Somaclonal variation in plantains (*Musa* spp, AAB group) derived from shoot-tip culture.

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ABSTRACT - The nature and extent of somaclonal variation in micropropagated plantains (*Musa* spp, AAB group), and factors influencing its incidence, were investigated. Plantain cultivars varied widely in terms of their *in vitro* stability as determined by the occurrence of variation in the phenotype of regenerated plants. Different cultivars also differed in the characters affected by phenotypic variation. Much of the somaclonal variation mimicked the variability occurring naturally, in that the variants of a cultivar were essentially copies of other plantain cultivars. Traits affected by phenotypic change were inflorescence morphology and associated female fertility, fruit shape, pseudostem, petiole and bract colour, and plant stature. Abnormal foliage was observed in some cultivars. Many of the phenotypic variants were stable through several cycles of vegetative propagation, suggesting that much of the variation was genetic in origin. Somaclonal variation was not correlated with time in culture. There was a trend of higher variation frequencies with increasing *in vitro* proliferation rates. Analysis of source identity of regenerated plants indicated that variation arose in culture, suggesting that the tissue culture environment imposed a stress that induced the changes. The narrow spectrum of somaclonal variation was of limited use in plantain improvement. Through its increased seed fertility, the French inflorescence-type variant may be of value in breeding the preferred, but highly sterile False Horn plantain, from which it was derived.

VARIATION SOMACLONALE CHEZ LES PLANTAINS (*MUSA* SPP. GROUPE AAB) DERIVEE DE LA CULTURE DE MERISTEME.

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RESUME - La nature et l'importance des variations somaclonales dans la micropropagation des plantains, ainsi que les facteurs influençant leur incidence sont étudiés. De grandes différences existent entre les cultivars quant à la fréquence et la nature des variations phénotypiques sur les plantes régénérées après culture *in vitro*. Une grande part de ces variations mime la variabilité naturelle et les variants apparaissent essentiellement comme des copies d'autres cultivars de plantains. Les caractéristiques affectées par ces modifications sont la morphologie de l'inflorescence et la fertilité femelle, la forme du fruit, la couleur du pétiole, du pseudo-tronc ainsi que la taille de la plante. On note dans quelques cas des anomalies du feuillage. Beaucoup de variants phénotypiques se révèlent stables au cours de plusieurs cycles de propagation, laissant à penser qu'une large part de cette variation est d'origine génétique. Il n'est pas établi de rapport entre le phénomène et le temps de culture. Ces variations ont tendance à être plus fréquentes avec l'accroissement des taux de multiplication. L'analyse de l'identité de l'origine des plants régénérés montre que la variation survient *in vitro* ce qui conduit à supposer que l'environnement provoque un stress, inducteur de changements. Le spectre des variations somaclonales est trop étroit pour être utile dans l'amélioration des plantains. Par sa fertilité femelle accrue le variant du type «inflorescence de French Plantain» pourrait être intéressant pour hybrider le Faux Corne dont il est issu et qui est hautement stérile.

INTRODUCTION

Somaclonal variation in *Musa*.

With the increasing use of *in vitro* culture for plant production over the past two decades, the occurrence of somaclonal variation, that is enhanced genetic variation among plants regenerated from *in vitro* culture, has been widespread (Larkin and Scowcroft, 1981 ; Scowcroft, 1984). Several reports on the occurrence of phenotypic variation among *in vitro* produced plants of *Musa* (bananas and plantains) confirm that it is a common phenomenon also in this genus. Most studies on the incidence of off-type plants were conducted with micropropagated bananas of the 'Cavendish' subgroup (*Musa* spp, AAA group), in which variation frequencies ranged from 2.5 to 30% (Hwang, 1986 ; Reuveni *et al.*, 1986 ; Arias and Valverde, 1987 ; Hwang and Ko, 1987 ; Pool and Irizarry, 1987 ; Stover, 1987 ; Drew and Smith, 1988 ; Smith, 1988). Little work has been done on plantains (*Musa* spp, AAB group) (Ram-

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charan *et al.*, 1987 ; Vuylsteke *et al.*, 1988 ; Sandoval *et al.*, 1989).

The genus *Musa* provides one of the foremost basic staple foods to large populations in the humid and subhumid tropical regions of the world. Recent concerns over declining yields in bananas and plantains, due to the spread of the virulent fungal leaf spot disease 'black sigatoka' (*Mycosphaerella fijiensis*), have given impetus to genetic improvement programmes (Persley and De Langhe, 1987 ; IITA, 1988). Hence attention has focused on the collection, movement and conservation of *Musa* germplasm, for which tissue culture is increasingly being used as an enabling technique (Vuylsteke, 1989 ; Vuylsteke *et al.*, 1990 a). Efforts to breed *Musa* for disease resistance using conventional methods are fraught with many obstacles (low fertility, triploidy, lack of variability) specific to the biology of the preferred parthenocarpic cultivars. This is illustrated by the absence, as yet, of a new man-bred banana cultivar that is commercially acceptable (Rowe , 1984). A wide array of plant tissue culture and molecular genetic techniques may be used to surmount some of the factors limiting traditional breeding approaches (Krikorian and Cronauer, 1984 ; Murfett and Clarke, 1987 ; Persley and De Langhe, 1987).

At the International Institute of Tropical Agriculture (IITA), and in collaboration with the International Network for the Improvement of Banana and Plantain (INIBAP), existing *in vitro* culture methods, such as shoot-tip culture for micropropagation and germplasm conservation and exchange, and embryo culture to enhance the germination of botanical hybrid seed, have been integrated with conventional multiplication and breeding activities (Vuylsteke *et al.*, 1990 a, b). The frequent use of *in vitro* culture techniques for the handling of *Musa* germplasm warrants investigations into the occurrence of somaclonal variation in this genus.

