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FIELD PERFORMANCE OF *IN VITRO* PROPAGATED BANANA PLANTS FROM 8TH AND 15TH SUBCULTURE.

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ABSTRACT

The field performance of *In vitro* propagated Grande naine banana variety from 8th subculture was compared with that of the 15th subculture plants. 8th subculture plants established and grew faster, taller and bigger than 15th subculture plants. The former produced more leaves resulting in a larger total leaf area (9622.74 cm^2 per plant) than the latter (9454.13 cm^2 per plant) and could be harvested earlier. The former also produces heavier bunches (25.158 kg for bunch) than the latter (21.137 kg for bunch). In this experiment, the 8th subculture plants started growth earlier & grew faster enabling them to intercept more light for photosynthesis than 15th subculture plants. The 8th subculture plants have more uniform growth than 15th subculture with very less variation. This may explain the higher yield in the 8th subculture plants. We found two variants in 8th subculture plant whereas 15th subculture plant have four variants. The frequency of occurrence of variants in 8th subculture plant is comparatively low. We conclude that 8th subculture plant perform better in terms of growth & yield than the 15th subculture plants under field condition.

Keywords: Field performance, Grande naine, leaf area, multiplication subculture, variation, yield

[I] INTRODUCTION

Banana and plantain (*Musa* sp.) is a crop of tremendous economic social importance in the humid and sub humid tropical region of the world [11]. Micropropagation or in-vitro techniques were established for fast multiplication of bananas [15]. Commercial production of micropropagated bananas is now common in many countries and it is estimated that 25 million plants are produced worldwide each year. Advantages of *In-vitro* micropropagation include high rates of multiplication, the small amount of space required to multiply large numbers of plants, production of diseases-free planting material and the homogeneity of their growth [17].

In general, micropropagated banana plants establish faster, grow more vigorously, are taller, have shorter and more uniform production periods and produce higher yields than conventional propagules [5][6][18][12][13].

The performance of micropropagated plants is also varied according to their multiplication subculture. The economics of banana tissue culture industry is directly depending on the number of multiplication subcultures. Generally after 8th subculture rooting is induced. As

number of subculture is increased (i.e. more than 8) then number of plantlets obviously increases which gives more profit. This study was conducted to understand the relationship of number of subculture & productivity of banana plants.

[II] MATERIALS AND METHODS

The study was carried out with Grande naine banana variety and it was carried out during 2006-08 at Kolhapur, which is situated at western parts of India. Healthy plants were selected and tagged according to their bunch, height, number of leaves etc. Suckers were separated from the parent plant at the point of attachment by using an earth chisel, the roots and the top of the shoots were trimmed off and they were washed under running tap water to clean material. The explants were reduced in size, sterilized with 70% alcohol & rinsed with distilled water.

The sub sequent sterilization procedures were carried out until it reaches into the laminar air flow unit.

2.1. Media Composition

MS medium [10] supplemented with & 6BAP (3mg/litre) was used for initiation and multiplication. For shooting MS medium supplemented with adenine sulphate is used. Rooting was done in MS medium supplemented with IBA (1mg/litre) & charcoal. After the pH was adjust to 5.8, medium was autoclaved at 121^0c for 15 min

2.2. Culture Condition

40 bottles, each with one explants was placed in a growth chamber kept at 25°C, a 16 hr photoperiod (3000 lux light). They were subcultured after three week & every two week thereafter [2]. At each subculture, multiple shoots were separated & placed in separate bottles.

2.3. Rooting and Hardening

Shoots with an average of 4-5 leaves were transferred into fresh MS medium with Charcoal and IBA (1mg/litre) to induce rooting [1]. After four weeks they were removed from the bottle, the medium was washed off from the roots. In prehardening plants were planted into plastic trays with cavities filled sterile cocopit. It is incubated in humid chamber at 80 – 90 % humidity and temperature is maintained at 30°C – 35°C for 45 days. For post hardening 70% sandy loam type soil obtained from river bank and 30% compost is used. These plants were acclimatized for two months in shade house.

