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Somatic Embryogenesis in Banana (*Musa* spp.)

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ABSTRACT

The present review summarizes the factors involved in the process of banana somatic embryogenesis and somaclonal variation during this process. Being a polyploid and vegetatively propagated crop, development of an efficient somatic embryogenesis system is critical for the application of genetic transformation or other biological technologies in genetic improvement of banana. Since the 1980s, considerable progress has been made in understanding and refining somatic embryogenesis and plant regeneration in banana, but there are still many bottlenecks that remain to be overcome. The low induction percentage of embryogenic callus is the major limitation in the process of somatic embryogenesis in banana. It strongly depends on genotype/cultivar, incubation condition and some other factors. Success rates for the initiation of good quality embryogenic cell suspensions depend largely on the quality of the selected embryogenic calli. The successful establishment of an embryogenic cell suspension in banana also relies on genotype/cultivar. The germination of somatic embryos into plants is not very efficient and needs to be further improved. This step is also highly variable and found to be affected by genotype/cultivar, regeneration system, and quality of embryogenic cell suspension among other factors. Fortunately, the proportion of somaclonal variants in banana regenerated through somatic embryogenesis obtained from most studies using field tests were low, which suggested that somatic embryogenesis could be used for genetic improvement of banana.

Keywords: embryogenic callus, embryogenic cell suspension, plant regeneration, somaclonal variation
Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; **2-ip**, isopentenyladenosine; **ALFP**, amplified fragment length polymorphism; **BAP**, 6-benzylaminopurine; **ECS**, embryogenic cell suspension; **FCM**, flow cytometry; **IAA**, indole-3-acetic acid; **ISSR**, inter-simple sequence repeat; **Morel**, Morel and Wetmore (1951); **MS**, Murashige and Skoog (1962); **NAA**, α-naphthaleneacetic acid; **PCV**, packed cell volume; **PGR**, Plant growth regulator; **RAPD**, randomly amplified polymorphic DNA; **SH**, Schenk and Hildebrandt (1972); **TDZ**, thidiazuron

CONTENTS

INTRODUCTION

Banana (including plantains) (*Musa* spp.) is a major tropical fruit in the world with an annual production of more than 100 million tons (FAO 2006). Banana also is the fourth most important food crop in the world (Moffat 1999; Tripathi *et al*. 2007), functioning as the staple food for at least 600 million people in the world (Arinaitwe *et al*. 2004). Banana production is threatened by many pests and diseases such as black Sigatoka (*Mycosphaerella fijiensis*), *Fusarium* wilt (*Fusarium oxysporum* var. *cubense*), viruses (*Bunchy top virus*, *Banana streak virus*, *Cucumber mosaic virus*), bacterial wilt (*Xanthomonas campestris* pv. *musacearum*) and nematodes (*Radopholus similes*). The most important constraints for the genetic improvement of banana through conventional breeding are sterility, long generation time, and triploidy of most cultivated bananas.

The integration of cellular biology and biotechnology, including mutation techniques into breeding programs may provide powerful tools to overcome limitations. However, these applications rely on the availability of highly regenerative embryogenic cell suspensions (ECSs; Krikorian and Cronauer-Mitra 1984). Banana plant regeneration via somatic embryogenesis using ECSs has the potential to produce non-chimeric plants because banana embryos have a single-cell origin (Roux *et al*. 2004a).

Banana plant regeneration has been achieved from ECSs that were initiated from several types of explants (Dhed'a *et al*. 1991; Escalant *et al*. 1994; Côte *et al*. 1996; Navarro *et al*. 1997; Grapin *et al*. 2000; Gómez *et al*. 2002;

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Khalil *et al*. 2002; Jalil *et al*. 2003; Xu *et al*. 2004a; Strosse *et al*. 2006), but many bottlenecks, such as poor embryogenic response (Escalant *et al*. 1994; Schoofs *et al*. 1999; Strosse *et al*. 2006) and low embryo germination percentage (Dhed'a *et al*. 1991; Côte *et al*. 1996; Grapin *et al*. 2000), remain to be overcome before an efficient banana regeneration protocol suitable for genetic improvement is developed.

