



Field Evaluation for Detection and Characterization of Off-types in Commercially Micropropagated Banana Plants cv. Grand Naine (AAA)

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Abstract: The field evaluation for detection and characterization of off-types in commercially micropropagated banana cv. Grand Naine was undertaken in this study to assess the magnitude of the variations in the farmer fields and to analyze the consequence of such variations. Around 15 private tissue culture units are supplying the planting material to the farmers through micropropagation. Field survey was taken up to identify and characterize more significant variants in order to understand the magnitude of the problem. Fields growing in-vitro Grand Naine banana plants in Hesaraghatta, Anekal, Kanakapura, Kadur were surveyed at the time of flowering and fruiting. Candidate plants showing distinct somaclonal variations were identified and recorded in data sheets along with the photographs. Different somaclonal off-types were identified and compiled depending on the morphological and agronomical characters and also by comparing with the true to type normal plant in the field. Our field study findings delineated that the morphological studies of commercially propagated in vitro banana cv. Grand Naine (AAA) in the field has shown the enormity of the off-types generated through micropropagation. Through field study 25 types of variants were identified and molecular markers for such variants have been tried for Dwarf variants and bunch variants which were the most common types of off-types causing heavy loss to the farmers as detected by present study. Furthermore, this field study has revealed the higher frequency of off-types in various fields which are unacceptable and needs to be addressed by proper methods.

Index Terms - Grand Naine (AAA), Somaclonal variation, Field evaluation, Vegetative characters, Reproductive characters.

I. INTRODUCTION

The advancements made in tissue culture techniques has made it possible to regenerate various horticultural species *in-vitro* as micropropagation protocols for commercial scale multiplication are available for a wide range of crops. Clonal propagation and preservation of elite genotypes, selected for their superior characteristics, require high degree of genetic uniformity amongst the regenerated plants. However, plant tissue culture may generate genetic variability, *i.e.*, somaclonal variations as a result of gene mutation or changes in epigenetic marks. Changes in the chromosome number, chromosomal rearrangements, single nucleotide changes, alteration of gene copy number, activation of transposable elements and sequence specific variation in DNA methylation etc., are some of the genetic changes due to which phenotypic variations are known to occur are heritable in nature (Hammerschlag, 1992).

During micropropagation of bananas and plantains, somaclonal variation can occur in regenerated plants. This variation may interfere with the use of these cultures in the commercial multiplication and in the production of stable lines for genetic transformation or physical or chemical mutagenesis. Somaclonal variation is not limited to any particular group of plants. It has been reported in ornamentals, vegetable and food crops, forest species, fruit, plantation crops. The economic consequences of somaclonal variation can be enormous for farmers who used tissue cultured plants in the field. It has been observed that extreme variability of tissue cultured banana plants in terms of yield, quality etc., resulting in heavy loss to the farmers. This has also resulted in legal dispute between the farmers and the companies that supplied the tissue culture plant material. The use of shoot tip culture for banana micropropagation, conservation and exchange of germplasm may be reduced by the occurrence of undesired somaclonal variants at high percentages. The off-types have delayed widespread industry acceptance of micropropagated banana (Smith et al. 1993).

It is becoming increasingly clear that somaclonal variation is usually undesirable. Somaclonal variations appears to be ubiquitous in *Musa*. It was commonly found in different genotypes of *Musa* including AAA, AAB groups (Hwang, 1986, Vuylsteke et al. 1991, Smith and Hamil, 1993, Reuveni and Israeli, 1989). Hence the present field evaluation study for detection and characterization of off-types in commercially micropropagated banana cv. Grand Naine was undertaken to assess the magnitude of the variations in the farmers' fields and to analyze the consequence of such variations.

