

Banana breeding: polyploidy, disease resistance and productivity.

R.H. STOVER and I.W. BUDDENHAGEN*

AMELIORATION DU BANANIER : POLYPLOIDIE, RESISTANCE AUX MALADIES ET PRODUCTIVITE.

R.H. STOVER et I.W. BUDDENHAGEN

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RESUME - L'amélioration du bananier a produit des tétraploïdes à partir de sacs embryonnaires, non réduits, du triploïde Gros Michel et de son mutant semi-nain Highgate pollinisés par des diploïdes *Musa acuminata* sauvages ou améliorés. Après 60 ans d'efforts aucun tétraploïde commercialement utilisable n'a pu être produit ; les tétraploïdes issus du Gros Michel ont un certain nombre de défauts propres dont la sensibilité à la maladie de Panama et aux cercosporioses. Suite aux progrès importants réalisés dans l'amélioration des diploïdes on suggère de synthétiser de nouveaux triploïdes à partir de diploïdes ; dans cette optique l'utilisation des techniques *in vitro* permettant de manipuler le potentiel génétique du genre *Musa*, paraît très prometteuse.

AVANT PROPOS

Les productions bananières (bananes douces, plantains et bananes à cuire) sont actuellement confrontées à de graves menaces parasitaires : Cercospora noir, maladie de Panama ...

L'amélioration génétique représente certainement une des seules stratégies d'adaptation qui puisse permettre, à terme, grâce à l'obtention de variétés plus tolérantes ou résistantes aux maladies, de maintenir ces productions essentielles pour les pays des zones intertropicales.

Devant l'urgence des problèmes, des axes prioritaires doivent être dégagés et une collaboration internationale développée. Cette prise de conscience, à l'échelle internationale, de la nécessité d'unir des efforts pour intervenir plus rapidement et plus efficacement, a été un des éléments décisifs dans la création du réseau international pour l'amélioration des Bananiers et des Plantains (INIBAP).

Un des premiers volets de cette collaboration consiste à établir un bilan critique des travaux réalisés dans le monde depuis le début du siècle, de faire le point des connaissances et de fixer les grands axes de recherches compte tenu des nouvelles problématiques, mais aussi des progrès récents réalisés dans le monde de la biologie.

* - Plant Pathologist, United Brands Company, La Lima, Honduras.
Professor, Department of Agronomy and Range Science, University of California, Davis.

C'est une contribution à ce premier volet que nous proposons Dr R.H. STOVER et Dr. I.W. BUD-DENHAGEN dans la présente publication. Nous tenons à les remercier, en espérant que leur initiative sera suivie par d'autres contributions, complémentaires ou contradictoires, ouvrant ainsi la voie à une réflexion constructive, orientée vers la mise en place d'un programme cohérent et efficace et mobilisant l'ensemble du réseau international.

J. GANRY

INTRODUCTION

Less than one-half of the banana clones are diploid and almost all the remainder are triploid. The diploids are utilized in some local production, but on a relatively small area. Triploids have been much more successful than diploids because of greater productivity, vigor, sterility and range of useful variability. During the past 60 years, the target in banana breeding has been to obtain a useful tetraploid from an initial addition to an unchanged triploid Gros Michel or a shorter version of it, of an «n» genome from a diploid. For several reasons, we believe the failure to obtain useful bred varieties has been due to an unwavering emphasis on this single strategy. However, tetraploidy may be the major evolutionary pathway between diploidy and triploidy in *Musa*. If so, tetraploidy could well be used for the purpose of breeding triploid varieties. Methods must be found to efficiently produce large numbers of triploids for selection of agronomically useful and resistant genotypes. This will necessitate new approaches to polyploid breeding including attempts at *in vitro* manipulation of the genetic potential of *Musa*. Here we examine diploids, triploids, tetraploids and tetraploid breeding and indicate why traditional tetraploidy is unlikely to yield clones that will replace comparable triploids.

Banana breeding began in 1922 at the Imperial College of Tropical Agriculture in Trinidad and in Jamaica in 1924. A program continued in Jamaica, with sometimes sporadic support, up to about 1980.

The United Fruit Company initiated an abortive breeding program in the late 1920s and began again vigorously in Honduras in 1960. The objective of all these programs was only to obtain, at first, a fusarial wilt (Panama disease) resistant Gros Michel, and later, resistance also to Sigatoka leaf spot, in a dessert banana for the export trades, to compete with the fusarial wilt resistant Cavendish group. Over the years, as the virtues of shorter plant stature became apparent, the standard to beat became shorter mutants of Cavendish (now Grand Nain) which have high agronomic productivity, and high resistance to fusarial wilt in the tropics. During this period and up until very recently, the problems of starchy plantains and bananas and the need for clones useful to small holders were not addressed by breeding.

About 1980 the chief breeder in Jamaica moved to Brazil where the primary emphasis is the improvement of the AAB dessert groups.

The very recent concern for a broader approach to breeding in *Musa* has been generated by four major factors : 1) The appearance in Central America (1972), Africa (1973) and Colombia (1981) of a more virulent form of Sigatoka leaf spot, termed black Sigatoka or black leaf streak, which also affects plantains ; 2) The increasing realization by the major donors to the International Agricultural Centers (IARC) that plantains and other cooking bananas are a major basic food source in many third world countries and the plantains and bananas had not been included in earlier IARC food crops ; 3) The appearance of fusarial wilt in Taiwan, South Africa and Australia which attacks Cavendish clones earlier considered to be immune. A new race of the pathogen is involved, and if this should develop in tropical regions in additions to subtropical, the great international banana trades would be endangered ; and 4) The realization by the major banana company which had invested in banana breeding that new superior varieties could not be protected by patents and therefore their breeding programs, without potential proprietary value, should be supported by governments and international agencies generally, for the welfare of all.

BREEDING SCHEMES

Nearly all banana breeding has been based on the approach of maintaining intact the genomes of Gros Michel or its shorter mutant Highgate and adding to it resistance genes from a diploid male parent. Gros Michel and Highgate produce a few unreduced eggs ($2n=3x=33$) (MENENDEZ and SHEPHERD, 1975 ; ROWE, 1984) which can produce seed upon pollination. This is in contrast to the members of the Cavendish group, which would be more desirable as female parents because of their fusarial wilt resistance, but which remain completely female sterile. By using Highgate, offspring have been obtained which are of intermediate height, resembling the Cavendish varieties Valery and Poyo.

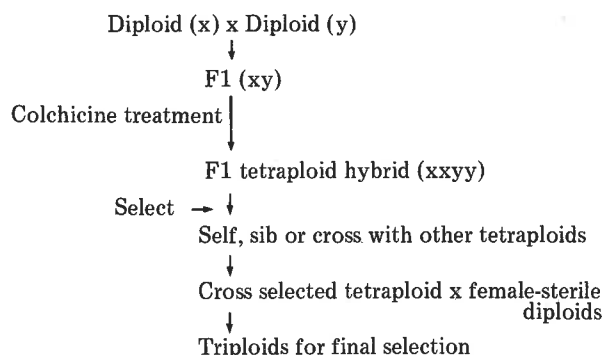
At first, wild diploids of *Musa acuminata* subsp. *malaccensis* were used as males. To improve the agronomic characteristics of the male, diploid breeding programs were initiated in Trinidad, Jamaica and Honduras that resulted in greatly improved bunches (see Diploid section). These diploids ($2n=22$), crossed onto Highgate, produce only tetraploids ($2n=44$) and a few heptaploids (see Tetraploid section).