A review on the bottlenecks in the generation and maintenance of morphogenic banana cell suspensions and plant regeneration via somatic embryogenesis was reported by Schoofs *et al*. (1999). A lot of progress has been made in banana somatic embryogenesis in recent years. The present review summarizes the factors affecting somatic embryogenesis in banana, and somaclonal variation during this process.

ESTABLISHMENT OF A SOMATIC EMBRYOGENIC SYSTEM IN BANANA

Being a polyploid and vegetatively propagated crop, banana's genetic improvement through conventional hybridization is complex and difficult. Plant regeneration via somatic embryogenesis from ECSs is used to overcome the limitation by mutation and genetic transformation methods for banana improvement because of its single cell origin.

In the 1980s, formation of spherical masses and embryo-like structures were reported from vegetative tissues (Cronauer-Mitra and Krikorian 1983; Jarret *et al*. 1985) and from inflorescence parts (Bakry *et al*. 1985), but somatic embryos were never obtained. Regeneration was mostly restricted to the formation of either shoots or roots through organogenesis. The first report on successful banana plant regeneration through somatic embryogenesis was that of Cronauer-Mitra and Krikorian (1988), who obtained somatic embryos from cell suspensions derived from apices cultured *in vitro*. One year later, the first successful plant regeneration via embryogenesis from embryogenic callus of banana was reported by Escalant and Teision (1989). In the same year, Novak *et al*. (1989) reported somatic embryogenesis in liquid medium using callus obtained from leaf bases. Since then, somatic embryogenesis techniques have advanced rapidly. Dhed'a *et al*. (1991) first developed a methodology to induce *Musa* ECSs from multiple meristem cultures in the cultivar 'Bluggoe' (ABB). In parallel, Ma (1991) developed a methodology to induce *Musa* ECSs from immature male flowers. A few years later, a temporary immersion culture system, which was originally developed for meristem propagation of banana, had been applied to enhance somatic embryogenesis in sweet and cooking triploid (Escalant *et al*. 1994). More recently, a temporary immersion bioreactor system was developed for the large-scale propagation of banana by Cuban researchers (Gómez *et al*. 2002). In recent years, some efforts have been made to try to improve embryogenesis in banana but no great progress has been obtained (Xu *et al*. 2004a, 2005; Wong *et al*. 2006; Sadik *et al*. 2007; Wei *et al*. 2007).

INDUCTION OF EMBRYOGENIC CALLUS

Explants used

At present, banana plant regeneration has been achieved from ECSs initiated from the following types of explants: leaf bases (Novak *et al*. 1989), multiple meristem cultures (Dhed'a *et al*. 1991; Xu *et al*. 2005; Strosse *et al*. 2006) or shoot tip sections (Ganapathi *et al*. 2001), young zygotic embryos (Escalant and Teision 1989; Marroquin *et al*. 1993); rhizome fragments (Lee *et al*. 1997; Navarro *et al*. 1997) and immature male flowers (Escalant *et al*. 1994; Côte *et al*. 1996; Gómez *et al*. 2002; Jalil *et al*. 2003; Xu *et al*. 2004a) and female flowers (Grapin *et al*. 1996, 2000). But the best results with respect to the quantity of the somatic embryos correspond to two types of explants, multiple meristem cultures and immature (fe)male flowers.

The methodology of developing *Musa* ECSs from multiple meristem cultures, which was referred to as the scalp-method, was first developed by Dhed'a *et al*. (1991). Since then, the genotype and cultivar list has been extended and many ECSs have been successfully established using this method (**Table 1**). The media used for somatic embryogenesis of this method are listed in **Table 2**. The scalp-method is applicable to a wide range of banana and plantain varieties. Scalps (explants derived from highly proliferating shoot-tip cultures), as starting material for initiating embryogenic callus, have several advantages over immature male or female flowers (Strosse *et al*. 2004). This starting material can be

Table 1 Establishment of embryogenic cell suspensions from scalp-derived embryogenic callus in different banana cultivars/varieties.