2.4. Field Planting

After secondary hardening, plantlets of Grande naine banana with average height of 0.5 m were planted at the Seema Biotech field. The very old leaves were removed from plants, leaving 4-5 leaves per plant. Each plot had 30 plants spaced

at 6 X 5 m. Plants were fertilized with 200 gm N, 70 gm P, 200 gm K per plant. The field was irrigated with the help of drip irrigation every day.

2.5. Growth and Yield Measurement

All plants were tagged for identification. Plant height and pseudostem circumference were measured at the time of planting and after the 10th month. Plant height was measured from the base of pseudostem to the top of the stem, the base of the central leaf and the circumference of pseudostem at the height 1 m from the base was measured.

[III] RESULTS

3.1. Growth Characteristics

The growth characteristic was assessed by measuring the following parameters: leaf number, plant height, leaf area, pseudostem circumference, sheathing leaf base & length of peduncle.

8th Subculture plants grew taller & pseudostem circumference is also more than 15th subculture plants. The former also had more leaves, larger leaf area, larger sheathing leaf base & length of peduncle than the latter (Table 1 & 2). These differences were significant as indicated in the table with the t-test values.

3.2. Yield Characteristics

The 8th subculture plants were harvested 20 days earlier & produced more fruits than the 15th subculture plants. The weight of bunch was significantly heavier in the 8th subculture plants than in the 15th subculture plants.

The length of bunch & length of peduncle was significantly larger in the 8th subculture plants than 15th subculture plants. The former also had more number of hands than the latter. However, there was no significant difference between them in the length of fingers and number of fingers (Table 3 & 4).

3.3. Variation in banana plants

Variation with regards to stature, pigmentation, growth peduncle, leaf size, flowering period, pseudostem circumference etc. were seen in 8th as well as 15th subcultures. We found two variants in 8th subculture plants whereas 15th subculture plants have four variants. These variants are physiologically distinguishable and have remarkable variation in flowering period (Table 5). Yield is measured by comparing it with 8th cycle normal healthy plants bunch weight

[Table-1]

Growth Parameters → / Statistical parameters ↓	Height (cms)	No. of Leaf	Pseudostem Circumference (cms)	Mean Leaf Area (cm^2)			Sheathing Leaf Base (cms)	Length of Peduncle (cms)
				B	M	T		
Average	209.8	10	62.47	8518.64	11154.5	9085.1	15.41	37
Standard Deviation	7.35	1.214	3.02				2.75	7.49
Standard Error of Mean	3.8	0.331	0.735				0.5	1.49
t- test	**	**	**	**	**	**	**	**

**Significantly different at P ‹ 0.01

Table: 1. Growth characteristics of 8th subculture plants.

[Table-2]

Growth Parameters → / Statistical parameters ↓	Height (cms)	No. of Leaf	Pseudostem Circumference (cms)	Mean Leaf Area (cm^2)			Sheathing Leaf Base (cms)	Length of Peduncle (cms)
				B	M	T		
Average	203.5	10	60.325	8236.4	11095.9	9030.1	14.56	34.5
Standard Deviation	20.85	1.876	4.54				2.8	9.81
Standard Error of Mean	1.47	0.286	0.635				0.52	1.92
t- test	**	**	**	**	**	**	**	**

**Significantly different at P ‹ 0.01

Table 2: Growth characteristics of 15th subculture plants.

[Table-3]

Yield Parameters → / Statistical parameters ↓	Length of Bunch (cms)	No of Hands	Length of Finger (cms)	Bunch Weight (Kg)	No of fingers (per bunch)
Average	65.04	10.64	16.59	25.158	17.315
Standard Deviation	7.84	0.637	1.97	2.467	1.867
Standard Error of Mean	1.56	0.125	0.38	0.485	0.387
t-test	**	Ns	**	**	Ns

**Significantly different at P ‹ 0.01

Table-3: Yield parameters of 8th subculture banana plants.