The major defects of this triploid x diploid approach are : (1) the low level of fertility of Highgate results in an average of only 1-3 seeds per pollinated bunch ; (2) the large contribution of the agronomically inferior and disease susceptible Highgate (when compared to the best Cavendish clones) to the tetraploid karyotype ; (3) the poor agronomic characteristics of the tetraploids when compared to the present commercial Cavendish clones, including an inherent defect in foliage architecture ; (4) the «dead end» target of a primary cross, in relation to further breeding and segregation ; and (5) the possibility of producing an occasional hard seed in the fruit since tetraploids undergo normal meiosis and produce abundant pollen.

When the primary tetraploids are crossed with the improved diploids, secondary triploids are produced. These were long considered to be useless (SIMMONDS, 1966), and the triploid approach to improving bananas received almost no attention until about 1980. Now that much superior diploids are available, and because of the inherent defects of tetraploids, triploid breeding schemes are being reconsidered.

A more fundamental problem with the tetraploid breeding strategy as proposed and followed is that it precluded recurrent selection and indeed precluded secondary cycles of crossing except at the diploid level for the single «n» donor. Thus, it relied upon a triploid clone selected in prehistory, with unknown antecedents, as the perfect clone except for disease resistance. Disease resistance genes were to be added in a single dose (n) to a 3n susceptible, forcing the need for both complete dominance and high penetrance of those genes and requiring the absence of negative effects of all other genes from the male. Moreover, by relying only on a primary cross, nothing could be learned of the genetics of the female parent. Even if one obtained the perfect clone, there would be no place to go if its resistance broke down or if new diseases appeared or new agronomic needs arose.

VAKILI (1967) found that tetraploidy could be readily induced with colchicine in *M. balbisiana* and *M. acuminata*. He proposed a colchicine induced tetraploid breeding scheme for producing tetraploids.



In contrast to the 3n x 2n method, which produces very few tetraploid hybrids, the proposed method could mass produce tetraploids from various improved diploids. These could be screened rigorously for disease resistance and all other characters and even undergo repeated crossing and selection cycles, as in diploids. The best could be crossed with superior diploids to yield triploids in large numbers. By having large numbers of triploids from diverse crosses available, and with rigorous disease screening at the seedling stage, the chances for obtaining a superior triploid clone would be greatly increased. The best triploids could even be reused as the present Highgate is used, for obtaining a new tetraploid for hybridizing with a superior diploid in a recurrent breeding system to initiate new tetraploid x diploid cycles with a triploid target. The basic scheme proposed by VAKILI, and the additions suggested here, have never been tried.

However, colchicine doubling and other forms of somatic doubling may be detrimental and results often have been disappointing (SANFORD, 1983). Heterozygosity is not increased, and homozygosity is increased by having more redundant alleles. Nevertheless, it is now realized that polyploid breeding can be more successful if a strategy is employed which builds up heterozygosity after the primary doubling. It seems clear that polysomic polyploidy (homologous genomes with random intergenomic pairing in meiosis) is likely to be successful only when it results in an increase in heterozygosity relative to related diploids (BINGHAM, 1980). This requires repeated crossing cycles.

BREEDING AND DISEASE RESISTANCE

Diseases and Sources of Resistance.

Important banana and plantain varieties are attacked by four major pathogens throughout the world's tropics (Table 1). By far the most serious of these pathogens is *Fusarium oxysporum* f. *cubense*. Varieties attacked by this pathogen must be replaced by resistant varieties. There are three races attacking dessert and cooking bananas and one race attacking *Heliconia* (Table 2). Race 4 of the fusarial wilt pathogen includes Race 1 virulence but it also attacks the Cavendish varieties ; so far it is destructive only in the subtropics. Thus far, the widely grown AAB plantains are immune to all four races.

The second most serious disease is leaf spot caused by *Mycosphaerella fijiensis*, including black Sigatoka and black leaf streak. In contrast to fusarial wilt, this disease causes severe defoliation but does not destroy the plant. In most areas some fruit is produced although yield can be reduced more than 50 percent, making production uneconomical. Varieties resistant to *M. fijiensis* (causing black leaf streak and black Sigatoka) are also resistant to *M. musicola* (causing Sigatoka, known also as yellow Sigatoka).

The recent appearance of black Sigatoka in Central

America and Colombia has greatly increased the cost of fungicidal control (5 to 10 fold) in plantation bananas. Moreover, this new form of Sigatoka attacks Horn plantains (AAB), previously affected by Sigatoka only at altitudes of 500-1000 meters, and it has severely affected plantain production in Central America and Colombia. This is a major setback for economical food production with this formerly low-input, easily-grown plant. Thus, the appearance of black Sigatoka and its threat to other areas poses added incentive for breeding not only commercial dessert bananas, but also plantains. Resistance is now badly needed by small holders and producers for in-country consumption, as well as for the export trades.

Bacterial wilt of bananas, also known as Moko disease, is more limited in distribution, being indigenous in Central and South America (on Heliconias) and introduced into Mindanao in the Philippines (BUDDENHAGEN, 1968). However, it can be devastating if sanitation control is not rigid, and the insect disseminated strain threatens production of some ABB cooking bananas, from where the disease is easily spread into commercial bananas.

The burrowing nematode (*Radopholus similis*) can reduce banana and plantain yields markedly in some soils, and other nematodes also can be damaging. Nematode resistance would be very useful especially as nematicide use becomes increasingly threatened due to environmental toxicity concerns.

Sources of resistance to the major diseases have been identified under Honduras conditions, but these are very limited in numbers (Table 1).

Tetraploid clones have been produced in Jamaica and Honduras with high resistance to Races 1 and 2 of fusarial wilt and moderate resistance to leaf spot. The first tetraploid clone released (IC2), produced in Jamaica around 1930, succumbed to fusarial wilt when grown in Honduras. The second clone (Bodles Altafort) is reported to be attacked by Race 2 of fusarial wilt in India (STOVER and SIMMONDS, 1987).

Two promising tetraploids from Honduras, resulting from a cross between Cocos (a semi-dwarf mutant of Gros Michel) with Pisang Lilin on the diploid side, resistant to fusarial wilt in Honduras, were sent to Taiwan for testing

TABLE 1 - Sources of resistance in Honduras to major world-wide diseases of *Musa*.

Pathogen	Resistance Sources
<i>Fusarium oxysporum</i> f. <i>cubense</i> (fusarial wilt, Panama disease) Race 1 Race 2 Race 4	<i>M. acuminata banksii</i> <i>M. acuminata errans</i> Diploid SH 3142
<i>Mycosphaerella fijiensis</i> (black Sigatoka, black leaf streak)	<i>M. acuminata</i> subsp. <i>burmannica</i> Diploid SH 2989
<i>Radopholus similis</i> (burrowing nematode)	<i>M. acuminata</i> 'Pisang Jari Buaya' Diploid SH 3142
<i>Pseudomonas solanacearum</i> (Moko, bacterial wilt)	Diploid SH 669

Notes : Some accessions of *M. acuminata* are resistant to Race 1 of *Fusarium*, others are susceptible or segregate for susceptibility on crossing. Race 4 data are from artificial inoculations in South Florida. Other data are from VAKILI (1965), ROWE (1984) and unpublished.

TABLE 2 - Known races of *Fusarium oxysporum* f. *cubense*.

Race	Host or Varieties attacked
Race 1	Gros Michel (AAA), Apple (AAB), Silk (AAB), Taiwan Latundan (AAB), IC2 (AAAA)
Race 2	Bluggoe (ABB) and close relatives, some Jamaica tetraploids (AAAA)
Race 3 *	<i>Heliconia</i> spp. in Honduras
Race 4 **	All Cavendish varieties (AAA), Taiwan Latundan (AAB), Gros Michel (AAA), Pisang Lilin (AA), Bluggoe (ABB).