Cultivar	Genotype	Embryogenic callus	Embryogenic cell suspension	Reference
Guyod	AA	Yes	No	Schoofs 1997
Kamaramasenge	AB	Yes	Yes	Schoofs 1997
Kisubi	AB	Yes	No	Schoofs 1997
Musa balbisiana 'tani'	BB	Yes	No	Schoofs 1997
Brasilero	AAA	Yes	Yes	Strosse *et al*. 2006
Cavendish 901	AAA	Yes	Yes	Schoofs 1997
Grande naine	AAA	Yes	Yes	Schoofs 1997; Xu *et al*. 2004a
Gran enano	AAA	Yes	Yes	Strosse *et al*. 2006
Highgate	AAA	Yes	No	Schoofs 1997
Williams	AAA	Yes	Yes	Schoofs 1997; Xu *et al*. 2005; Strosse *et al*. 2006
Igitsiri	AAAh	Yes	No	Schoofs 1997
Nakitengwa	AAAh	Yes	Yes	Schoofs 1997
Agbagba	AAB	Yes	Yes	Schoofs 1997; Strosse *et al*. 2006
Bise egome	AAB	Yes	Yes	Schoofs 1997
Guoshanxiang	AAB	Yes	Yes	Wei *et al*. 2005a
Lady finger	AAB	Yes	No	Schoofs 1997
Navolean	AAB	Yes	Yes	López *et al*. 2004
Obino l'Ewai	AAB	Yes	Yes	Strosse *et al*. 2006
Orishele	AAB	Yes	Yes	Strosse *et al*. 2006
Prata	AAB	Yes	No	Schoofs 1997
Three hand planty	AAB	Yes	Yes	Schoofs 1997
Bluggoe	ABB	Yes	Yes	Dhed'a *et al*. 1991
Cachaco	ABB	Yes	Yes	Strosse *et al*. 2006
Cacambou	ABB	Yes	Yes	Strosse *et al*. 2006
Cardaba	ABB	Yes	No	Dhed'a 1992
Dole	ABB	Yes	Yes	Strosse *et al*. 2006
Saba	ABB	Yes	Yes	Dhed'a 1992

Table 2 Composition of the culture media used in somatic embryogenesis of *Musa* spp. (scalp-method, based on Dhed'a *et al*. 1991, Xu *et al*. 2005 and Strosse *et al*. 2006).

	P4	ZZss	ZZl	RD1	RD2	REG	MSAK
Macronutrients	MS	1/2MS	1/2MS	1/2MS	1/2MS	1/2MS	MS
Micronutrients	MS	MS	MS	MS	MS	MS	MS
Vitamins	MS	MS	MS	MS	MS	MS	MS
Ascorbic acid (mg/L)	10	10	10	10	10	10	10
Myo-inositol (mg/L)				100	100	100	100
2,4-D (mg/L)		1.16	1.16				
Zeatin (mg/L)		0.22	0.22				
BAP (mg/L)	22.73				0.23		
IAA	0.18					0.18	
Active carbon (mg/L)							1000
Sucrose (g/L)	30	30	30	30	30	30	30
Gelrite (g/L)	2	3		2	2	3	2
pH	6.2	6.2	6.2	5.8	5.8	5.8	5.8
Photoperiod (day/night)*	0/24 h	0/24 h or 24/0 h	24/0 h	0/24 h	16/8 h	16/8 h	12/12 h
Duration (m)	5~14	3~8	3~12	2	1	1	1

P4, for the preparation of scalps; ZZss, for callus induction; ZZl, for the establishment of embryogenic cell suspension; RD1, for the regeneration of embryos; RD2, for the maturation of embryos; REG, for germination; MASK, for rooting and shooting.
2,4-D, 2,4-dichlorophenoxyacetic acid; BAP, 6-benzylaminopurine; IAA, indole-3-acetic acid; MS, Murashige and Skoog (1962).
* Where 0/24 h or 24/0 h is shown, very faint continuous light is used; For 16/8 h or 12/12 h, photoperiod is not strict and almost any combination of day/night can be used.

multiplied *in vitro* and can be obtained from any landrace. Explants are always available, independent of season. Scalps (like all *in vitro* tissues) can be started from virus-indexed material, unlike explants from field-grown plants. The major shortcoming of the scalp-method is the lengthy material preparation phase (Schoofs *et al*. 1999; Strosse *et al*. 2006).