II. MATERIALS AND METHODS

Grand Naine banana cv. belongs to Cavendish subgroup, is one of the most important commercially propagated fruit crops of Karnataka State. Around 15 private tissue culture units are supplying the planting material to the farmers through micropropagation. Table 1 shows the list of units supplying *in-vitro* banana plants to the farmers. Field survey was taken up to identify and characterize more significant variants in order to understand the magnitude of the problem. Fields growing *in-vitro* Grand Naine banana plants in Hesaraghatta, Anekal, Kanakapura, Kadur were surveyed at the time of flowering and fruiting. Candidate plants showing distinct somaclonal variations were identified and recorded in data sheets along with the photographs. Different somaclonal off-types were identified and compiled depending on the morphological and agronomical characters as described by Pekmezci et al and also by comparing with the true type normal plant in the field (Pekmezci et al. 1998).

Table 1: List of Tissue Culture Units in Karnataka

S. No	Name of the unit and address	Annual Banana production (approx.)
1	Sri Ramco Biotech, Sabari Complex, Residency Road, Bangalore-25.	2 million
2	Prathibha Ankur Biotech, B60, KSIDC Indl. Area, Bommasandra Estate, Bengaluru-99.	0.5 million
3	Khoday Biotek Palmgrove Nurseries, 14 th Mile, Kanakapura Road, Tataguni Post, Vaderahalli, Bengaluru-62.	2 million
4	Global Greens Pvt. Ltd., No. 8A , Kaggalipura Uttarahalli Hobli, Bangalore-62.	0.2 million
5	EK Plant Technology Pvt. Ltd. Adabanasavahalli, Makali Post 21 st KM, Tumkur Road, Bengaluru-23.	1.0 million
6	Venkatesh Kumar, Rajashree Biotech, 112, 4 th Cross, R. Nagar, Bangalore-10.	0.2 million
7	Suvarnamukhi Biotech Sakalavara, Kaggalipura Bangalore-62.	0.3 million
8	MS Ramaiah Biotechnologies Pvt. Ltd. Manchanakurchi Village, Sodekoppa Road Nelamangala, Bangalore.	1.0 million
9	MV Shailesh Kothariya, Googley Biotech, Flat-121, D-5 Block, Kendriya Vidyalaya, Yelahanka, Bengaluru	1 million
10	Green Earth Biotechnologies Ltd., 22, Central Street, 8 th Cross Road, Kumarakrupa West, Bangalore.	3.0 million
11	MV Sundaraj, Verdant Biotechnologies Ltd. Jigani Indl. Area, 2 nd Stage, Anekal Tq. Bengaluru	0.2 million
12	Riddhi Biotech Pvt. Ltd., Siddarth Colony, PB Road Dharwad.	0.05 million
13	Biotechnology Centre, Bannerghatta Road, PO Box No. 7648 Hulimavu, Bengaluru-76	1.0 million

Source: Horticultural Department, Hulimavu, Bangalore.

III. RESULTS

Field survey was taken up with an objective to characterize and to evaluate the percentage of somaclonal variants (off-types) in the farmer fields, growing *in-vitro* banana plants supplied by commercial biotech companies. In the present survey, somaclonal variations in vegetative characters as well as reproductive characters were observed. Table 2 shows the list of off-types observed in all the fields surveyed. In the somaclonal variations affecting vegetative characters' dwarf off-type was the most common variant observed in all the fields we surveyed, ranging from 3 to 60% (Plate 1, Figure c, d, e), followed by leaf variants of rosette orientation (Table 2) or smaller leaf blades. In the majority of dwarf off-types, bunch formation was absent or improper. But overall variation in the vegetative characters like giant, narrow leaf blades, less leaves, pigmentation in the pseudo stem had little implication on the productivity of healthy bunch other than dwarf off-types without bunch. We have also observed variation in normal looking plant with delayed bunch formation up to 9% in the present study. Most of normal off-type plants (Plate 2, Figure i) formed the bunch after 16-18 months (normally 9-12 months) in different durations and few normal off-types did not produce bunch even after 18 months.