* - WAITE, 1963.

** - HWANG *et al.*, 1980.

in an area where Cavendish varieties were devastated by Race 4. Both clones were susceptible (HWANG *et al.*, 1980).

Cavendish varieties are the basis of all the export trades and there is no known adequate substitute variety if they succumb to a new race of fusarial wilt in the tropics. At present, Cavendish varieties are being attacked by fusarial wilt only in the subtropics (Taiwan, Southern Queensland, South Africa). Preliminary screening of breeding material using the Taiwan fungus, designated as Race 4, was carried out in containers in South Florida (Table 3). Results indicate that the diploid SH 2095, widely used in the Honduras

of vertical or horizontal resistance, or to know the meaning of «races» in this host-pathogen system. It should be recognized that the word «race» used in this context has no genetic meaning, unlike «races» in rusts, determined by specific genes for resistance in differential hosts.

What is apparent from the limited information is that «Race 4» from Taiwan has additional virulence to the tropical wilt *Fusarium* from Honduras since it readily attacks a diploid used as a resistant parent in Honduras. With the present availability of diploids it should be possible to establish differentials to each race and to carry out crossing and determine the genetics of resistance.

TABLE 3 - Reaction of four genotypes resistant to Race 1 to Race 4 of *Fusarium oxysporum* f. *cubense* in containers in South Florida.

Variety	Infection index	Rating
SH 2095	28	Highly susceptible
Grand Nain-Valery	10	Susceptible
SH 2989	4	Moderately resistant
SH 3142	0	Resistant

Notes : Rhizome infection 70 days after inoculation classified as light = 1 ; medium = 2 ; and severe = 3. Ten plants of each variety were inoculated and the maximum infection score is 30. The three SH clones are diploids bred for resistance to *Fusarium* in Honduras. All 4 entries were derived, for this experiment, from rhizomes.

breeding program, is susceptible, whereas the diploid SH 3142 is resistant. Much more widespread testing is needed in Race 4 areas to identify other sources of resistance to this highly destructive race that could evolve in the tropics.

The three genomes of fusarial-wilt susceptible Gros Michel in tetraploids, and the eventual appearance of fusarial wilt in many tetraploids, indicates horizontal resistance (ROBINSON, 1973) or tolerance (BUDDENHAGEN and DE PONTI, 1983) is not at a high level in Gros Michel-derived tetraploids. Cavendish varieties have been considered to have remained resistant to fusarial wilt due to horizontal resistance. However, Cavendish varieties are devastated in some subtropical countries by Race 4 of fusarial wilt, a strain with a broadened host range, since it can also attack Gros Michel, Bluggoe, Pisang Lilin and Latundan. In Taiwan, young Cavendish plants are infected without being subjected to the several months of cold stress occurring in the subtropics. Race 4 was probably introduced into tropical Mindanao on rhizomes imported from Taiwan in the 1960s since fusarial wilt persists at a chronically low level on Cavendish varieties in certain localized areas in Mindanao. This is the only area in the tropics where localized cases of fusarial wilt can be found on Cavendish varieties. This suggests Race 4 is not epidemiologically competent in the tropics. However, in the absence of comparative pathogenicity tests in the tropics and subtropics with the different so-called races, and without work on the genetics of resistance, it is impossible to resolve questions

With a diploid breeding program and a coordinated screening program involving locations with the different known races and stresses, it should be possible to specify and recombine the genes conferring resistance. Once the genes are identified, the abundant triploids derived from the scheme proposed above could be assessed for presence of the recombined genes. Research needs to be carried out to determine the similarity of the pathogens lumped into «Race 4» from such wide ranging locations as Taiwan, South Africa, and Queensland. They could well have different genes for virulence.

It is a major mystery why the Cavendish group remains resistant in the tropics, on many thousands of hectares of soils infested with the old Gros Michel pathogen population. Probably much is to be learned by studying fusarial wilt/genotype interaction in Asian areas such as Thailand where great host genotype variability has evolved. Probably more «races» of *Fusarium* exist there.

Varieties highly resistant to *Mycosphaerella musicola* (common Sigatoka) also show some resistance, although at a lower level, to *M. fijiensis* (black Sigatoka). High levels of resistance to *M. fijiensis* have been incorporated into advanced diploids in Honduras. However, on crossing with Highgate to produce tetraploids, the resistance is eroded to some extent, although it remains much higher than in Cavendish varieties. It is not known if this resistance will be sufficiently stable to be maintained when these tetraploids are challenged by the pathogen over a wide geogra-

phical range. The breeder concerned with plantains must now consider black Sigatoka resistance as a major target. The genetics of resistance in various diploids needs to be determined so that different genes, if they exist, may be combined. With this easily-disseminated aerial pathogen with its common sexual stage, the development, selection and spread of new more virulent races may occur readily following deployment of «resistant» clones. The rapid spread of black Sigatoka in Central America and elsewhere indicates the potential. Since the pathogen has a sexual stage, the study of genetics of virulence/avirulence and resistance/susceptibility (in diploids) should be an ideal and important research objective. By concentrating breeding on deriving triploids from diploids, determining the genetics of host/pathogen resistance and virulence genes and their number, in relation to Sigatoka, black Sigatoka and potential races, becomes a realizable goal. It is important to be able to recombine the different resistance genes through repeated crossing and accurate testing.

High levels of resistance to the burrowing nematode have been identified and transferred to improved diploids, including SH 3142 (PINOCHET and ROWE, 1979). However, some of the diploids resistant to *Radopholus similis* were susceptible to *Pratylenchus coffeae* (PINOCHET and ROWE, 1979). *P. coffeae* is not a serious pest of AAA bananas, but it does cause considerable damage to plantain roots. *P. goodeyi* attacks Cavendish varieties in some subtropical areas and other varieties in Tanzania. The sources of resistance to the burrowing nematode are few and since these have not been tested on extensive areas their stability when confronted with the variable pathogen remains unknown.

Screening for resistance to fusarial wilt.

Screening for resistance to the three races of fusarial wilt is the most crucial first step in evaluating a potential new variety. Apparently, early screening procedures were not adequate, as some of the first supposedly resistant tetraploids were later attacked by fusarial wilt. Also, prior to 1965 the importance of Race 2 was not realized, and Race 4, as a potential threat to new varieties, began to be considered only about 1980.

There are two basic systems of screening : (1) rhizomes planted in the field with or without artificial inoculation and (2) seedlings, small rhizomes, or *in vitro* meristem plants planted in containers with artificial inoculation. Up to 1965, the Jamaica program screened tetraploids by planting at sites where a diseased Gros Michel plant was removed (SIMMONDS, 1966). The clone was discarded if more than two of ten plants became diseased. If there were only one or two diseased plants, the clone was maintained but closely observed for subsequent reaction to fusarial wilt.

In Honduras, rhizomes of the tetraploid clone to be tested are planted in holes in which chopped tissue from plants infected with Races 1 and 2 has been added. It

requires 18-24 months to evaluate disease reaction (ROWE and RICHARDSON, 1975).

When Race 2, and possibly another unidentified race, was detected in Jamaica, screening was augmented by pot tests under controlled conditions in a glass house (MENENDEZ and SHEPHERD, 1975). However, pot tests did not always agree with tests on older plants in the field. Field sites had to be prepared in which infectivity was made very high and uniform by growing successive stands of diseased, highly susceptible varieties. Even then, it was stated that resistance to Panama disease is not clear-cut and clones were classified on the frequency and extent of overt symptoms or of internal corm symptoms on outwardly healthy plants. In each case these were compared with standard reference clones (MENENDEZ and SHEPHERD, 1975).