Reported here are our results of a study on 3500 field-grown plants derived from shoot-tip cultures of seven plantain cultivars that represent the spectrum of cultivar variability within the plantain subgroup. In this paper we only assess the nature and extent of somaclonal variation in plantains and its potential contribution to plantain breeding. One can as yet only speculate on the causes and origins of the variation, but research carried out elsewhere (Jarret, 1990) may soon elucidate the underlying mechanisms. Nevertheless, an account of the phenotypic characteristics of somaclonal variation is essential to any explanation of its origins (Karp and Bright, 1985).

Natural variability in plantain.

This necessitates a prior brief discussion on natural variability in plantains. The plantain subgroup manifests a wide and unique morphological variation despite botanical homogeneity. Variability in West and Central Africa far exceeds that found in its putative centre of origin, South India, reflecting a long history of cultivation and suggesting that the region is a secondary centre of plantain diversity. De Langhe (1961, 1964 a) recognized that the wide spectrum of African plantain cultivars, of which 113 have been identified (Swennen, 1990), make up a supposedly homogeneous group (i.e., derived from a very limited number of botanically different clonal sources) which greatly diversified by accumulated somatic mutations to give a complex reticulate pattern of variability. Characters of seemingly parental origin are plant size and orientation of the inflorescence (De Langhe, 1964 a, b). A large number of somatic mutations affected pseudostem colour and dwarfism, fruit colour, shape and apex, and inflorescence morphology (De Langhe, 1964 a ; Tezenas du Montcel *et al.*, 1983). The latter trait affects the most striking morphological difference among plantain cultivars, which are categorized into four types according to the degree of inflorescence degeneration : the French plantain, the French Horn plantain, the False Horn plantain, and the Horn plantain (Figures 1-3) (De Langhe, 1961 ; Tezenas du Montcel *et al.*, 1983 ; Swennen and Vuylsteke, 1987). These four inflorescence types represent typical steps in the so-called «plantain inflorescence degeneration line» (De Langhe, 1964 c), which is the term used to describe the phenomenon of continuous variation from inflorescences with many small fruits and persistent male flowers (French plantains) to inflorescences with few, but large, strongly parthenocarpic fruits, and male bud absent at maturity (French Horn, False Horn and Horn plantains).

MATERIALS AND METHODS

Plant material.

Seven plantain cultivars (Table 1) were selected as representatives of the major taxonomic groups that embrace the bulk of phenotypic variability in the plantain subgroup (*Musa* spp, AAB group) (De Langhe, 1961 ; Simmonds, 1966 ; Tezenas du Montcel *et al.*, 1983). Their taxonomic classification is given in Table 1. These cultivars have been described (Swennen and Vuylsteke, 1987 ; Swennen, 1990).

Shoot-tip culture.

The seven plantain cultivars were micropropagated following standard methodology of aseptic shoot-tip culture (Vuylsteke and De Langhe, 1985 ; Vuylsteke, 1989). In brief, explants were obtained from lateral shoots, mostly small buds, of true-to-type flowering donor plants in the field genebank and surface disinfected in 0.5% NaOCl (15 min). Shoot tips (about 5 mm) were isolated aseptically, cultured on 20 ml of proliferation medium in 150 x 25 mm test tubes, and kept in a culture room with 26-32°C, 14 h light/10 h dark cycle and 3000 lux.

The proliferation medium consisted of modified MS-medium (Murashige and Skoog, 1962) with 0.4 mg l^{-1} thiamine, 20 mg l^{-1} ascorbic acid, 0.18 mg l^{-1} indole-3 acetic acid (IAA), 4.5 mg l^{-1} 6-benzyladenine (BA), 5 g l^{-1} agar, and without inositol (pH 5.8). Multiplication of shoot cultures proceeded through serial subcultures about every 10 weeks on the same medium. Plantlets were regenerated from proliferating shoot cultures by culturing small clusters of 2-4 shoots/buds on the MS medium described above, but without IAA and with 0.19 mg l^{-1} 1-naphthaleneacetic acid (NAA) and 0.23 mg l^{-1} BA. Rooted plantlets were transplanted to the nursery to acclimatize for 2-3 months before field planting.

Plants were regenerated from 13 months old shoot

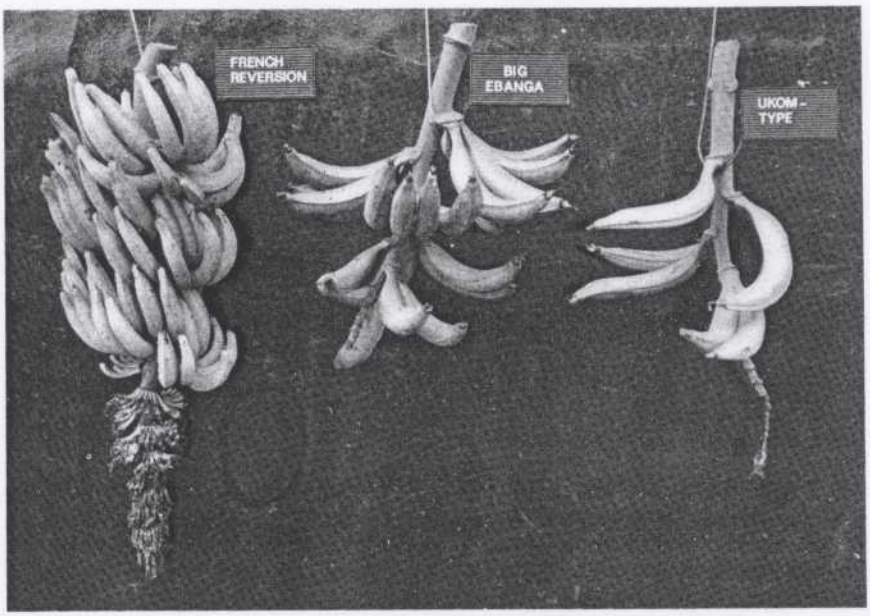

FIGURE 1 - Somaclonal variation in the form of inflorescence-type variation in the giant False Horn plantain cv 'Big Ebanga'. From left to right : the 'French reversion' off-type, the original type, and the unstable, more degenerated 'Ukom'-type variant. Inflorescence-type variation in the medium False Horn plantain 'Agbagba' is very similar to this.