Table 2: Types of somaclonal variations observed in the field

S. No.	Variation observed in vegetative characters	
1	Gigantic plants with normal bunch	
2	Variation observed in vegetative characters	
3	Dwarfs with normal formation of bunch	
4	Dwarfs without bunch flowers	
5	Dwarfs with malformed fingered fruits	
6	Dwarfs with small bunch	
7	Dwarf with small bunch and fruits fall even at slight touch	
8	Dwarf with lesser leaves and no bunch	
9	Extra ordinary dwarfs with small bunch	
10	Extra ordinary dwarfs without bunch	
11	Normal plants with rosette type of leaf arrangement	
12	Normal plants with narrow leaf blades	
13	Normal plants with leaves vertically facing the sky	
14	Dwarf with lesser number of leaves	
15	Bigger or smaller girth of the plant with various types of pigmentation	
S. No.	Variations observed in reproductive characters	
1	Bunch Orientation	'S' Shaped; 'C' Shaped; Curved
2	Inflorescence Axis	Small; Long
3	Length of the Bunch	Small; Long
4	Fruits	Malformed or smaller / bitter fruits Bunch facing the sky Lesser fruits in each hand (less than 8) Lesser hand in each bunch (less than 8) Smaller fruits in all the hands or fingers.
5	Inflorescence	Bigger spadix

Table 3 shows the list of various fields surveyed to evaluate off types of Grand Naine banana cv. Before characterizing, plants were thoroughly checked for symptoms of viral, bacterial or fusarium wilt to avoid the infectious plants in the present study. Healthy variants were selected for evaluation of off-types in all the fields. Even minute details like cultivation method, distance between two crops, date of plantation along with time of flowering and fruiting were taken into consideration for better assessment. Observations were carried out in open field. Stem circumference, height, leaf number, length of the bunch stalk, hands and fruit number were recorded.

Table 3: Field Study showing Percentage of somaclonal variations

S. No.	Farm Name	Place	Source of tissue culture plants	No. of G-9 plants grown	% Somaclones
1	Parvathaiah's Farm	Bilijaji Hesaraghatta, Bangalore	M.S.R. Biotech	400	62.75%
2	Mohandas Farm	Hesaraghatta Bangalore	M.S.R. Biotech	2000	22.6%
3	Gopal Farm	Taralu Kanakapura Road Bangalore	Khoday's Biotech	1500	31%
4	Guruva Reddy Farm	Tali Road Anekal	M.S.R. Biotech	I Ratoon	26.5%
			Ramco Biotech	II Ratoon 500 III Ratoon 500	14.8% 9%
5	Rudraya's Farm	B.H. Road Kadur	Green Earth	500	24%