SHEPHERD and LACY (1968) screened diploid seedlings in flats heavily inoculated with a spore suspension of the pathogen. This was considered necessary because tetraploids produced from routinely screened diploids had demonstrated an insufficient level of wilt resistance. According to SHEPHERD and LACY, the apparently polygenic resistance of the diploids was clearly insufficient. They found most wild clones showed resistance or high resistance to Race 1, with susceptibility or high susceptibility to Race 2. Two Borneo accessions were susceptible to both races. Only one clone from Annam was highly resistant to both races.

VAKILI (1965) also screened seedlings in flats. His techniques were the most severe of all tests described. First, the seedling roots were immersed overnight in a microconidial spore suspension and then transplanted to flats. After one month, the surviving seedlings were re-inoculated by infesting the soil and cutting the seedling roots *in situ*. This technique greatly increased the amount of infection. Even so, about 10 percent of the screened seedlings succumbed to disease in the field compared with 70 percent of unscreened seedlings. There was great genetic variability in differential wilt resistance to Races 1 and 2 in *M. acuminata* subsp. *errans*, *banksii* and *microcarpa* and among hybrids. *Musa balbisiana* was the only species tested which displayed seedling susceptibility and mature plant resistance.

SUN and SU (1984) have tested the reaction of small meristem-derived plants to Race 1 and Race 4 in 150 ml breakers containing a mixture of decomposed tree fern tissue and sand. Plants were uprooted from the breakers, washed with tap water and placed in a conidial spore suspension for one minute. The inoculated plants were replanted and incubated at 28°C in a growth chamber. Most plants showed external symptoms of leaf yellowing within two weeks and collapse and death of leaves and pseudostem occurred within four weeks. With this technique, the differential reaction of Cocos (a short Gros Michel) and Cavendish varieties to Race 4 (and not to Race 1)

was readily established. SUN and SU stated that tissue-culture plants are promising material for screening for wilt resistance.

HWANG *et al.* (1984) have mass-produced *in vitro* more than one million plants of a Cavendish variety in Taiwan for use by farmers and for research. These plants show up to five per cent somaclonal variation (see table 10). HWANG set up a mass-screening program for detecting resistance to Race 4 by planting the *in vitro*-produced plants in a nursery soil heavily infested with diseased tissue. After 3-4 months, depending on seasonal temperature, the surviving plants are dug up and the rhizomes examined for infection. Those free of infection are again multiplied *in vitro* for additional tests. Of more than 13,000 plants screened thus far 17 or 0.12 per cent have remained healthy (personal communication from S.C. HWANG).

Clearly, with such a variety of screening techniques, a comparative study of screening methods is needed for all three races of *Fusarium oxysporum* f. *cubense* and for different clones within each race. The use of meristem plants would greatly speed up the field screening process from more than a year to a month or two, provided the field and container tests give comparable results. It would appear that with a severe container test, the number of escapes would be less than in a field test, but to what degree useful field resistance might be discarded by any particular seedling test can only be established by research.

The problem of realistic screening has many facets, and for a systemic soil-borne pathogen such as *Fusarium oxysporum* and a large perennial plant such as banana, it is especially difficult.

First, resistance is an expression of tolerance (BUDDENHAGEN, 1981, 1983). The roots are invaded, the pathogen reaches the xylem and the degree of its proliferation and systemic spread determines the degree of symptom expression and our interpretation of resistance or susceptibility. With the development of an extensive corm in mature plants, potential barriers are developed which may not be so effective in seedlings.

The expression of resistance with systemic invasion in bananas is especially vulnerable to modification by environmental conditions affecting defense mechanisms, and by the inoculum dose. Temperature, nutritional status, light and soil pH are probably major variables affecting resistance expression. It is already clear that plant age and low temperature stress also influence the expression. Thus, even without considering pathogen variability, the assessment of resistance expression requires considerable standardization and comparative studies between quick, efficient seedling tests and field performance.

Second, is a consideration of the inoculum dose in relation to disease expression. Since the degree of systemicity and blocking of the vessels is the key to resistance/suscep-

tibility evaluation, challenge should not be so severe that it precludes systemicity-blocking reactions. This is probably what occurred where VAKILI cut roots of young seedlings of *M. balbisiana* and immersed them in a concentrated suspension of conidia. The plants became systematically invaded through simple spore uptake, and their normal field reaction of resistance, exerted through limiting systemicity from few invasion sites, could not be expressed. Thus, the need for a realistic challenge, allowing expression of defense reactions, with environmental conditions covering the normal field range.

Third, the fungal pathogen variability in the region where a clone is to be grown must be taken into account. So far, our concept of resistance to fusarial wilt is largely limited to the interaction of breeding material with the fungal population at three limited sites, one each in the small experimental fields in Honduras, Jamaica and Trinidad. It is still uncertain if the original pathogen was introduced into these areas (in seed pieces) but it is highly likely that it was and that much greater variability exists in Asia. Moreover, with the spread and invasion of tropical American soils generally by this pathogen, the probability exists that mutants with different virulence genes and different degrees of aggressiveness exist within the region. The lack of widespread testing of potential parents supposedly resistant is probably a major limitation to eventual success in breeding bananas for resistance to fusarial wilt. The differential performance of IC2 in Jamaica and Honduras and of the «resistant» diploid SH 2095 in Honduras being highly susceptible to the Taiwan fungus are cases in point.

It is highly probable that both major and minor genes control the host/pathogen interactions governing eventual expression of sufficient resistance in this system. It will be a major challenge of research to develop screening procedures which reveal the minor as well as major genes. Past banana breeding programs have been deficient in not determining the genetics of host/pathogen interaction and of resistance genes with specific pathogen races. If one is considering a recurrent selection program based on diploids for resynthesis of the ideal triploid, the genetics should be straightforward.

It should be recognized that we know essentially nothing of the virulence relationships of the four known races. No virulence genes have been identified and comparative tests with diploid differentials which could be intercrossed for straightforward genetic analysis are almost entirely lacking. So, the word «race» here is used in a quite different way from its use, for resistance, in wheat rust race terminology, where races are defined on the basis of known numbers of different virulence genes. What can be inferred from the limited information, however, is most significant in that Race 4 is virulent to both Gros Michel, Cocos and Cavendish clones (SUN and SU, 1984). Thus, it appears to be a derivative of race 1 with a new additional virulence gene (s), selected naturally in Taiwan by the planting of

Race 1-resistant Cavendish varieties. The presumption is strong, with the appearance of Cavendish-attacking fusaria in three widely separated sub-tropical locations, that Race 1 can acquire additional virulence gene(s) and these can be selected for by formerly-resistant Cavendish where it is grown under seasonal cold-stress. The key question is whether these new forms with additional virulence genes will be environmentally competent in the tropics. It is of interest that Race 2, which appeared in Honduras and elsewhere on ABB Bluggoe, is not also carrying the virulence gene(s) of Race 1. Although it would appear to have been derived from Race 1 on the basis of origin, it has lost Race 1 virulence. The logical inference is a mutation of the Race 1 virulence gene itself. This has occurred independently at several locations (WAITE, 1977).

With bananas, where replacement of clones is a major and expensive undertaking, it is imperative that the answers on resistance evaluation and genetics of resistance/virulence be correct.

DIPLOIDS

With respect to the Jamaican breeding program, no published literature or reports are available on the diploids used for producing tetraploids during the last 15 years. The two tetraploids released prior to 1970 were from *Musa acuminata* subsp. *malaccensis* (IC2) and Pisang Lilin (Bodles Altafort), crossed with Gros Michel.