The methodology to establish *Musa* ECSs from immature male flowers was first developed by Ma (1991). The development of this methodology was considered as a major breakthrough in the development of a somatic embryogenesis system for bananas and inspired numerous studies. Nowadays, immature male flowers are the most widely used explants for ECSs in banana (**Table 3**). The media used for somatic embryogenesis of this method are referenced in **Table 4**. When compared to the scalp-method, the male flower-derived method does not need a long time to prepare the explants, which reduces the possibility of variation during the long subculture on P4 medium (**Table 2**). On the other hand, though bananas can produce male flowers all year around, they are produced in large amount within a few months (Xu *et al*. 2005). Moreover, this method is only suitable for genotypes having a male bud. In various types of *Musa* the male part of the axis is short-lived, and no male buds or only a degenerating male bud, is present. Numerous cultivars belong to this type such as the AAB False Horn Plantains. Hence, the male flower-method has also been used with immature female flowers for those cultivars that do not produce male flowers (Grapin *et al*. 2000). The use of female flowers may extend the application of this method to more types of *Musa*.

Induction percentage of embryogenic callus

Banana is a highly recalcitrant crop in somatic embryogenesis, with extremely low embryogenic response. Escalant *et al*. (1994) reported 0~7% embryogenic callus induction using immature male flowers as explants. Strosse *et al*. (2006) tested 24,375 scalps and found only 3.3% resulted in an embryogenic response. The response of Cavendish types and highland bananas is usually lower than 1% (Schoofs *et al*. 1999; Xu *et al*. 2004a). Most importantly, the ideal embryogenic callus which is suitable for the establishment of ECSs is extremely lower. In contrast, the embryogenic callus induction percentage of some other monocots, such as wheat and barley, sometimes is as high as 100% (Benkirane *et al*. 2000). The embryogenic response of banana is not only low, but also varies with the following factors.

Genotype/cultivar

Escalant *et al*. (1994) cultured male flowers of 5 banana cultivars belonging to 3 different genotypes to obtain embryogenic clusters and found that the percentage of embryogenic clusters was dependent on the genomic group, varying from 0 of *Musa* ABB cv. 'Pelopita' to 7% of *Musa* AAB cv. 'Silk'. Grapin *et al*. (1998) reported values of 1.9% and 2.9% in 'Curaré Enano' (AAB) and 'Curaré' (AAB), respectively, using female flowers as explants. Scalps of 18 varieties belonging to 5 genome types (*Musa* AA, AAA, AAA-h, AAB, ABB) were induced for embryogenesis and the average embryogenic frequency was 6.0% for cooking bananas (ABB), 3.8% for Cavendish-type bananas (AAA), 1.8% for plantains (AAB), and 0 for *Musa* AAA-h (Strosse *et al*. 2006). Some other similar results have also been reported (Ganapathi *et al*. 1999; Grapin *et al*. 2000; Xu *et al*. 2004a). Not only was the frequency of embryogenic callus highly dependent on genotype/cultivar, so were meristematic globules and the number of meristematic globules obtained from each rank of male flower (Xu *et al*. 2003).

Incubation condition

The frequency of embryogenic callus induction from scalps was dependent on the incubation condition. The frequency of embryogenic callus induction of 'Williams' (AAA) obtained in dark was 10.8%, which was 1.44-fold higher than that obtained under light (Xu *et al*. 2005) while inverse result was observed on one clone of 'Grande Naine' (Xu *et al*. 2004a, 2005). For male flower explants, no embryogenic callus could be obtained when incubated under light (Xu *et al*. 2004a, 2004b).

