	0.64	0.51	0.20	0.17

S= Shade house; SH1= Shade house 1; SH2= Shade house 2; the medium used in the treatments comprises of soil: press mud cake: vermicompost (1:1:1, v/v/v) and modified by the addition of nitrogen fixer, phosphate solubilizing microbes and VAM as indicated below: N= Nitrogen fixer (*Azotobacter*); P= Phosphate solubilizer (*Aspergillus*); NP_0= without nitrogen fixer and phosphate solubilizing microbes; NP_1= with nitrogen fixer and phosphate solubilizing microbes, each @ 0.5 g/plant; NP_2= with nitrogen fixer and phosphate solubilizing microbes, each @ 1 g/plant; V_o= without VAM; V_1= 5 g VAM/plant; V_2= 10 g VAM/plant; V_3= 15 g VAM/plant, Treatments 1-9 did neither receive chemical fertilizer, nor fungicide. Treatment 10 did not receive any bio-fertilizer and plants were hardened by using only water soluble chemical fertilizers on weekly basis (0.5 g/L) and fungicidal treatments applied @ 1 g/L at alternate wk; Treatment 11 served as a control (without any chemical and bio-fertilizers).

trend was followed in case of morphological characters, viz. height, girth, number of leaves, leaf length and width, and chlorophyll content.

Viewed in context of the observations in Table 2 (which may be treated as a control), observations in Table 3 (which may be treated as an experimental) unambiguously reflect the contribution of bio-fertilizers on the growth of *Grand Naine* plantlets. Percent survival increase by 3.2% is statistically significant and could have tangible bearing on the farm yield/income. Increase in root length has potential to collect maximum plant nutrients from the soil. Similarly, an increase in biomass (height, girth, number of leaves and their surface area) indicated plant vigour, which could have bearing on banana output.

Profiles of Macro- and Micro-nutrient Uptake as Functions of Biofertilizers

The data from Table 4 show (i) no significant difference in the uptake of N_2 by plantlets as a function of media, (ii) significant variation in the uptake of P, and (iii) maximum uptake of K in SNP_2V_2, followed by SNP_2V_1 and SNP_1V_1 in both the shade houses. Prasad and Kumar[16] in their studies have shown that there was (a) little requirement of N for banana, which is basically a carbohydrate-rich crop, (b) greater absorption of P due to mycorrhizae for carbohydrate metabolism[17] and (c) need for higher K uptake for stomatal regulation during summer. Since NPK profiles are not studied earlier for banana plantlets during secondary hardening, we have no data for comparison.

Mg content was found significantly higher in SNP_2V_1 in both the shade houses (Table 4). In all probability, this is a reflection of intense carbohydrate metabolism in *Grand Naine* plants, where large amount of ATP is generated and utilized by virtue of Mg^{++} being an integral part of chlorophyll and an activator of enzymatic processes synthesizing and utilizing ATP[18]. The same trend was followed by Mn^{++} which is interchangeable with Mg^{++}, Zn^{++} and Fe^{++} as per Prasad and Kumar[16]. Almost comparable trend was seen in the uptake of Fe^{++}, Cu^{++} and Zn^{++} also.

VAM was found to play an important role in the survival and growth of various micro-propagated plantlets, such as avocado[19], pineapple[20], pear and peach[21], citrus[22], and guava [23]. Similarly, the effect of VAM on *Shrimanti* variety of banana has been positive on growth and yield[24]. Singh and Singh[25] observed a marked increase in the uptake of P, Ca, Mg, Zn and Cu in the mycorrhizal plants in banana. Aneesa Rani *et al* [26] too noted that application of 2 g *Azotobacter*, PSBs and 10 g VAM gave maximum height, girth, root length, and number of roots/root fibres in cashew seedlings. Thus, the above findings on the effect of VAM are in concurrence with the observations made on other horticultural crops, besides substantiating earlier findings[1,27].