The Honduran diploids are listed in Table 4. The three most important are SH 2095, SH 3142, and SH 2989. SH 2095 has the best agronomic features (large bunch, long fingers) but is susceptible to black Sigatoka and Race 4 of fusarial wilt. SH 3142 incorporates the burrowing nematode resistance of Pisang Jari Buaya and is resistant to black Sigatoka and Race 4 of fusarial wilt. SH 2989 is a source of high level *burmannica*-derived resistance to black Sigatoka. Almost all diploids are grown in Race 1 infested soil and have been tested for resistance to Race 2. None have been tested yet for resistance to Race 4 under

TABLE 4 - Diploids used as males in banana breeding.

Clones and year developed	Origin and salient features
TRINIDAD-JAMAICA	
<i>Musa acuminata</i> subsp. <i>malaccensis</i>	Wild diploid resistant to fusarial wilt and leaf spot in Trinidad, crossed with Gros Michel, and IC2 (AAAA) selected.
Pisang Lilin (1940s)	Wild edible diploid resistant to fusarial wilt and leaf spot ; crossed with Gros Michel ; Bodles Altafort (AAAA) selected
HONDURAS	
SH 2095 (1973)	(Sinwobogi x Tjau Lagada) x (wild <i>malaccensis</i> x Guyod) susceptible to black Sigatoka ; excellent agronomic features ; poor pollen
SH 2989 (1976)	<i>burmannica</i> -derived resistance to black Sigatoka
SH 3142 (1977)	Resistance to burrowing nematode and black Sigatoka from Pisang Jari Buaya
SH 3105 (1977)	SH-2095 x SH 2741 ; dwarf character from SH 2741.
SH-3176 (1978)	SH 2095 x SH 2989 ; <i>burmannica</i> -derived resistance to black Sigatoka
SH 3248 (1979)	SH 3142 x SH 2989 ; slightly susceptible to black Sigatoka
SH 3249 (1979)	SH 2095 x SH 3049 ; dwarf
SH 3217 (1979)	SH 2095 x SH 2766 ; excellent agronomic features
SH 3273 (1980)	SH 3142 ; SH 3026 ; black Sigatoka resistant
SH 3350 (1980)	SH 3142 x SH 3176 ; black Sigatoka resistant
SH 3351 (1980)	SH 3142 x SH 3176
SH 3352	
SH 3354	
SH 3358	
SH 3359	
SH 3362 (1981)	SH 3142 x SH 3217 ; high level resistance to black Sigatoka
SH 3371 (1981)	SH 2095 x SH 3142 ; excellent agronomic features
SH 3393 (1981)	SH 3142 x SH 3217 ; high level resistance to black Sigatoka
SH 3397 (1981)	SH 3142 x SH 3217 ; high level resistance to black Sigatoka
SH 3320 (1981)	SH 3142 x SH 3180 (derived from SH 2989) ; immune to black Sigatoka
SH 3437 (1983)	SH 2989 x SH 3217 ; high level resistance to black Sigatoka

field conditions in the subtropics. All the diploids are subject, in recent years, to challenge by natural infection by black Sigatoka in Honduras. However, none of the diploids has been widely tested. In fact, they are hardly examined outside the breeding plot in Honduras (or Jamaica). Thus, their general adaptability, or their weaknesses to other pathogens, other races of pathogens, to insects, and to other factors occurring naturally outside the breeding plots, are not assessed. The intent has been for wider assessment only of the tetraploids to be derived from crossing the diploids onto the triploid Highgate. But this wider assessment of tetraploids has also not been carried out because none has been considered competitive to Grand Nain on a commercial scale at the large company plantation level, although they have not been so tested.

The Honduran program (and the Jamaican) has thus carried out recurrent selection breeding at the diploid level, and the improvement over natural diploids has been very considerable. The program in Honduras is now generally at the 5th cycle for diploids and much effort now is to use the best diploids as male parent with Highgate to obtain tetraploids with an intact Highgate genome ($2n=3x=33$) plus an n genome from the tetraploid.

Although the Honduras program has produced many excellent diploids, it has only recently released the first tetraploid (SH 3436) derived from the cross Highgate x SH 3142. This clone is available to the public and is being tested for resistance to Race 4 of fusarial wilt in Queensland, Taiwan, and South Africa ; it is not being planted commercially as a replacement for Grand Nain.

TRIPLOIDS

Triploids comprise all the major clones that are widely cultivated. SIMMONDS (1966) lists only two diploids (AA and AB) as common dessert bananas. Only a few natural tetraploids have been recorded and none are much cultivated (RICHARDSON *et al.*, 1965 ; VAKILI, 1967 ; SHEPHERD and FERREIRA, 1982).

From the breeding standpoint, the only important triploids have been Gros Michel and its shorter mutant Highgate. Gros Michel was devastated by fusarial wilt and replaced with Cavendish varieties for the export trade in the 1950s and 1960s. At first, intermediate-height clones (Valery, Giant Cavendish) were used, later, the semi-dwarf Grand Nain. Since semi-dwarf Cavendish varieties are now the standard for export in Central America, and Highgate has been the vehicle that hopefully could be altered to reach that standard, we will confine our discussion to these varieties. Highgate was never grown as a commercial variety because of short fingers and a somewhat smaller bunch compared with Gros Michel. It was selected by plant breeders to reduce the height of tetraploids derived from Gros Michel.

The Cavendish varieties tested for seed fertility by pollinating with pollen from diploids have never yielded seed. Such pollination attempts were most extensively done with the variety Valery, where a few hundred bunches were pollinated. Several other Cavendish clones have been pollinated to a more limited extent, with no success. It is this apparent seed sterility (without any research to determine or overcome the blocks) that has precluded the use of the «ideal triploids» as parents. The only exception to the absence of Cavendish input into banana breeding was the successful yield of seed from a wild *Musa acuminata* subsp. *malaccensis* when pollinated with Valery pollen in 1964. Several dwarfs were obtained (the dwarf character from Valery) and these were used further in diploid breeding and are now in the pedigree of all the advanced dwarf diploids.

Because of the female seed sterility of the Cavendish varieties, all banana breeding, until recently, has depended on Gros Michel, or its shorter version, Highgate, for the contribution of its intact entire genome to a new variety. This has been the most serious obstacle to producing a variety that can compete with the short Cavendish varieties, because they are agronomically inferior as well as carrying susceptibility to the major fusarial wilt race. Moreover, as stated elsewhere, due to the rarity of female restitution, few tetraploid seeds are obtained, limiting the progeny numbers for selection. The superior diploids are undoubtedly highly heterozygous, necessitating large numbers of progenies to explore the potential of the « n » genome to be added to the triploid number. Also, recurrent selection at the tetraploid level is essentially ruled out by this approach.

Some salient plant and yield characteristics of Gros Michel and one of the best yielding Cavendish varieties, Grand Nain, are outlined in Table 5. Several features stand out that make Gros Michel a poor competitor with Grand Nain : massive size with respect to foliage and height, a slow ratooning rate, high losses and low yields. STOVER (1982) proposed that Grand Nain should be the export banana ideotype. In addition to agronomic defects, tetraploid progenies derived from present breeding schemes will be carrying three genomes from the fusarial-wilt susceptible and Sigatoka susceptible female. This does not bode well for durability (JOHNSON, 1984) of resistance to fusarial wilt and Sigatoka, as these pathogens are pressured to develop new races if the new tetraploids are introduced and planted on a wide scale.

The architecture of Gros Michel suggests that in partitioning, the foliage and pseudostem receive a much greater proportion of the assimilates than in Grand Nain. This is precisely so ; 27 per cent of the dry matter at harvest of Gros Michel and Highgate is composed of fruit compared with 40-44 percent for Grand Nain (Table 6). Gros Michel and Highgate are poor representatives of an ideal triploid with respect to plant architecture, partitioning, yield and susceptibility to disease.