Plant growth regulators (PGRs)

PGRs play an important role in the induction of embryogenic callus. Daniels *et al*. (2002) reported that 4 mg/L 2,4-D (2,4-dichlorophenoxyacetic acid) was the best concentration for banana callus induction. Similar result was observed by Xu *et al*. (2004b): an increase in the concentration of 2,4-D resulted in a higher percentage of died explants, e.g. the percentage of died explants obtained at 8 mg/L 2,4-D (more than 40%) was about two times of that obtained at 4 mg/L 2,4-D. The embryogenic callus induction percentage of 'Mas' (AA) reached 15.16% when 2,4-D was substituted by 2 mg/L picloram (Wei *et al*. 2007). Scalp formation was achieved earlier and at much lower concentrations of combined BAP (6-benzylaminopurine) and TDZ (thidiazuron) than when applied alone, and combinations of 2.80/1.00 and 2.30/1.25 mg/L BAP/TDZ produced the best scalps (Sadik *et al*. 2007).

Table 3 Establishment of embryogenic cell suspensions from flower-derived embryogenic callus in different banana cultivars/varieties.

Cultivar	Genotype	Embryogenic callus	Embryogenic cell suspension	Reference
Col.49	AA	Yes	Yes	Grapin *et al*. 1998
SF265	AA	Yes	Yes	Grapin *et al*. 1998
IRFA 903	AA	Yes	Yes	Côte *et al*. 2000a
Mas	AA	Yes	Yes	Jalil *et al*. 2003; Wei *et al*. 2005b
Basrai	AAA	Yes	No	Ganapathi *et al*. 1999
Baxi	AAA	Yes	Yes	Xu *et al*. 2003
Grande naine	AAA	Yes	Yes	Escalant *et al*. 1994; Côte *et al*. 1996; Navarro *et al*. 1997; Becker *et al*. 2000; Kulkarni *et al*. 2004; Chong *et al*. 2005[1]
Gros Michel	AAA	Yes	Yes	Grapin *et al*. 1998
Guangdong No. 2	AAA	Yes	Yes	Xu *et al*. 2004a
Huanong No. 7	AAA	Yes	Yes	Xu *et al*. 2004a
Lokhandi	AAA?	Yes	No	Ganapathi *et al*. 1999
Shreemanti	AAA	Yes	No	Ganapathi *et al*. 1999
Trikoni	AAA	Yes	No	Ganapathi *et al*. 1999
Williams	AAA	Yes	Yes	Xu *et al*. 2003
Yangami km 5	AAA	Yes	No	Grapin *et al*. 1998
Curare	AAB	Yes	Yes	Grapin *et al*. 2000
Curare enano	AAB	Yes	Yes	Grapin *et al*. 2000
Dwarf Brazilian	AAB	Yes	Yes	Khalil *et al*. 2002
Dominico	AAB	Yes	Yes	Grapin *et al*. 1998
French sombre	AAB	Yes	Yes	Grapin *et al*. 1996
Mysore	AAB	Yes	No	Grapin *et al*. 1998
Rasthali	AAB	Yes	No	Ganapathi *et al*. 1999
Silk	AAB	Yes	No	Grapin *et al*. 1998
FHIA-01	AAAB	Yes	Yes	Grapin *et al*. 1998
FHIA-02	AAAB	Yes	Yes	Grapin *et al*. 1998
FHIA-18	AAAB	Yes	Yes	Gómez *et al*. 2002
FHIA-21	AAAB	Yes	Yes	Daniels *et al*. 2002

1 Cultivating immature male flowers directly in liquid culture media

Other factors

Season greatly influenced the induction of embryogenic callus, e.g. more than 13% of flowers gave embryogenic response when inoculated in September and October, while less than 2% in the following December and January (Escalant *et al*. 1994). The response of hands of male flowers was found to depend strongly on the position on the floral bud. Escalant *et al*. (1994) mentioned that in 'Grande Naine' (AAA), 74% of the embryogenic clusters obtained were distributed between positions 7 and 13. Daniels *et al*. (2002) reported that the more differentiated floral buds (10~14) had a lower embryogenic response compared to floral bud positions 5~9. The frequency of embryogenic callus induction in banana sometimes even was different from one experiment to another. For example, the embryogenic response of 'Grande Naine' scalps belonging to the same clone inoculated on January 31 was 4.17% (incubated in dark) and 0.83% (incubated under light), while that of those inoculated on February 19 was 5.83% and 7.5%, respectively (Xu *et al*. 2004a). So, the embryogenic response of banana is far from being understood.