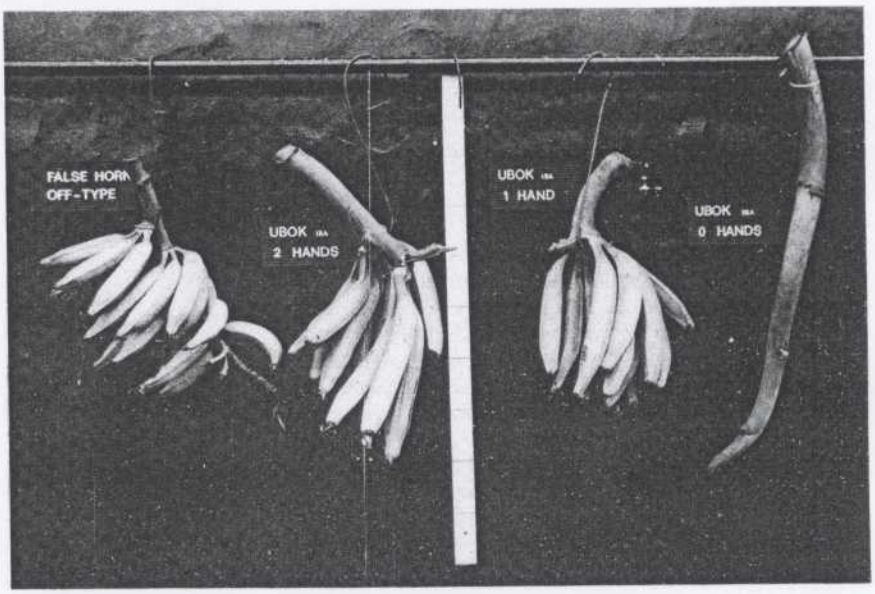

FIGURE 2 - Somaclonal variation in the form of inflorescence-type variation in the Horn plantain cv 'Ubok Iba'. From left to right : False Horn variant, two true-to-type bunches with 1-2 hands, and unstable variant showing total inflorescence degeneration.

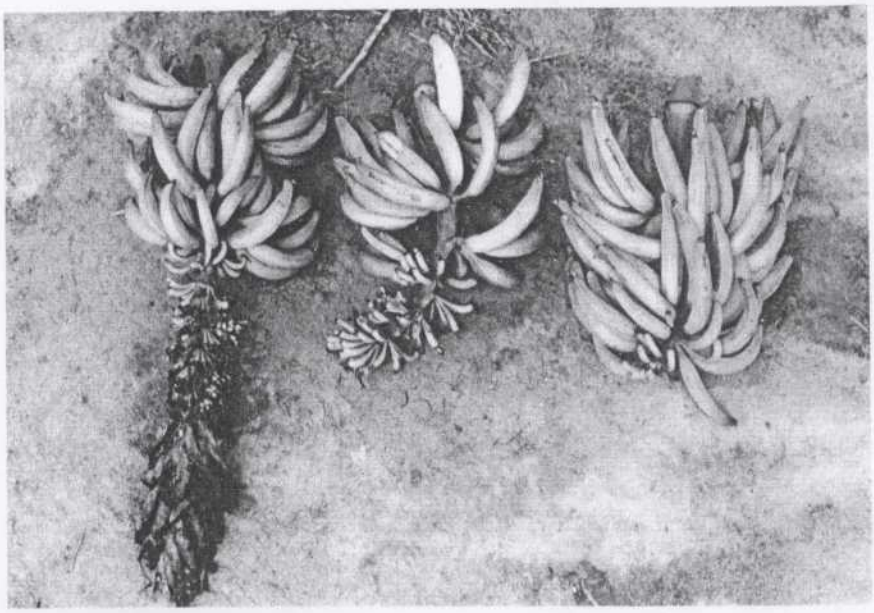

FIGURE 3 - Somaclonal variation in inflorescence morphology of the highly unstable cv 'Bise Egome 2'. From left to right : 'French reversion' off-type, true-to-type French Horn bunch, and Horn off-type.

TABLE 1 - Somaclonal variation (SV) in micropropagated plantain (*Musa* spp, AAB group).

Cultivar name	Plantain category	Number of plants screened	Somaclonal variation (%)	SV that mimics natural variability (%)
Bobby Tannap	Medium French	391	0.0	-
Ntanga 2	Giant French	393	0.5	50
Obino l'Ewai	Medium French	388	2.1	25
Agbagba [a]	Medium False Horn	520	4.4	61
Ubok Iba	Medium Horn	248	12.5	90
Big Ebanga	Giant False Horn	369	35.0	100
Bise Egome 2	Medium French Horn	181	69.1	99

a : see also Table 2.

cultures, i.e. after 5 subcultures on proliferation medium, except for the cultivars 'Agbagba' and 'Obino l'Ewai', in which plants were produced from 28 months old cultures (12 subcultures). The cv 'Bise Egome 2' was monitored in a separate experiment, in which plants were regenerated from 7 months old cultures (3 subcultures).

Source identity of regenerated plants.