TABLE 5 - Some plant characteristics of ratoon crops of Gros Michel and Grand Nain.

	Gros Michel		Grand Nain
	Height, cm	658 567	(HARTMAN) (BUTLER)
Adult leaf, length x width, cm	316 x 85 343 x 110	(HARTMAN) (BUTLER)	224 x 93
Adult leaf size m ²	2.10 2.80	(HARTMAN) (MOREAU)	1.67
Foliage per plant at shooting m ²	28.7 38.4	(HARTMAN) (MOREAU)	16.9
No. leaves at shooting (excluding bract leaf)	13.7	(MOREAU)	11.5-12.5
Monthly growth rate, cm	42.4 46	(HARTMAN) (BUTLER)	21.8
Ratooning rate (peeper to shoot), days	573		236
Shooting in May to harvest time, days	90.5 98-104	(HARTMAN) (BUTLER)	91.5
Yield potential (no losses) 18.14 kg boxes per ha	1962		3700
Average field losses %	34		10
Population ha	700-900		1800-2000
Fusarial wilt resistance			resistant
Race 1	susceptible		field resistant
Race 4	susceptible		in tropics

Notes : Gros Michel data from HARTMAN (1929), BUTLER (1960) and MOREAU (1965). Grand Nain data from STOVER (1979, 1982, 1986), STOVER and SIMMONDS (1987). Grand Nain shoot to harvest time is for Grade 14 (1 $\frac{14}{32}$ inch) harvested in May.

TABLE 6 - Dry matter partitioning at harvest (per cent) in Grand Nain, Gros Michel and Highgate.

	Grand Nain		Gros Michel	Highgate
	Cameroun	Honduras	Honduras	Honduras
	Foliage	16.8	12.1	18.7
Pseudostem	22.2	23.9	36.9	34.4
Rhizome	10.5	16.9	13.0	19.7
Roots	-	1.0	3.2	1.3
Fingers	43.7	38.9	27.1	27.5
Suckers	5.9	7.2	-	-

Notes : Foliage includes petioles ; fruit stalk included in pseudostem ; suckers not measured for Gros Michel and Highgate. Grand Nain data from MARCHAL and MALLESSARD (1978) and STOVER (1986) ; Gros Michel data from A.F. BUTLER (unpublished) ; Highgate data from breeding area in Honduras

Up until recently, it has been stated, that there is no approach other than via Gros Michel for producing a banana suitable for the export trades. We consider that it is most unlikely for a variety to come via Gros Michel, or Highgate, that can compete with Grand Nain or other Cavendish varieties such as Poyo, Valery and Giant Cavendish. This indicates that the requirements of the export trades will change only when forced to do so by the demise of the Cavendish varieties. If these varieties are devastated by a new race of fusarial wilt, then, possibly, some tetraploid

that is less-than-ideal could be taken out of storage to fill the void.

However, the increasing cost of black Sigatoka control is also a major force influencing the need for a new commercial banana. This high cost (up to \$900 ha/yr) must be weighed against the potential disadvantages of existing tetraploids. There may be situations where these tetraploids could be immediately useful, such as for local production for internal markets and for home use.

TABLE 7 - Naturally occurring tetraploids.

Group name	Genome	Distribution
Klue Teparod (tiparot, Balonkawe)	ABBB	Luzon (Philippines), Malaya, Siam, Thailand, Burma.
Atan	AAAB	New Britain
Kalamazol	AABB	Solomon Islands
Number of accessions		
11	AAAB AABB	Papua New Guinea
1	ABBB	Papua New Guinea
1	AAAA	Papua New Guinea

Notes : RICHARDSON *et al.* (1965) except Papua New Guinea (SHEPHERD and FERREIRA 1982) ; Papua New Guinea accessions not studied in detail and all may not be tetraploids.

TETRAPLOIDS

Prior to 1965 only one natural tetraploid was known (Klue Teparod). When the 648 accessions of the United Fruit Company were examined cytologically, only six natural tetraploids were found (RICHARDSON *et al.*, 1965). These consisted of three distinct clonal groups including the previously described Klue Teparod (Table 7). In Papua New Guinea, 13 accessions among a little more than 200, were listed as tetraploids ; some of these may be duplicates and some, on cytological examination, may not be tetraploids (SHEPHERD and FERREIRA, 1982). Thus, the few natural tetraploids existing are confined to South East Asia, and are mostly from primitive agricultural areas. RICHARDSON *et al.* (1965) stated that although uncommon, it is not unlikely that many natural tetraploids exist. Those known are mostly tall plants (3-5 m) and have the drooping weak-petioled characteristic of bred tetraploids. Consequently, they are less likely to have been selected for edible types by local peoples in primitive agriculture. It is of interest, however, that only one natural pure *acuminata* tetraploid (AAAA) is known, and this from Papua New Guinea (SHEPHERD and FERREIRA, 1982).

VAKILI (1967) noted that Klue Teparod tetraploids were grown and maintained with care in Luzon because of the pleasant taste. Members of the other two groups (Table 7) were found in semi-primitive cultivations, where they were not growing in competition with the more vigorous and advanced diploid and triploid clones. VAKILI considered tetraploidy in the evolution of the edible banana as either an intermediate transitory phase or a waste product. Since most tetraploids were found in areas where edible cultivars were grown haphazardly in mixture under very primitive agriculture, he suggested that tetraploidy is one of the intermediary evolutionary pathways between diploidy and triploidy. It would appear that where bananas were domesticated, tetraploids did occur ; some still do, mostly where agriculture is in its more primitive stages. However, primitive man did not propagate these tetraploids

widely, opting instead for triploids. Thus, to the primitive farmer/gatherer, triploids were better. In addition to their superior agronomic performance, both under primitive and advanced agriculture, triploids (AAA) would have had the added advantage under primitive agriculture, where highly pollen-viable diploids were present, of absence of seed in the fruit. It would, indeed, be of considerable interest to compare seed occurrence in the interspecific tetraploids and triploids and in the one presumed *acuminata* tetraploid growing in New Guinea and New Britain.

VAKILI (1967) studied the experimental formation of tetraploids using colchicine treatment of the seed of diploid *M. balbisiana* and *M. acuminata* (subsp. *banksii*, *errans*, *microcarpa*). Tetraploids of *M. balbisiana* in comparison with diploids, produced fewer roots, had droopy, fragile leaves, produced fewer suckers that took longer to emerge, were slower ratooning, and had smaller bunches but, usually, larger fruit (Table 8). The most unfavorable characteristic of tetraploids was that they took three months longer than diploids to produce a bunch.

In general, the effects of tetraploidy on *M. acuminata* were similar to those of *M. balbisiana*, with a greater range in variation. The exception was that fruit length was shorter in *acuminata* tetraploids than in the diploid 'parent', whereas for *balbisiana* tetraploids it ranged from 0.5-2, relative to *balbisiana* diploid fruit. Mature primary tetraploids were slow in producing suckers and had thick and drooping leaves which doubled easily, and pseudostems were weak when compared to diploids.

The morphological characteristics displayed by colchicine-induced polyploids of *M. balbisiana* and *M. balbisiana* x *M. acuminata* hybrids led VAKILI (1967) to believe that some supposed ABB varieties such as Bluggoe and Saba were parthenocarpic BBB. The characteristics of component parts of male buds in different polyploid seeded *M. balbisiana* plants closely resembled their counterparts in Bluggoe and Saba. The only missing component was

TABLE 8 - Growth habit of colchicine-induced tetraploids of *Musa balbisiana* compared with diploids (from VAKILI, 1967).