[Table-4]

Yield Parameters → / Statistical parameters ↓	Length of Bunch (cms)	No of Hands	Length of Finger (cms)	Bunch Weight (Kg)	No of fingers (per bunch)
Average	60.36	9.92	16.4	21.137	17.238
Standard Deviation	4.96	0.562	2.28	5.197	2.046
Standard Error of Mean	0.96	0.108	0.454	0.986	0.563
t-test	**	Ns	**	**	Ns

**Significantly different at P ‹ 0.01

Table 4 : Yield parameters of 15th subculture banana plants.

[Table-5]

Subculture	Variants	Description	Yield (%)	Frequency (%)
8th Subculture	Variant 1	Tall vigorous plant, stem reddish green, peduncle pale green, late flowering	99.37	2
	Variant 2	Dwarf plant, short peduncle, stem pale green, short broad leaves, proper flowering	71.54	2
15th Subculture	Variant 1	Tall plant, pseudostem circumference is more than normal & blackish in colour , green leaves, more leaf area long peduncle, late flowering	119.24	4
	Variant 2	Dwarf plant, short broad green leaves with red margin, pseudostem circumference is more, short reddish peduncle, proper flowering	71.54	3
	Variant 3	Tall plant, pseudostem circumference is small & greenish black in colour, long narrow leaves, leafs are arranged one below the other on both sides, pale green colour leaves, proper flowering	71.54	10
	Variant 4	Normal height as healthy plant, pseudostem circumference is normal with reddish pigments, leaves are black green in colour, proper flowering	95.39	2

Table 5: Spectrum of variants clones observed in tissue-cultured plants of Grande neine

[IV] DISCUSSION

Several authors have reported the superior growth and yield of *In vitro*-propagated banana plants compared to the conventional suckers derived plants in various types of banana and plantain [18][4][12][3]. But none of the researcher had compared *In vitro*-propagated banana plants belonging to 8th & 15th multiplication subcultures. Our results are in agreement with the results obtained for *In vitro*-propagated banana plants of above mentioned researchers.

Kwa & Ganry (1990) & Robinson & Anderson (1991) reported that faster growth & high yield are not always observed in the *In vitro*-propagated banana plants. Our results suggest that as number of multiplication subculture increases,

growth and yield is decreases. We observed that growth and yield of *In vitro*-propagated plants is also depending on the soil and environmental conditions.

In vitro-propagated 8th subculture & 15th subculture plants both produced without diseases pathogens. Israeli *et. al* (1988) also found similar results in case of *In vitro*-propagated banana plants.

8th subculture plants show superior growth characteristics than 15th subculture plants. We got significant difference in height, pseudostem circumference, mean leaf area & length of peduncle. But we got almost same number of leaf in both cases & sheathing leaf base with negligible difference. This may be the result of growth hormones & number of subcultures. The same number of leaf indicate the carry-over effect of this hormone [18][4][3]. But we got greater leaf area in case of 8th subculture plants. Therefore they begin active photosynthesis earlier than 15th subculture plants. The result of active photosynthesis is good growth characters and final fruit yield. It is also clearly observed from yield characteristics like bunch weight, length of bunch etc. We also get more standard deviation from mean value for all growth & yield characteristics for 15th subculture plants than 8th subculture plants. This deviation from mean value shows non uniformity in growth & yield characters of 15th subculture plants.

Some plantlets which are conspicuously distinct from the parental clones were observed in the population of hardened plants in 8th subculture as well as in 15th subculture (Table 5). Jambhale *et. al.* (2001) also reported similar result in case of different banana clones from 10th, 12th & 14th subcultures. Variant 3 from 15th subculture exhibited higher variant frequency (10%). Variant 1 from 15th subculture shows higher yield as compared to normal healthy plant from 8th cycle, but it has late flowering and takes more time for fruit harvesting. Variants with late flowering shows more final yield as compared with normal healthy plant, whereas variants with proper flowering period shows less yield.

We found 24.934 % decrease in yield of 15th subculture plants. This decrease in yield is due to the increase in the number of variations in the 15th subculture plants.