Cultures were multiplied from 8 initial shoot tips through random, serial subcultures until a stock of 120 cultures was obtained for each cultivar. Each culture was then numbered from 1 to 120. Plants were regenerated by randomly taking some of the numbered cultures and placing all shoots and buds of these selected explants on regeneration medium. Source identity of regenerated plants was thus maintained by keeping the number of each set of sister plants produced from the same explant of the last subculture. Enough cultures on plant regeneration medium were made so as to enable the planting of four blocks of 98 plants for each cultivar (except 'Bise Egome 2'). The unequal numbers of observed plants (Table 1) is due to loss of some plants upon field establishment. In cv 'Ubok Iba', one block could not be planted due to slow regeneration of rooted plants (caused by continued proliferation of shoots and buds on the regeneration medium). This missing block was then filled in by 'Agbagba' plants.

In the case of cv 'Agbagba' in which plants were regenerated at several intervals (see below), source identity was maintained only in the plants derived from the last subculture.

Effect of time in culture.

In the cv 'Agbagba', the effect of time in culture was investigated by regenerating plants from different subcultures (Table 2) through an open-ended system : some proliferating cultures were randomly taken to regeneration medium, while the remaining ones were used to maintain a stock of multiplying cultures through subculturing on proliferation medium. The earlier part of this work has been published (Vuylsteke *et al.*, 1988).

TABLE 2 - Somaclonal variation in micropropagated plants of the False Horn plantain cv 'Agbagba' (*Musa* spp, AAB group) as affected by time in culture.

In vitro multiplication cycles	Number of plants regenerated	Number of variants						Frequency (%)
		French inflorescence	'Monganga' inflorescence	Distorted laminae	Drooping leaves	Leaf variegation	Total	
0	39	0	0	1	0	0	1	2.6
2	367	23	2	3	0	0	28	7.6
3	157	1	0	0	22	0	23	14.7
4	107	0	0	3	0	0	3	2.8
5	55	0	0	0	0	0	0	0.0
6	240	1	1	0	0	1	3	1.3
7	70	4	3	0	0	0	7	10.0
12	520	13	1	2	7	0	23	4.4
Total	1555	42	7	9	29	1	88	
Frequency of variants (%)		2.7	0.5	0.6	1.9	0.1	5.7	

Effect of *in vitro* proliferation rates.

In order to investigate a possible influence of proliferation rate on frequency of somaclonal variation, the number of shoots and buds produced per explant was counted during the five consecutive subcultures (on proliferation medium) prior to plant regeneration (not in cv 'Bise Egome 2'). Chi-square test of the data on *in vitro* proliferation rates indicated variance heterogeneity. Examination of the data revealed that the variances were proportional to the means and consequently a square-root transformation was applied for the analysis of variance.

Screening for somaclonal variation.

This was performed by monitoring typical morphological descriptors of plantain (De Langhe, 1961, 1964 a ; Tezenas du Montcel *et al.*, 1983) and measuring standard growth parameters (Swennen and De Langhe, 1985 ; Swennen and Vuylsteke, 1987) on field-grown plants. Phenotypic variation that remained stable in the two ratoon crops was considered as somaclonal variation (except in cv 'Bise Egome 2' which is discussed separately).

RESULTS AND DISCUSSION

Effect of genotype.

Within the homogeneous plantain subgroup, the rate of occurrence of somaclonal variation was cultivar specific, ranging from 0 to 69% (Table 1). The False Horn plantain cultivars 'Big Ebanga' and 'Agbagba', which have an analogous phenotype except that the former is of giant stature and the latter is medium-sized, both showed similar variations in inflorescence type, but with an eightfold difference in the extent of total variation (35 and 4.4%, respectively). The three French plantain cultivars had very low frequencies of variation. The cv 'Bise Egome 2' had the highest incidence of variation ever recorded in *Musa*. Such large cultivar-dependent effects suggest that there are inherent differences in stability between plantain clones. This challenges the commonly assumed homogeneity of the African plantain complex.

The prospect of clonal uniformity from asexual propagation is based on the expectation that mitosis is a conservative process, but it has been argued that this may not be as conservative as once thought under stressful conditions, such as in tissue culture (McClintock, 1984 ; Walbot and Cullis, 1985). This seems to apply also to plantains, since frequencies of phenotypic changes in *in vitro* propagated plantains can be high in certain clones.

The nature of somaclonal variation.

For the larger part, somaclonal variation in plantain was not unique as much of the somaclonal variation mimicked the variability occurring naturally (Table 1). Somaclonal variation thus should not be considered as being generated by *in vitro* culture *per se*. The frequency of this variation, however, seems to be greatly amplified *in vitro* (Vuylsteke *et al.*, 1988).

Somaclonal variation in plantain mainly affected the inflorescence, with true-to-type and off-type plants representing all typical steps of the «plantain inflorescence degeneration line» (Figures 1-3). For example, in the micropropagated False Horn plantain 'Agbagba', which is the most common clone in West Africa, inflorescence-type variation in the form of reversion to a typical French plantain bunch type, i.e., with complete inflorescence, was observed at a frequency of 2.7% (Table 2). The occurrence of such variation, called 'French reversion', has also been reported among conventional propagules, but at a lower frequency of 0.7% (Vuylsteke *et al.*, 1988). In addition, the *in vitro* generated 'French reversion' variant manifested an associated change in the level of female fertility. Seed production was higher in the French variant than in the original 'Agbagba' cultivar (Table 3), which agrees with the general observation that French plantains are more fertile than False Horn plantains (Swennen and Vuylsteke, 1991). Also in cv 'Agbagba', *in vitro* culture resulted in 0.5% variants with a more degenerated inflorescence which resembled the bunch of the cultivar 'Monganga' (De Langhe, 1961) (Table 2). Similarly, the cv 'Big Ebanga' showed 35% 'French reversion' and 2.4% degenerative shift to an 'Ukom'-type bunch (Swennen and Vuylsteke, 1987) (Figure 1), although the latter variant was unstable in the ratoon crop. Inflorescence degeneration, as observed here in micropropagated 'Agbagba' and 'Big Ebanga' plants, as well as in the French Horn cv 'Bise Egome 2' (discussed separately below), has never been reported in a plantain clone *in situ*. Inflorescence-type variation was widespread among False Horn plantains, which is illustrated by its reoccurrence in most plantings (Table 2).