ESTABLISHMENT OF ECS

Success rates for the initiation of good quality ECSs depend largely on the quality of the selected embryogenic calli. Embryogenic complexes are often very heterogeneous, only a very small fraction of which is suitable for transfer to liquid medium. "Ideal" embryogenic callus, which is friable, transparent and also of the right size and in the right developmental stage, is preferably used for the initiation of ECS. Organized embryogenic cell clusters and other non-embryogenic components are not good for the establishment of ECS. So, if possible, only "ideal" embryogenic callus should be selected for the establishment of an ECS. If there is no "ideal" embryogenic callus available, the removal of large embryos (length exceeding 0.5 mm) and compact structures is recommended, and only the embryogenic callus and very small embryos (less than 0.2 mm in length) remained for the initiation of ECS (Strosse *et al*. 2006). The result of transfer of embryogenic complexes to a fresh semi-solid induction medium for proliferation of embryogenic cells is unpredictable (Schoofs *et al*. 1999). Alternatively, a cell suspension which has not yet been established should be inoculated onto a fresh semi-solid induction medium from time to time for proliferation of embryogenic cells.

Not every good complex will lead to a good ECS, and whether an "ideal" embryogenic callus will result in a good ECS was also variable. The success rate from "ideal" embryogenic callus to an ECS from scalp-derived complexes was two out of three to one out of nine, dependent of cultivars and lines (Xu *et al*. 2004a). Strosse *et al*. (2006) reported a frequency of 34.1% on average scalp-derived embryogenic calli successfully giving rise to established ECSs, also genotype and cultivar dependent. In our laboratory, almost every "ideal" embryogenic cell clusters from male flowers has been able to result in the establishment of an ECS, but the regeneration capacity of these ECSs was cultivar dependent.

PLANT REGENERATION FROM CELL SUSPENSION VIA SOMATIC EMBRYOGENESIS

In banana the conversion process from somatic embryos into plants is not efficient enough. The number of somatic embryos formed per ml of plated PCV (packed cell volume) banana ECS was comparable with some other crops, such as alfalfa and coffee (Côte *et al*. 1996). But the germination percentage of banana embryos was relatively lower, especially in early studies. For example, the plant recovery frequency was only 1.5~2% from ECS of dessert and cooking bananas obtained from rhizome tissue culture (Novak *et al*. 1989), 10~23% from scalp-derived ECS of ABB type cv. 'Bluggoe' (Dhed'a *et al*. 1991), and 3~20% from male flower-derived ECS of *Musa* AAA cv. 'Grande Naine' (Côte *et al*. 1996).

The conversion process from somatic embryos into plants in banana is also highly variable.

Besides genotype and cultivar (Xu *et al*. 2004c; Strosse *et al*. 2006), the quality of ECS is the most important factor affecting the germination percentage of embryos and the re-

Table 4 Composition of the culture media used in somatic embryogenesis of *Musa* spp. (flower-method, based on Côte *et al*. 1996 and Navarro *et al*. 1997).

	MA1	MA2	MA3	MA4	MA5
Macronutrients	MS	MS	SH	MS	MS
Micronutrients	MS	MS	SH	MS	MS
Vitamins	MS	MS	MS	Morel	MS
Myo-inositol	100	100	100	100	100
IAA (mg/L)	1			0.2	
NAA (mg/L)	1		0.20		
Biotin (mg/L)	1	1	1		
2,4-D (mg/L)	4	1			
Zeatin (mg/L)			0.05		
2-iP (mg/L)			0.20		
Kinetin (mg/L)			0.10		
BAP (mg/L)				0.05	
Proline (mg/L)			230		
Glutamine (mg/L)	100	100	100		
Malt extract (mg/L)	100	100	100		
Lactose (g/L)			10		
Sucrose (g/L)	30	45	45	30	30
Gelling agent (g/L)	Agarose 7		Gelrite 2	Gelrite 2	Gelrite 2
pH	5.7	5.3	5.3	5.7	5.7
Photoperiod (day/night)	0/24h		0/24h	16h/8h	12h/12h
Duration	5~6 m		80 d	60 d	30~40 d