Table 4—Profiles of NPK and micronutrient uptake during secondary hardening

No.	Treatment	N (%)		P_2O_5 (%)		K_2O (%)		Mg (ppm)		Mn (ppm)		Fe (ppm)		Cu (ppm)		Zn (ppm)	
		SH1	SH2	SH1	SH2	SH1	SH2	SH1	SH1	SH2	SH2	SH1	SH2	SH1	SH2	SH1	SH2
1	SNP_1V_I	3.8	3.4	0.9	0.9	4.2	4.4	4105.0	4098.0	147.5	139.2	455.0	426.0	29.1	41.1	125.3	126.4
2	SNP_1V_2	3.4	3.4	0.9	1.1	3.6	4.2	4070.0	4066.0	139.3	136.1	350.0	401.0	23.7	40.2	131.2	132.3
3	SNP_1V_3	3.2	3.2	0.8	0.9	3.2	3.6	4102.0	4061.0	133.3	139.3	333.7	313.8	17.2	33.6	119.3	120.4
4	SNP_2V_I	3.4	3.6	1.3	1.3	4.3	4.5	4186.0	4174.0	162.2	166.8	425.0	423.2	43.4	40.3	117.6	119.6
5	SNP_2V_2	3.8	3.7	1.1	1.2	4.6	4.2	4129.0	4101.0	151.2	152.2	401.3	422.8	31.7	36.8	131.1	133.9
6	SNP_2V_3	3.6	3.2	0.9	0.9	3.4	3.6	4040.0	4020.0	133.5	139.4	317.5	319.9	22.4	26.6	119.3	126.3
7	SNP_3V_I	3.9	3.4	1.1	1.1	3.6	3.5	4111.0	4100.0	143.2	141.1	401.0	419.0	29.0	40.1	130.0	130.0
8	SNP_3V_2	3.8	3.6	0.9	1.1	3.9	3.9	4070.0	4053.0	141.1	138.3	301.0	302.3	24.0	28.1	104.4	110.3
9	SNP_3V_3	3.4	3.2	0.5	0.7	3.2	3.4	4035.0	4026.0	131.5	132.3	300.0	306.2	15.7	19.1	99.8	101.3
10	SNP_0V_0	3.2	3.4	0.8	0.8	3.3	3.3	4010.0	4011.0	129.3	126.4	273.9	285.2	17.2	19.3	99.4	101.8
11	S_0	2.0	2.2	0.1	0.2	2.4	2.8	3865.0	3798.0	125.6	119.8	159.7	198.5	9.1	13.2	96.4	95.1
	CD at 5%	0.17	0.12	0.15	0.14	0.21	0.21	1.56	3.19	1.68	1.87	2.09	2.16	1.81	1.91	1.99	1.91

S= Shade house; SH1= Shade house 1; SH2= Shade house 2; the medium used in the treatments comprises of soil: press mud cake: vermicompost (1:1:1, v/v/v) and modified by the addition of nitrogen fixer, phosphate solubilizing microbes and VAM as indicated below: N= Nitrogen fixer (*Azotobacter*); P= Phosphate solubilizer (*Aspergillus*); NP_0= without nitrogen fixer and phosphate solubilizing microbes; NP_1= with nitrogen fixer and phosphate solubilizing microbes, each @ 0.5 g/plant; NP_2= with nitrogen fixer and phosphate solubilizing microbes, each @ 1 g/plant; V_0= without VAM; V_1= 5 g VAM/plant; V_2= 10 g VAM/plant; V_3= 15 g VAM/plant, Treatments 1-9 did neither receive chemical fertilizer, nor fungicide. Treatment 10 did not receive any bio-fertilizer and plants were hardened by using only water soluble chemical fertilizers on weekly basis (0.5 g/L) and fungicidal treatments applied @ 1 g/L at alternate wk; Treatment 11 served as a control (without any chemical and bio-fertilizers).

Conclusion

In the light of profiles of (i) survival, (ii) root system (number, length and colonization), (iii) height and girth, (iv) leaves and (v) chlorophyll content, GNP_2V_1 was optimal for primary hardening. Besides the above parameters, macro- as well as micro-nutrient uptake was monitored during secondary hardening, where SNP_2V_1 medium has emerged as the optimum medium for *Grand Naine* variety of banana. These observations cannot be looked in isolation as they have been an "positive feedback" of the parameters monitored during primary and secondary hardening. Cumulatively, these observations have left no doubt in finalizing the composition of GNP_2V_1 for optimal primary hardening and SNP_2V_1 for optimal secondary hardening of banana plantlets in future.

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