Inflorescence-type variation was also observed in the Horn plantain cultivar 'Ubok Iba', which has a bunch typically consisting of only 1-2 hands. Micropropagated 'Ubok Iba' produced an off-type False Horn bunch with 6-8 hands (Figure 2), resembling the cultivar 'Agbagba', at a frequency of 4%. Such variation has never before been observed in Horn plantains. Variation in petiole colour was also encountered in 'Ubok Iba'. Normal plants have a red petiole margin ; a green mutant, which also occurs among conventionally propagated plants, appeared in 6.9% of the micropropagated plants.

TABLE 3 - Number of seeds per bunch in the False Horn plantain 'Agbagba' (*Musa* spp, AAB group) and its 'French reversion' variant upon pollination with two diploid bananas (*Musa* spp, AA group).

Female plantain parent	Diploid male parent					
	Pisang lilin			Calcutta 4		
	No. of bunches pollinated	No. of seeds		No. of bunches pollinated	No. of seeds	
		Mean	Max		Mean	Max
Agbagba	86	0.02	2	189	0.03	2
French off-type	131	0.17	3	204	2.33	20

In the French plantain cultivar 'Obino l'Ewai', a somaclonal variant, which resembled the French plantain cultivar 'Amou' (Swennen, 1990), was observed at the rate of 0.5%. The 'Amou'-type variant had a brillant green-yellow pseudostem and pinkish bracts in contrast to the dull yellow-green pseudostem colour and the dark blue-purplish male bud of the original 'Obino l'Ewai' cultivar.

In the giant French plantain cv 'Ntanga 2', one plant with variant plant size was noted. Normal plants of this cultivar produced 48 ± 0.6 foliage leaves before flowering and reached a height of 495 ± 6 cm with a pseudostem circumference of 99 ± 0.4 cm (confidence limits at 5%). The off-type plant counted 31 leaves and was 330 cm high with pseudostem girth at 61 cm. Although the leaf length/width ratio decreased from 3.5 to 3.0, dwarfism is excluded since such mutation does not generally affect leaf number and pseudostem girth. This variant was unexpected because plant size variation is considered to be of parental origin and not a somatic mutation (De Langhe, 1964 a).

The French plantains in our study did not exhibit inflorescence-type variation. Apparently the interconversion between plantain inflorescence types occurs only in the False Horn and Horn plantains. This is evidence of discontinuity in plantain variability and hence challenges the hypothesis that all French and Horn types intergrade (Simmonds, 1966).

Much of the array of natural morphological variability of plantains was thus recovered in the somaclonal variation : variation in inflorescence and fruit morphology, pseudostem, petiole and bract colour, and plant stature. In fact, somaclonal variation essentially involved the acquisition of another cultivar's phenotype, as observed in the cvs 'Agbagba', 'Big Ebanga', 'Ubok Iba', 'Obino l'Ewai' and 'Ntanga 2'. This, however, also involved some rapid changes never before observed *in situ*. Variation in bunch orientation and in fruit apex form was observed in later plantings of micropropagated 'Obino l'Ewai' (unpublished results) Dwarf mutants were not identified during our investigations, but have been noted elsewhere (Sandoval *et al.*, 1989).

The observation that somaclonal variation is not unique also emerges from reports on phenotypic variation in micropropagated cultivars of the 'Cavendish' subgroup (*Musa* spp, AAA group), in which dwarfism is the single most common variant trait (50-75% of off-types) (Hwang, 1986 ; Reuveni *et al.*, 1986 ; Stover, 1987 ; Smith, 1988). Dwarf mutations are also known to occur in Cavendish clones *in situ* (Simmonds, 1966), but at much lower frequencies than *in vitro*.

In spite of the high incidence of somaclonal variation in some cultivars, the spectrum of variant phenotypes was not diverse. In a single cultivar, the number of variant types ranged from one to five only. For example, in the cv 'Agbagba', the 5.7% phenotypic variation (Table 2) was expressed only in five off-types, four of which are undesirable and agronomically inferior (Vuylsteke *et al.*, 1988). However, a single off-type could be variant for several morphological traits at a time. In cv 'Obino l'Ewai', the 'Amou'-like off-type displayed a change in pseudostem and bract colour. In the Horn plantain cv 'Ubok Iba', the False Horn variant (Figure 2) also showed an altered petiole colour. The inflorescence-type variants in cvs 'Agbagba', 'Big Ebanga' and 'Ubok Iba' (Figures 1 and 2) showed concurrent changes in a series of pomological characteristics that are closely related to plantain inflorescence type. It seems that a whole set of apparently tightly linked traits changed simultaneously, unless these are under the same regulatory gene control.

Notwithstanding the narrow spectrum of variation, which implies that some characters are not prone to change, somaclonal variation involved both quantitative and qualitative traits. Variation in inflorescence type, a character that is likely to be polygenically controlled, as well as in petiole colour, a trait under more simple genetic control, were noticed.

Aside from the variants in inflorescence-type and petiole colour, the next most frequent off-types were those exhibiting abnormal foliage (e.g., Table 2). Plants with the right lamina-half narrow and distorted were most common and have been found in all the cultivars under study, except 'Big Ebanga', at a range of 0.25-1.2%. Drooping leaf habit, associated with lesser vigour and slow growth, was observed only in 'Agbagba' (1.9%) and 'Obino l'Ewai' (1.0%). Based on a report by Simmonds (1948) on the effects of ploidy on banana leaves, we speculate that these aberrant foliage off-types could be the result of ploidy variation. Leaf variegation was a rare event.