MA1, for callus induction; MA2, for the establishment of embryogenic cell suspension; M3, for the regeneration and maturation of embryos; M4, for germination; M5, for rooting and shooting

2,4-D, 2,4-dichlorophenoxyacetic acid; 2-iP, isopentenyladenosine; BAP, 6-benzylaminopurine; IAA, indole-3-acetic acid; Morel, Morel and Wetmore (1951); MS, Murashige and Skoog (1962); NAA, α-naphthaleneacetic acid; SH, Schenk and Hildebrandt (1972)

generation capacity of ECS. Georget *et al*. (2000) characterized five types of cellular aggregates in ECS of banana of *Musa* AAA, cv. 'Grande Naine', and found that different types of cell aggregates had different embryogenic potential: very few embryos could regenerated from type I (isolated cells or small cell aggregates) with 48% germination percentage, Types II (embryogenic cells) and III (embryogenic cells with peripheral proliferation zones) were the most highly embryogenic, with 90% and 80% germination percentage respectively, while no plants could regenerate form type V (nodules composed of a central zone of meristematic cells and of an external zone of starchy cells).

Plant regeneration system may also affect germination percentage and plant regeneration capacity of ECS. Nowadays, there are two systems used, semi-solid culture system and temporary immersion system. Plant regeneration capacity obtained in temporary immersion system was 60~70%, which was much higher than that obtained from semi-solid culture system (Escalant *et al*. 1994). In FHIA-18 (AAAB), a much higher germination rate was obtained in a temporary immersion system of a bioreactor than in semi-solid medium control, because the temporary immersion system could avoid oxidation of embryos (Gómez *et al*. 2002). A very high regeneration rate (approx. 32,000 plants ml^{-1} settled cell volume) was obtained via incorporating a liquid-based, embryo-development medium in the process of recovering plants from banana ECSs (Wong *et al*. 2006).

Moreover, the frequencies of embryo germination and plant recovery are affected by some other factors. After a piece of filter paper was inserted between the semi-solid medium and the somatic embryos, the germination percentage of embryos germinated in the first germination process (Type I) went from 26% to 59.5% (Escalant *et al*. 1994). Grapin *et al*. (1996) reported that prolonging the culture time on M3 medium (**Table 4**) could increase the germination percentage of embryos. Côte *et al*. (1996) found that germination percentage of embryos depended on the size of the embryos at the stage of transfer to M4 medium (**Table 4**), e.g. the average germination rate of embryos 800~1000 μm in diameter was nearly 20% while embryos of 100~250 μm was only 3%. The incubation condition for embryos regeneration significantly influenced regeneration capacity of ECS, and darkness was better for 'Grande Naine' (AAA), 'Agbagba' (AAB) and 'Orishele' (AAB) (Xu *et al*. 2004c, 2004d), but there was no significant different between darkness and light for 'Williams' (Xu *et al*. 2005). The cell density was also found to have an influential effect on the number of somatic embryos formed, the size of embryos, and the number of embryos germinated (Daniels *et al*. 2002). In a bioreactor, the number of globular embryos obtained varied not only with initial cell density, but also with concentration of dissolved oxygen and the pH control of the medium (Gómez *et al*. 2002).