	Diploids	Tetraploids
<i>M. balbisiana</i>		
Roots per 1000 cc rhizome	114	36
Pseudostem		
Height in m	4.3	4.9
Circumference		
1 m above ground (cm)	63	79
Leaf		
Habit	Erect	Drooping
No. doubling per plant at base	0.2	2.0
No. emerged/plant/month	3.8	4.3
Suckers		
No. per plant	5.06	3.0
Emergence time (months)	5.9	8.4
Bunch		
Shooting time (months)	13	15.5
Avg. No. hands	10	8
Fruit		
Relative size	1	0.5-2

parthenocarpy. In a classification of Philippine clones, VALMAYOR *et al.* (1981) have classified Saba as BBB but Bluggoe as ABB, thus adding weight to the hypothesis that parthenocarpy has evolved also in *M. balbisiana*.

Of the edible tetraploid clones produced in the Trinidad-Jamaica program, only three have been evaluated outside of Jamaica. IC2 was attacked by fusarial wilt when planted on a commercial scale in Honduras but to what extent is not recorded. WAITE (1977) classified IC2 as susceptible, with at least 50 percent of the plants wilting. RICHARDSON (personal communication) suggested that the loss of a chromosome segment in IC2 may have been the cause of loss of resistance. Polyploids are known to withstand chromosome losses. IC2 still exists in collections but has had no acceptance as a producing clone. Bodles Altafort was widely distributed in the 1960s. Tetraploids 2390 and Bodles Altafort were recently evaluated in Australia with 24 triploid varieties (TURNER, 1984). The major defects of Bodles Altafort were excessive height (and consequently the highest losses), small bunch, short fingers and a slow ratooning speed (Table 9). Highgate had the slowest ratooning rate of all 24 triploids tested. Tetraploid 2390 was

similar to Robusta (a Cavendish clone) but with a smaller bunch and smaller fingers. It is noteworthy that the ratooning rate of 2390 was similar to Robusta even though Highgate is one of the parents of this tetraploid. Hence, the diploid parent can strongly influence ratooning rate.

In the early 1970s, 27 tetraploids were evaluated in Jamaica and England for fruit quality factors (NEW and MARRIOTT, 1976). No information is available on agronomic characteristics except for one unnamed tetraploid (MENENDEZ and SHEPHERD, 1975). Data presented for the plant and first ratoon crops indicate bunch size, finger weight and yield were not much different between the tetraploid and Valery. However, the interval between the first and second ratoon was 81 days longer for the tetraploid at 1700 plants per ha and 105 days longer at 2000 plants per ha. No tetraploid was identified which did not have an inherent tendency to drop the fingers when ripe. All tetraploid fruit (and Highgate) had weak necks. Thus, it is likely that the trait is inherited rather than a consequence of polyploidy. Inheritance is indicated since, in Honduras, some tetraploid fruit from Highgate as 3n female had strong necks (but agronomic defects).

TABLE 9 - Comparison of two tetraploid varieties with triploid Robusta in New South Wales (from TURNER, 1984).

	AAA Robusta	AAAA 2390	AAAA Bodles Altafort
Height at shooting (cm)	227	250	375
Bunch weight (kg)	19	17	9
Finger weight (g)	120	117	85
Time from planting to harvest of second ratoon (months)	38	38	47

Recently, 31 tetraploid clones were compared with Valery for consumer acceptability in England (BALDRY *et al.*, 1981). Two of the tetraploid clones were similar in acceptability to Valery. Valery, even when fully ripe, was distinctively harder than many of the panel members liked, while the tetraploids tended to be soft.

In summary, no tetraploid generated by the only breeding strategy employed to date, has the ideal quality, agronomic and resistance characteristics required by international commerce.

IN VITRO PROPAGATION AND SOMACLONAL VARIATION

The first *in vitro* culture of banana tissue was reported by COX *et al.* (1960) who grew embryos from *Musa balbisiana* seed. Since then embryo culture is routinely used by banana breeders to increase the viability of embryos obtained in crosses producing few seed. This was followed by culture of free and clumped cells from banana fruit pulp (RAM and STEWARD, 1964). Shoot tip culture (apical meristem) was first reported by MA and SKII (1972). Since then shoot tip or meristem culture has become a routine procedure for the rapid *in vitro* multiplication of banana and plantain clones (CRONAUER and KRIKORIAN, 1983, 1984).

In vitro reproduction of crop plants has shown the potential for producing substantial variability in itself and without mutagens. SNOWCROFT and LARKIN (1982) have called this «somaclonal variation». Cavendish variety banana plants have been produced *in vitro* on a large commercial scale since 1982 for planting projects in Jamaica and Taiwan. Variability, described as somaclonal variation, has ranged from 20 per cent in Jamaica to 5 per cent in Taiwan (personal observations).

The method of *in vitro* reproduction in Taiwan has been described by HWANG *et al.* (1984). Prior to this KAO (1979) subjected *in vitro* plants to gama radiation and obtained mutations affecting stature, pigmentation, leaf shape and size, and suckering. The range of mutants found by HWANG in Taiwan is shown in Table 10. Some are detectable when plants are young and others after flowering. Many of these mutants are being screened for resistance to Race 4 of fusarial wilt (see section on Screening for Resistance to Fusarial wilt).

Growth of banana protoplasts and somatic embryos has been reported (CRONAUER and KRIKORIAN, 1983) but there has been no success as yet in regeneration of plants from protoplasts and embryos.

With the exception of shoot-tip culture, *in vitro* techniques of cell culture and manipulation are in the very early

TABLE 10 - Some somaclonal mutations observed in Taiwan Cavendish bananas mass-produced *in vitro* without mutagens

Stature

- a) Various degrees of dwarfism ; about 65% of mutants are dwarfs with small bunches, short fingers, short petioles and leaves, short internodes, upright leaves with a tendency to «choke throat».
- b) Giantism
Excessively tall plants with long distance between internodes.

Foliage

- a) Drooping leaves, weak petioles, wide spacing of internodes (resemble tetraploids)
- b) Narrow leaves, usually more upright.
- c) Irregular shape to lamina, sometimes tattered or a portion missing.
- d) Shorter, smaller leaves.
- e) Waffled or wavy edges of lamina with changes in leaf thickness.
- f) Increased waxiness.

Plant colors

- a) Darker green or different shades of green.
- b) Rose shades in petiole and leaf sheaths.
- c) Purple to black shades or black spotting in petioles and leaf sheath.
- d) Variegated leaves (shades of yellow and pale green) sometimes resembling «mosaic».

Fruit characteristics

- a) Small bunches with short fingers.
- b) Narrow and elongate male bud.
- c) Sweetish flavour.

Pseudostem characteristics

- a) Thicker pseudostem.
- b) Thinner pseudostem.
- c) «Woody» texture.

stages of development. Current results, especially with embryo formation, are encouraging. Even if protoplast fusion cannot be obtained, the use of mutagens in free-cell cultures could yield some useful or at least interesting variability. Modern techniques of assessing variability have been infrequently applied. A recent electrophoretic study with 23 proteins of *acuminata* and *balbisiana* and 17 cooking bananas revealed that ploidy levels had no effect on numbers of bands resolved and that Saba (believed to be BBB) could be differentiated from Bluggoe (ABB) (RIVERA and CORONEL, 1983).

ALTERNATIVE BREEDING APPROACHES

Since the tetraploids generated to date by restitution from the triploid Highgate appear to have agronomic, resistance, or quality deficiencies, it is reasonable to consider other approaches.