A somaclonal variant with resistance or tolerance to black sigatoka has not been observed among the 3500 micropropagated plantains. The narrow spectrum of variant but novel phenotypes limits the possibility or revealing useful variability for plantain improvement.

Stability of phenotypic variants.

In vegetatively propagated crops, conventional genetic analysis of somaclonal variants is difficult or impossible, but it is generally accepted that transmission of off-type traits through at least two successive clonal field generations indicates a true genetic basis for the variation (Scowcroft 1984 ; Karp and Bright, 1985).

We have evaluated the stability of phenotypic variants of plantains during three successive production cycles (plant crop and two ratoon crops). In the cultivars 'Obino l'Ewai', 'Agbagba' and 'Ubok Iba', all off-type traits were transmitted consistently to the two following clonal generations in the field. Three of the five variants of 'Ntanga 2' showed a transient leaf distortion. In 'Bobby Tannap', the three variants with distorted laminae were unstable as the first ratoon grew a normal phenotype. All 129 'French reversion' variants of 'Big Ebanga' remained stable through the two ratoons, but the nine degenerated 'Ukom' off-types were not stable. Temporary abnormalities and unstable phenotypic variation have not been included under somaclonal variation in Table 1. True-to type regenerants of these six cultivars were also stable in the two successive ratoons, except in cv 'Agbagba' where three out of the 496 normal plants showed a phenotypic switch from False Horn to French inflorescence in the first ratoon. Cultivar 'Bise Egome 2' is considered separately.

The stability of 'Agbagba' variants (Table 2) was tested more thoroughly by monitoring the transmission of off-type traits to sucker progeny, following a cycle of conventional clonal propagation, and after another *in vitro* passage, i.e., by reintroducing off-types from the field to *in vitro* culture. The French variant of 'Agbagba' was stable among sucker progeny (72 plants) and *in vitro* progeny (197 plants). The 'Monganga' variant was also stable in sucker progeny (10 plants). In contrast, foliage variants showed consistent off-type traits when propagated by suckers, but were unstable following further *in vitro* multiplication. Among 29 *in vitro* plants of the distorted lamina variant and 45 plants of the drooping leaf variant, 12 and 9, respectively, reverted to the normal phenotype of 'Agbagba'. Astonishingly, one regenerant of the drooping leaf variant adopted the phenotype of the distorted lamina variant. This demonstrates that the putative ploidy variation, which causes this foliage variation, occurs at random and can go in both directions.

The stability of the plantain inflorescence-type variants in the ratoons and among sucker progeny suggests that this variation is genetic in origin. From cytological observations of pollen mother cells, the 'French reversion' variant of cv 'Agbagba' proved to be euploid (K.V. Bai, IITA, personal communication, 1990). Thus, this type of phenotypic variation was not the result of numerical chromosome variation. We have recently been able to produce sexual progeny of the 'French reversion' variant of 'Agbagba'. Ten of the eleven different field-established hybrids have so far flowered and produced a complete (French) inflorescence. These 'French reversion' hybrids are currently being selfed. Assessment of selfed progeny can demonstrate heritability of variant traits and will establish the true basis of this variation.

Effect of time in culture.

The production of micropropagated plants of cv 'Agbagba' has been separated per cycle of *in vitro* multiplication in Table 2. Bearing in mind that each subculture is grown for about 10 weeks, frequency of variation does not appear to increase with time in culture. This is in contradiction with the general opinion that somaclonal variation is likely to be enhanced with prolongation of the culture period (Scowcroft, 1984).

The reason for this unusual observation is not clear. Some variants may be unstable during further *in vitro* passages and revert to the original phenotype. This has been demonstrated in the case of the foliage variants of 'Agbagba' (see above). Also, the sample size of some subcultures may not have been large enough. The suggestion of lack of correlation between culture time and variation frequency should thus be taken with caution. It is generally considered to be good practice to limit the length of time in culture in order to minimize variation, although this may be difficult to achieve in *in vitro* genebanks.

Effect of *in vitro* proliferation rate.

Significant differences in shoot/bud proliferation were found, both between cultivars and between subcultures (Table 4). Variation in multiplication rates among cultivars most likely reflected inherent genotypic differences in endogenous growth substance levels in shoot tips, because concentrations of exogenously applied growth regulators were identical for all cultivars. Comparison of Tables 1 and 4 shows that there was a tendency of higher somaclonal variation frequencies with increasing proliferation rates in culture. The correlation is, however, not significant largely due to one clone (cv 'Big Ebanga'). Henshaw *et al.*, (1980) had already suggested that mutation frequencies are likely to be proportional to the rate of cell division. In plantains this relation was not clearcut and other factors such as genotype (intrinsic instability) may be overriding in some cases.

Conversely, Reuveni *et al.* (1986) concluded from their observations on 'Cavendish' bananas (*Musa* spp, AAA group) that *in vitro* multiplication rate did not influence the degree of variation.

TABLE 4 - Proliferation rates in shoot-tip cultures of plantains (*Musa* spp, AAB group).

Plantain cultivars	Proliferation rates in serial subcultures					Cultivar mean [1]	Transformed mean	Sample size
	1st	2nd	3rd	4th	5th			
Bobby Tannap	21.1	11.4	15.3	13.5	10.8	12.4 b	3.523	147
Ntanga 2	15.8	10.9	13.6	12.5	12.1	12.1 b	3.474	152
Obino l'Ewai	-	-	-	7.4	11.8	9.2 a	3.028	90
Agbagba	16.6	14.7	15.2	19.6	18.7	16.5 c	4.065	87
Ubok Iba	27.6	21.3	21.2	22.7	19.1	20.8 d	4.559	134
Big Ebanga	14.8	13.8	12.3	20.4	14.8	15.3 c	3.916	153
							(SE ± 0.085)	
Subculture mean [2]	20.1 c	15.4 a	16.6 ab	17.9 b	16.5 ab			
Transformed mean	4.483	3.925	4.076	4.234	4.065	(SE ± 0.116)		
Sample size	36	112	110	230	275			

1 : Data shown are backtransformed means after square-root transformation of original counts, adjusted for subculture differences. Mean separation by LSD at 1% level.