SOMACLONAL VARIATION IN BANANA REGENARETED THROUGH SOMATIC EMBRYOGENESIS

The term somaclonal variation was introduced by Larkin and Scowcroft (1981) to describe the genetic variation in plants regenerated from any form of cell culture. In banana and plantain, many factors such as biological (genotypes, explant types), physical (duration of culture), and chemical (growth regulators) factors result in somaclonal variation in banana tissue culture process. But the reports on the effects of these factors seem to be inconsistent. Reuveni *et al*. (1993) found that the rate of variation in Cavendish banana was not affected by both the medium composition and rate of multiplication. The rate of multiplication and variation was, however, strongly correlated in Bairu and his co-authors' study ($\gamma = 0.725$; $n = 6$): as the concentration of BAP and level of sub-culture increased, so did the amount of variation (Bairu *et al*. 2006). Damasco *et al*. (1998) also reported a similar result and demonstrated that the frequency of variation was genotype dependent. While Zaffari *et al*. (2000) stated that not enough work has been done in this regard to reach a logical conclusion.

Dhed'a (1992) first reported somaclonal variation in banana plants regenerated from ECSs. He observed 5~10% abnormal somatic embryos recovered from a 'Bluggoe' suspension, which could grow into normal plants in spite of their abnormality. True-to-typeness of plants of three cultivars regenerated directly from somatic embryos present in embryogenic complexes and from established cell suspensions was evaluated by Schoofs (1997). He showed that the variation ratio was highly genotype/cultivar- and line-dependent: all plants of 'Three Hand Planty' (AAB) and 'Agbagba' (AAB) were vegetatively normal; for 'Williams' (AAA) except line E4000, 1.8% off-types were found among plants regenerated from suspension cultures, which was

much lower than that found among plants from clonal propagation (15%). Roux *et al*. (2004b) assumed that two factors resulted in somaclonal variation from ECS: 1) some non-embryogenic cells with possible abnormal chromosome numbers could have been co-transferred with embryogenic cell cultures from embryogenic calli into liquid medium; 2) some abnormal cells emerged under the effect of *in vitro* culture condition could overgrow embryogenic cells. They found that the effect of the number of subculture on the ploidy of ECS was genotype-dependent.

Côte *et al*. (2000b) evaluated the variance of 500 plants derived from 'Grande Naine' cell suspensions. During the acclimatization phase, only two types of variants were observed. However, when these plants were planted in the field, the morphological abnormalities disappeared. They showed similar agronomical behavior to plants obtained from shoot tip culture, without finding statistical differences in 11 morphological parameters studied. A population of 1,500 plants propagated via somatic embryogenesis in the tetraploid 'FHIA-18' showed similar characteristics to plants propagated from shoot tip cultures both in the acclimatization stage and in field experiments. Only 0.13% somaclonal variants was observed in the plants arising from somatic embryogenesis, which was low taking into consideration that other propagated methods accept up to 5% variants in field conditions (Gómez *et al*. 2006).

Flow cytometry (FCM) analysis, a very powerful technique for ploidy assessment (euploidy/aneuploidy) of suspensions, was used for rapid detection of aneuploidy in *Musa* species (Roux *et al*. 2003, 2004b). In addition, molecular techniques, such as randomly amplified polymorphic DNA (RAPD) (Damasco *et al*. 1996; Ray *et al*. 2006; Deepthi *et al*. 2007), amplified fragment length polymorphism (AFLP) (Engelborghs *et al*. 2004) and inter-simple sequence repeats (ISSR) (Ray *et al*. 2006) were used to detect somaclonal variation.

The percentage of off-types detected by molecular methods was much higher than that from field tests. The reason may be that most off-type embryogenic cells could not regenerate into true-to-type embryos or plantlets, and most regenerated plants are from true-to-type embryogenic cells.

CONCLUSION

As demonstrated above, the major constraint in the process of somatic embryogenesis in banana is the low induction percentage of embryogenic callus. There are not enough ideal initial materials for the establishment of *Musa* ECS. The method which can significantly improve embryogenic callus induction percentage will be another major breakthrough in the development of a somatic embryogenesis system for bananas and inspire numerous studies on genetic improvement through cellular biology and biotechnology.

Though somatic variation is considered as a major constraint for present day micropropagation and plant regeneration via somatic embryos (Gómez *et al*. 2002), the proportion of variants in somatic embryogenesis obtained from most studies using field-test was low enough, which suggested that somatic embryogenesis could be used for genetic improvement of banana.

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