[V] CONCLUSION

8th subculture plants are more superior to 15th subculture plants. It is proved from their comparative study of growth & yield characteristics. 15th subculture plants were exposed to plant hormones for 425 days in lab whereas 8th subculture plants were exposed to plant hormones only for 250 days. Due to this additional hormone

exposure more variations were observed in 15th subculture plants. Variants with late flowering were having good comparative yield. Undoubtedly 8th subculture plants have uniform growth characters, better yield & less variation, hence they are more productive.

REFERENCES

[1] Buah, J.N., Kawamitsu, Y. and Murayama, S. 1998. Nursery growth of banana cultured on auxin free and auxin supplemented media. Plant Prod. Sci. 1 : 207-210

[2] Buah, J.N., Kawamitsu, Y., Sato, S. and Murayama, S. 1999. Effects of different types and concentrations of gelling agents on the physical and chemical properties of media and the growth and multiplication of banana (*Musa* spp.) *In vitro*. Plant Prod. Sci. 2 : 138-145

[3] Buah, J.N., Kawamitsu, Y., Yonemori, S. and Murayama, S. 2000. Field performance of *In vitro*-propagated and sucker-derived plants of Banana (*Musa* spp.). Plant Prod. Sci. 3 (2) : 124-128.

[4] Drew, R.A. and Smith, M.K. 1990. Field evaluation of tissue cultured banana in south-eastern Queensland. Aust. J. Exp. Agric. 30 : 569-574.

[5] Hwang, J.S., Chen, C.L. and Lin, H.L. 1984. Cultivation of banana using plantlets from meristem culture. Hortscience 19 : 231-233.

[6] Israeli, Y., Reuveni, O. and Nameri, N. 1988. Genetic variability and performance of *In vitro* propagated banana plants. In C.J.A. Guzman and C.R. Romero eds., Memoria de la IV congreso international sobre agrofisiologia del banana. 94-104. San Jose, Costa Rica.

[7] Israeli, Y., Reuveni, O. and Lahav, E. 1996. *In vitro* culture of bananas. In S. Gowen ed., Bananas and plantains. Chapman and Hall, New York. 1-612

[8] Jambhale, N.D. Patil, S.T. Jadhav, A.S. Pawar, S.V. Waghmode, B.D.(2001) Effect of number of subcultures on *in vitro* multiplication of four banana clones. INFO*MUSA* 10 (1): 38-39

[9] Kwa, M. And Ganry, J. 1990. Utilisation agronomique de vitro-plants de bananier. Fruits. Nomeru (Special) : 107-111

[10] Murashige, T. and skoog, F. 1962. A revised medium for the rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant. 15 : 437-497

[11] Robinson, J.C. 1996. Bananas and plantains. CAB International Wallingford. 128

[12] Robinson, J.C. Connie, F. and Eckstein, K. 1993. A field comparison of conventional suckers with tissue culture planting material over three crop subcultures. J. Hort. Sci. 68 : 831-836.

[13] Robinson, J.C. 1996. Banana nutrition In s. Gowen ed., plantains and bananas. Chapman and Hall, New York. 1-612

[14] Robinson, J.C. and Anderson, T. 1991. Repeat trials confirm advantages of banana tissue culture in plant crop. Citrus subtrop. Fruit Res. Inst. Inf. Bull. 288: 10-11

[15] Vuylsteke D, 1989. Shoot tip culture for the propagation, conservation, and exchange of *Musa* germplasm. Practical manuals for handling crop germplasm *In vitro*. International Board for Plant Genetic Resources, Rome. 56.

[16] Vuylsteke D, 1998. Shoot tip culture for the propagation, conservation, and distribution of *Musa* germplasm. International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria. 82

[17] Vuylsteke D. Ortiz R, 1996. Field performance of conventional vs. *In vitro* propagules of plantain (*Musa* spp., AAB group). Hortscience 31: 862-865

[18] Zamora, A.B., Damasco, O.P., Estano, E.S., Barba, R.C. and Patena, L.F. 1989. Growth and yield of *In vitro* and sucker derived banana plants (*Musa* spp.) cvs. Lakatan, Bungulan and Saba. Philippine Agroculturist 72 : 458-465.