2 : Data shown are backtransformed means after square-root transformation of original counts, adjusted for cultivar differences. Mean separation by LSD at 1% level.

TABLE 5 - Summary of source identity analysis of regenerated plantain (*Musa* spp, AAB group) plants as produced from their respective explants.

	Number of explants in the last subculture					Explants producing variants (%)
	producing true-to-type plants only	producing variant plants only	producing mixture 1 a	producing mixture 2 b	Total	
Bobby Tannap	45	0	0	0	45	0.0
Ntanga 2	57	0	2	0	59	3.4
Obino l'Ewai	60	0	5	0	65	7.7
Agbagba	36	1	11	1	49	26.5
Ubok Iba	16	1	6	4	27	40.7
Big Ebanga	14	15	10	0	39	64.1
Total number of explants	228	17	34	5	284	

a : mixture of true-to-type plants and plants of one variant phenotype.
b : mixture of true-to-type plants and plants of two different variant phenotypes.

Origin of somaclonal variation.

Analysis of the source identity of regenerated plants demonstrated that several individual explants gave rise to both normal and variant phenotypes (Table 5). A whole spectrum of variation can thus emanate from a single explant. Most importantly, the fact that a population of sister plants can contain both normal and variant plants, and variant plants of different types, indicates that these variants arose during the *in vitro* culture phase. Apparently, not all the genotypic configurations observed as later phenotypic variations existed in the shoot tips collected for initiation of the culture.

It has been argued that culture media components, particularly the growth regulators, may be mutagenic (for review, see Karp and Bright, 1985 ; Gould, 1986). In our study all seven plantain cultivars were multiplied under identical *in vitro* conditions, characterized by a high concentration of benzyladenine (4.5 mg l^{-1} BA) in the medium. Yet three cultivars showed little phenotypic variation (Table 1). The high cytokinin level in the medium is used to maintain high multiplication rates, the principle reason for using *in vitro* propagation methods. Reuveni *et al.* (1986) also concluded from their study on somaclonal variation in 'Grande Naine' and 'Williams' bananas that the mutation rate is not affected by the medium composition. Rather, genotypic effects make some cultivars more prone to somaclonal variation than others. Smith (1988) advocated the multiplication of (unstable) cultivars on low cytokinin media, but it remains to be seen whether this will result in less somaclonal variation.

It is important to stress that an off-type was obtained by direct regeneration (on medium with BA at 0.23 mg l^{-1}) of a plant from a shoot tip, i.e., without going through a multiplication cycle (Table 2). This suggests that the tissue culture phase is important, rather than the media components (Karp and Bright, 1985).

Somaclonal variation in cv 'Bise Egome 2' : a special case.

The plantain cultivar 'Bise Egome 2' is a particular case. This clone, commonly classified as a French Horn, was included in our study because it regularly produced a French bunch type in the field genebank (five plants only per cultivar) and thus seemed to be very unstable.

The 69% somaclonal variation rate (Table 1) underscores the highly unstable nature of this cultivar. The population of 181 'Bise Egome 2' plants, produced *in vitro*, was a potpourri of all possible inflorescence types in the continuum of the «plantain inflorescence degeneration line». The extremes were represented by the French inflorescence type (34.3%), on the one hand, and the true Horn type (3.3%), on the other (Figure 3). The remaining plants showed a variety of French Horn and False Horn inflorescence types, with a great variation in the number of hands bearing intermediate flowers and in the total number of intermediate flowers. This emphasizes the continuous nature of variation in inflorescence type, which makes classification into discrete categories difficult.

In the first ratoon, there was also a high frequency of inflorescence-type flux (Table 6). Most importantly, 41% of the French variants of the plant crop produced a degenerated inflorescence, with 4.5% true Horn bunches. The interconversion of plantain inflorescence types is not uncommon, both *in vitro* and *in situ*, in the case of reversion from False Horn to French. However, here a degenerative shift from French to False Horn and Horn was also encountered. To our knowledge, this is the first report of such degenerative inflorescence-type variation. In one plant with a French bunch, a helicoid flower arrangement was observed from the 5th to the 10th hand (approximately), instead of the normal pattern of nodal clusters.

In 'Bise Egome 2', the *in vitro* passage caused a sudden burst of instability in inflorescence morphology which continued in the first ratoon of the regenerated plants (Table 6). This is in sharp contrast with the six other plantain cultivars. In maize and alfalfa, the occurrence of unstable mutants has been one of the hallmarks associated with transposable-element activity (Walbot and Cullis, 1985 ; Groose and Bingham, 1986 ; Lee and Phillips, 1988). It is tempting to ascribe the somaclonal variation in 'Bise Egome 2' to the deletion/insertion of mobile

TABLE 6 - Variation in inflorescence-type in the first ratoon of the *in vitro* propagated plantain cv 'Bise Egome 2' (*Musa* spp, AAB group).

Inflorescence type in the plant crop	Number of plants	Number of plants per inflorescence type in the 1st ratoon			
		French	French Horn	False Horn	Horn
French	44	26	9	7	2
French Horn	41	17	17	6	1
False Horn	39	14	16	8	1
Horn	5	3	0	2	0
Total	129	60	42	23	4

genetic elements, because of the instability of the variant phenotypes.