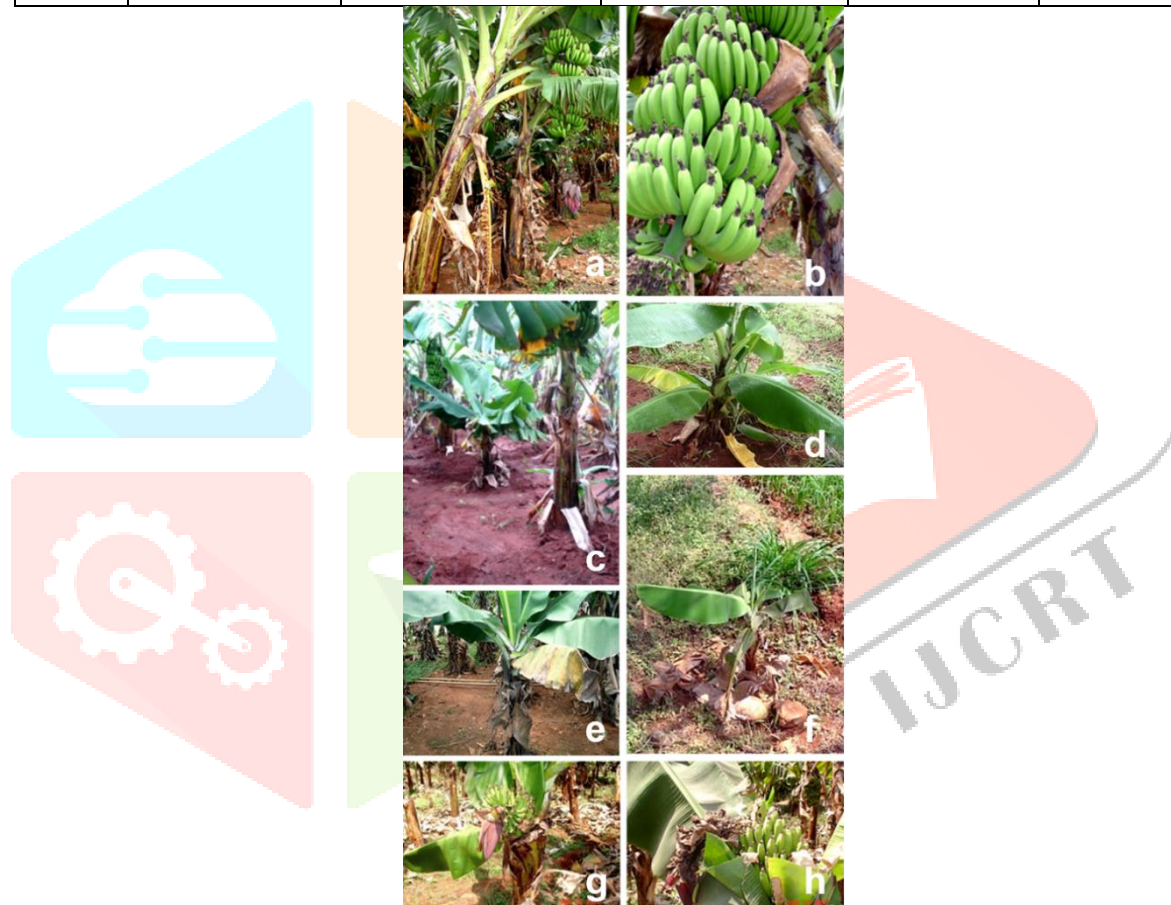
**Plate 1**

Figure a-h: Field detection of morphological somaclonal variants in commercially in vitro propagated banana cv. Grand Naine at various forms.

- True-to-type in vitro propagated banana plant with healthy looking bunch.
- Normal healthy bunch showing proper hands and fingers and correct orientation of fingers.
- Dwarf off-type variant among normal banana plants.
- Extra ordinary dwarf without bunch.
- III Ratoon crop showing dwarf off-type in vitro grown banana plant.
- Non-flowering extraordinary dwarf off-type in Parvathia's farm.
- Bunch variant showing small, abnormally looking, with less hands and fingers inside dwarf off-type banana.
- Bunch variant facing sky with less hands and fingers.



Plate 2

Figure a-I: Field detection of morphological bunch variants of commercially propagated in vitro plants of banana cv. Grand Naine at various fields.

- a. Small bunch inside the plant with less hands and less fruits.
- b. Differently oriented bunch with less hands and fruits.
- c. Bunch oriented straightly with less hands and fingers.
- d. Compact bunch variant with reduced yield.
- e. Hands and fruits in the bunch facing different directions.
- f. Malformed fruits in the bunch (bitter taste)
- g. C-shaped bunch with fruits facing side wise.
- h. Abnormally oriented bunch with small fruits and less hands.
- i. Normal banana plants showing delayed flowering.

A crucial parameter for the banana growers is the quality and quantity of the fruit characters and in the present field survey highest incidence of variations affecting the fruit quality and yield were observed. Table 4 gives the list of variations affecting the bunch character in various fields. Dwarf off-type without flowering ranged up to 52% in Parvathaiiah's farm in Hesaraghatta, which caused heavy economic loss to the farmer. Out of 400 G-9 plants planted 211 plants were off-types without bunch even after 20 months of planting (Plate 1, Figure c & f). This inferior off-type phenotype resulted in severe economic loss with wasted investment of time, field space and other resources in the cultivation of deleterious type of plants to the farmer. This has led to the legal dispute between the farmer and M.S.R. biotech company (the supplier) in the consumer court. Various types of off-types were more in Parvathaiiah's farm like bunch inside, small bunch, bunch facing the sky, improper bunch, less hands in the bunch, malformed fingers, less fingers in each hand etc. (Plate 2, Figure d, e, f, h, Table 4).