It seems difficult to believe that all modern bananas and plantains were selected in prehistory from natural crosses and ploidy changes without any effort to improve the diploid progenitors. With the great progress in cyclic improvement of diploids by scientific breeding in recent years, it would seem that resynthesis of the original triploid types from the improved diploids would be the reasonable route to take. The strategy would then be not to keep intact the triploid Highgate genome but to remake a triploid from scratch, using many of the improved diploids in the right combinations. By breeding at the diploid level, both the genetics of resistance and of other characters, and specific, and general combining ability can be assessed. Resistance genes can be pyramided. Repeated cycles and recurrent selection can be practiced. By expanding the diploid breeding program to include appropriate balbisianas, resynthesis of all the commercially useful genomes (AAB, ABB, BBB) can be attempted as well.

The logical approach would be to double the chromosomes of many of the improved diploids (VAKILI, 1967) and then to intercross with superior but not closely related diploids, to retain maximum heterozygosity. Doubling the chromosome numbers of a diploid could increase the capability to hybridize with other species especially if both are diploids (DEWEY, 1980). Parental choice could be sought for maximizing both heterozygosity and the combining of appropriate resistances, quality factors, and agronomic characters. The object would be to obtain large numbers of triploid progeny for seedling screening for disease resistance with subsequent field screening of the smaller number of survivors for more general characters. A crossing center could even distribute seeds or embryos worldwide for screening for different environments with different pathogens or different altitudes, temperatures, latitudes and rainfall regimes.

It may well be that the limitations of Highgate-derived tetraploids will not be shown to be a universal generality for tetraploids and that superior tetraploids will be obtained from doubled diploid x doubled diploid combinations. These could either be utilized directly or used as parents in both tetraploid x tetraploid and tetraploid x diploid combinations. It should be recognized that the suggestions here to develop triploids is by genetically different routes from the idea of extracting «secondary triploids» from a Highgate-derived tetraploid. In this latter case there would be the first meiosis involving the breakup of the $3n$ Highgate genomes, wherein these genomes have never been separated or tested for their breeding potential. It is not surprising that such «secondary triploids», when attempted, have revealed many poor recombinations. With the construction of primary triploids by breeding tested diploids or by tetraploids derived from doubled diploids and their tested tetraploid progeny, the results should be very different. This approach, however, will probably require a departure from the requirement of having the diploids be highly parthenocarpic, as has been the case in the past.

Efforts should be made to back up the resynthesis approach with a review of the most likely geographical areas and most likely progenitors of the present most desirable triploid clones. The major pathogens in those areas should be carefully assessed for variability and virulence on a set of diploid differentials.

It would be of interest to attempt to separate the genomes of the major triploids in order to better assess those genomes individually, especially for resistance/susceptibility genes. Apparently, VALERY (AAA) did provide an n gamete through pollen, to a haploid gamete of a diploid (ROWE, 1984). Further attempts using pollen from major triploids should be tried. Additionally, cell and tissue culture and other modern methods should be explored for extracting single genomes out of the common triploids. It would also be of interest to attempt to double the Cavendish triploids to obtain viable gametes of various ploidy levels from the hexaploid. Since dwarfness is a requirement in commercial bananas and should be desirable in plantains as well, more flexibility in breeding would be provided by having more dwarf diploids. Tissue culture and mutagens should be employed to develop more such stocks.

The approaches suggested here lend themselves to taking advantage of fixing high levels of heterozygosity by two alternatives to normal sexual reproduction: 1) somatic fusion of hybrid diploid cells, and 2) hybridization of gametes with the unreduced chromosome number ($2n$ gametes) from diploid hybrids (BINGHAM, 1980). Banana breeders and geneticists could well examine the lead of research on potato in relation to genetic manipulation and modern genetic engineering applications.

Modern research in genetic engineering, regeneration

from cells and tissues, microinjection and even recombinant DNA methodologies would find a fertile area in application to *Musa* genetics and breeding. Classical cytogenetics and classical genetics of characters need to be revitalized for *Musa*, and with a diploid approach to major breeding targets, these should be straightforward.

CONCLUSIONS

After 60 years of attempting unsuccessfully to breed a useful tetraploid from Gros Michel, new innovative approaches to banana breeding are needed if progress is to be made and sustained towards replacing present disease-susceptible and low-yielding banana varieties. Moreover, breeding of disease resistant AAB plantains is now required, since they have become susceptible to black Sigatoka.

Fusarial wilt and leaf spot diseases are causing severe losses in many important clones in all the major triploid genomes (AAA, AAB*, ABB). Sources of resistance to these pathogens have been identified in the breeding areas, but they are limited in numbers and have not been tested widely. However, resistant tetraploids, when distributed beyond their area of selection, have succumbed to fusarial wilt. Resistance to fusarial wilt, in Cavendish varieties, once thought to be horizontal or durable, has succumbed to Race 4 in the subtropics. Race 4 has virulence additional to Race 1 virulence, broadening its host range to include Cavendish varieties. The presumption of major gene virulence is very strong, and of selection of such fungal genes on deployment of formerly resistant genotypes in areas subject to host stress. It is not known if horizontal resistance functions in response to *Mycosphaerella fijiensis* because genetic studies of virulence/avirulence, resistance/susceptibility have hardly been conducted. Great research opportunities exist for understanding the genetics of resistance/virulence of these pathosystems. Such information is sorely needed if banana and plantain breeding is to progress successfully. Resistance to other diseases, such as Moko, nematodes, and fruit spotting pathogens, is even less understood. Progress in banana and plantain breeding requires a great deal more emphasis, on host/parasite interaction and genetics, at several levels, including factors influencing epidemiological progression of disease. The need for greater in-depth work involving modern plant pathology and modern genetics, with plant breeders in team efforts, is very great.

Triploidy has evolved in *Musa* as the most productive and useful ploidy level. Diploids and tetraploids developed to date have serious defects that have excluded them from widespread usefulness. Tetraploids derived from the present strategy of adding a n genome from an improved diploid to an intact 3n genome from Highgate vary in characteris-

tics, but they all have deficiencies. It is not absolutely impossible that a commercially competitive tetraploid for international commerce might be obtained by this approach, but it is unlikely. However, tetraploids of this type may now exist that are useful in some situations, such as for local use and for internal consumption where black Sigatoka control is prohibitively costly. Such use should be explored vigorously.

The more basic problem of such a breeding strategy, however, remains. That is, the «dead end» nature of the approach, precluding recurrent selection and cyclic improvement. Additionally, no cushion is provided as pathogens evolve new races or aggressiveness. It remains to be seen, however, if tetraploidy can be used to produce useful triploids. Triploids that «closely approach commercial standards» are said to be produced by the $4n \times 2n$ cross (ROWE, 1984). However, none of these have been examined by horticulturists or agronomists. An approach we believe to be much more promising is to resynthesize new triploids from doubled diploids \times diploids, rather than by the traditional approach involving the Gros Michel genomes as a unit.

Very little information is available about the crop physiology of bred tetraploids and triploids. When the clones produced by banana breeders, even if of no commercial significance, are made available to crop physiologists and plant pathologists for thorough study, then some guidelines will be available to banana breeders as to where the defects lie. These could be in canopy structure, suckering, ratooning speed, partitioning, fruit development, or susceptibility to races of pathogens not present in the breeding area. Up to now the banana breeder, in addition to screening against local pathogen races, has looked mainly at fruit characteristics (ripening behavior, flavor, bunch size and seed fertility) and little is known in-depth about plant behavior.

The use of *in vitro* techniques of manipulating the genetic potential of *Musa* warrants a great deal more support. *In vitro* methods, successful with other crops, could be one way of overcoming the inherent difficulties in triploid breeding. These techniques should supplement new approaches to the classical methods. Additionally, classical genetics and cytogenetics need to be revitalized for *Musa*, if sustained progress is to be made in improving bananas and plantains, an urgent need in tropical agriculture.

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