CONCLUSIONS

We have observed that high frequencies of phenotypic changes can occur in micropropagated plantain plants that have been regenerated directly from shoot cultures, i.e., without an intervening callus phase (Banerjee *et al.*, 1986). There is controversy as to whether the newly formed meristems that arise directly from the shoot-tip explant originate through a strict axillary budding system or as adventitious growths. However, it has been demonstrated that the mode of shoot bud regeneration in highly proliferating *Musa* cultures, such as in plantains, is adventitious (Banerjee *et al.*, 1986). Scowcroft (1984) ranked adventitious shoots as having higher instability than axillary shoot multiplication.

Large differences in the degree of somaclonal variation have been observed among plantain cultivars. French plantains were stable, with off-types barely exceeding 2%. False Horn plantains ranged from moderately unstable (5%) to highly unstable (> 25%). This suggest that the genomes of the stable French plantains and the unstable False Horn plantains are fundamentally different. Likewise, different cultivars differed in the characters affected by *in vitro* instability and such differences may be related to botanical origins (Simmonds, 1966).

The spectrum of somaclonal variation was rather narrow. Micropropagated plantains displayed variation in inflorescence type and associated degrees of female fertility, fruit shape, pseudostem, petiole and bract colour, plant stature, and leaf and growth habit. Much of this somaclonal variation corresponds to natural inflorescence-type variation, but whose occurrence is enhanced *in vitro*. Evidently, the loci in the plantain genome that regulate inflorescence morphology in the False Horn and Horn plantains are unstable, especially under *in vitro* conditions, but also *in vivo*. Analysis of the molecular basis for this hypervariable region of the genome will contribute to our understanding of the organization of the plantain genome.

The similarities between somaclonal and natural variation suggests that similar mechanisms operate to generate the genomic changes in both instances. This supports the hypothesis that the mechanisms responsible for the generation of rapid genomic changes are akin to those generating variation on an evolutionary time scale (Walbot and Cullis, 1985). Somaclonal variation can thus provide a tool to infer a process of genetic change (Roth *et al.*, 1989). Knowlegde of the mechanism(s) involved in plantain inflorescence-type change could prove useful in plantain improvement. Indeed, the ensuing potential control over this process of change may provide a way to breed the preferred, but highly sterile False Horn types, because their corresponding 'French reversion' variants manifest increased female fertility. Seeds have been produced from crosses of the French variant with black sigatoka resistant diploid bananas (Swennen and Vuylsteke, 1991) and some of the hybrids resulting showed resistance to the disease. If these improved 'French reversion' hybrids were to convert back to the original False Horn type during another *in vitro* passage, a practical scheme for breeding the 'Agbagba' plantain ideotype would then be available. The possible switch back of 'French reversion' variants to the original, more degenerated inflorescence type has been demonstrated in the unstable cultivar 'Bise Egome 2'.

Somaclonal variation arising through shoot bud proliferation *in vitro* should not be overestimated as a source of new useful variability in plantains, and probably in *Musa* at large. Screening at the whole plant level for somaclonal variants with disease resistance requires considerable space and labour (Hwang and Ko, 1987). But if screening could be performed at the cellular level, with selection pressure applied *in vitro*, this technique could be particularly useful.

Following the proposal of McClintock (1984), we speculate that the somaclonal variation which we observed in micropropagated plantains occurred in response to stress imposed by the tissue culture environment. *In vitro* culture is a highly stressful situation for the individual cells in the cultured tissues, in contrast to the highly integrated and balanced environment of the whole, intact plant. Rapid genomic change in the form of somaclonal variation may ensue from mechanisms of genetic instability that are activated by 'genome shock' through tissue culture (McClintock 1984 ; Karp and Bright, 1985 ; Walbot and Cullis, 1985). Therefore, the use of *in vitro* culture techniques for the propagation and conservation of *Musa* germplasm should be considered carefully, especially when handling unstable genotypes. A redeeming feature, however, is that in unstable plantain clones, variations seem to occur in both directions along a series of true-to-type and off-type characters (as in the cv 'Bise Egome 2', Table 6), thereby conserving the (inherently unstable) nature of the genotype.

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VARIACION SOMACLONAL EN PLATANOS (*MUSA* SPP. GRUPO AAB) OBTENIDA DE CULTIVO DE MERISTEMOS.

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RESUMEN - La naturaleza y la importancia de las variaciones somaclonales en la micropropagación del plátano, así como los factores que influencian su incidencia son estudiados. Grandes diferencias existen entre los cultivares en cuanto a la frecuencia y la naturaleza de las variaciones fenotípicas en las plantas regeneradas después del cultivo *in vitro*. Una gran parte de esas variaciones imita la variabilidad natural y las variantes aparecen esencialmente como copias de otros cultivares de plátanos. Las características afectadas por esas modificaciones son la morfología de la inflorescencia y la fertilidad femenina, la forma del fruto, el color del pecíolo, del seudo-tallo así como la altura de la planta. Se notan en ciertos casos, anomalías del follaje. Muchas de las variantes fenotípicas se presentan estables durante varios ciclos de propagación, haciendo pensar que una gran parte de esta variación es de origen genético. No se ha establecido una relación entre el fenotipo y el tiempo de cultivo. Esas variaciones tienen tendencia a ser más frecuentes con el aumento de las tasas de multiplicación. El análisis de identidad del origen de las plantas regeneradas muestra que la variación aparece *in vitro* lo que haría suponer que el ambiente provoca un stress, inductor de cambios. La gama de las variaciones somaclonales es muy estrecha para poder ser de utilidad en el mejoramiento del plátano. Por su fertilidad femenina aumentada, la variante del tipo «inflorescencia de French Plantain» podría ser interesante para hibridar el Falso Cuerno del cual ella misma proviene, y que es altamente estéril.