Table 4: Type of Somaclones observed in various fields

S. No.	Name of the plant	No. of Plants	Dwarf off-type	Gigantic	Dwarf off-type non flowering	Malformed fingers	Improper bunch/ less hands fruits	Normal non Flowering
1.	Parvathaiah Farm	400	--	--	211	20	10	10
2.	Mohan Das Farm	2000	50	03	02	30	20	350
3.	Gopal Farm	1500	11	20	05	104	25	300
4.	Guruva Reddy Farm	I 800	20	05	10	120	57	26.5%
		II 500	07	03	12	30	22	14.8%
		III 500	04	--	11	20	10	9%
5.	Rudraya Farm	500	03	--	--	97	20	24%

Another significant observation made in the present investigation was improper bunch with lesser hands and fruits (Plate 3, Figure a & b). This off-type variant (20%) was observed in all the fields along with malformed small fingers (Plate 3, Figure h & i). The percentage of this variation was too high which drastically reduced the yield in all the fields. This type of somaclonal variation had severe implication on quality and quantity of the yield, which was deleterious and unwanted by the growers. Present field survey of second and third ratoons G-9 *in-vitro* plants indicated the genetic stability of off-types such as dwarf and improper bunch even in the third generation. This field study has disclosed the higher frequency of off-types in various fields which are unacceptable and needs to be addressed by proper methods (Table 2).

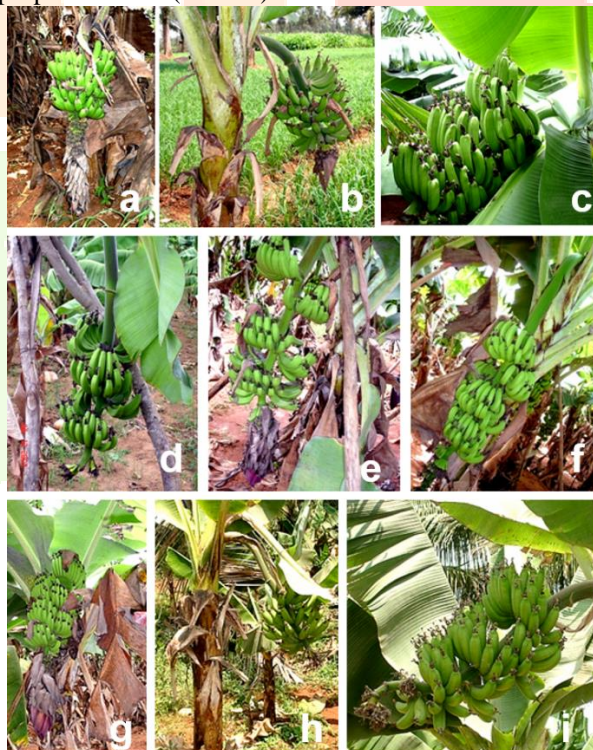
**Plate 3**

Figure a-i: Field identification of morphological bunch variants of commercially propagated *in vitro* plants of banana cv. Grand Naine at various fields.

- Bunch with less hands (minimum yield)
- Sick looking bunch with less hands and fingers
- Small bunch formed inside the plant (without dropping dam)
- Elongated peduncle axis with small bunch with less hands and fingers
- Elongated peduncle axis showing more distance between hands.
- Long peduncle axis with less yield.
- Bunch inside the plant.
- Abnormal bunch with less hands and fingers.
- Bunch oriented in upward direction with less hands and fingers.

IV. DISCUSSION

Banana is one of the horticultural plants, multiplied through micropropagation compared with conventional planting material. The large-scale propagation of banana through *in-vitro* methods has led to the development of off-types with little commercial value (Israeli et al. 1991). Present field evaluation for characterization of *in-vitro* off-types reveals the serious implications of such undesirable variants for potential benefits. Sandoval et al reported that the somaclonal variant index varies in a wide range between 0.1 to 60% depending on different parameters such as number of subcultures, genetic mosaic, growth regulator concentration, donor genotype. Similar observation was done in this study in various fields growing banana clones supplied by various tissue culture units. Somaclonal variation ranging from 1-62% was observed in different fields owing to the different source of donor genotype (Sandoval et al. 1996).

According to Vuylsteke, somaclonal variations has so far had a limited direct contribution to the genetic enhancement of *Musa* and hence are undesirable which limits the widespread use of *in-vitro* techniques (Vuylsteke et al. 1996). In present field study, the severity of variation was observed in one of the fields (Parvathaiyah) where 62% of plants were off-types without bunch. Even after one year of legal dispute in the consumer court, banana grower still has not got any compensation from the commercial unit. Many studies have documented the somaclonal variation in Cavendish group (AAA group) (Hwang, 1986, Vuylsteke et al. 1991, Smith and Hamill, 1993, Reuveni and Israeli, 1989). According to Ventura et al the frequencies of variations in AAA genome are more than ABB group. In this study variation index ranged from 1-62% which according to Smith in unacceptable level (Ventura et al. 1988, Smith, 1988). According to Daniell et al well managed tissue culture units should keep the variation level to 2-3% which is acceptable to most growers (Daniell et al. 1999). James et al points out that occurrence of off-types through tissue culture is higher than random mutation, chemical or radiation induced mutation (James et al. 2007).

Morphological variations affecting reproductive as well vegetative organs were seen in this field evaluation. Most of the variations like dwarfism and leaf variants were inferior to the original plant but variations in the reproductive structures like bunch variants caused heavy economic implications. Similar observation was made by Smith and Drew in Cavendish bananas, where bunch variants were often smaller than the normal plants (Smith and Drew, 1990). Dwarf off-type was one of the common variants observed in the present study ranging from 1-52% in different fields. Duvdevani et al reported 40% dwarf off-type in Cavendish cvs., (Duvdevani et al. 19980. Israeli et al reported 80% dwarf variants in Cavendish Grand Naine banana (Israeli et al. 1996). Many authors have reported the dwarf off-type to be the common morphological variant in the field (Walther et al. 1997, Smith et al. 1993, Martin et al. 1997).

Improper bunch with malformed fingers were the most common and important variants observed in this study which caused low productivity in the yield to the farmers. Similar observations were done by Johns in Chinese Cavendish *in-vitro* grown banana plants. According to him the marketable yield was below average (Johns, 1994). Mantled flowers in oil palm are also one of the main variants amounting to 10%, which decreases the yield of the oil (CIRAD, 2003). Larkin et al reported the extensive morphological variation in wheat line (Larkin et al. 1984). Podwyszynska et al reported morphological flower colour variation in *in-vitro* tulips (Podwyszynska et al. 2006).

In our study many abnormalities occurring in the bunch like less hand, less fingers, reduced fingers, longer inflorescence axis, S-shaped bunch, C-shaped bunch etc., were observed. Uma et al has also described some of the bunch variants in banana cvs. (Uma et al. 2002) Zaffari et al reported 90% variation (Nanism) in Cavendish group of banana cvs. Furthermore, various research investigators reported the use of somaclonal variation for better clonal selection but most of the authors endorse the inferior quality of variants which can cause serious impediment to the *in-vitro* handling and improvement of *Musa* (Zaffari, 2002, Budak, 2004, Hwang et al. 1986, El-dough et al. 2007, Mohamed, 2000).

V. CONCLUSIONS

This study emphasizes the credibility of planting materials supplied by the tissue culture companies to the farmers across Karnataka. The commercial industry should take foremost concern for maintenance of true-to-type nature of micropropagated plants. The tissue culture units should take multidisciplinary approach to reduce the off-types and to get genetically true-to-type of banana plants without any ambiguity. If not done early, serious economic consequences have to be faced either by the company or by the farmer. Morphological studies of commercially propagated *in-vitro* banana cv. Grand Naine (AAA) in the field has shown the enormity of the off-types generated through micropropagation. Through field study 25 types of variants were identified which were the most common types of off-types causing heavy loss to the farmers as detected by present study.

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