CHARACTERIZATION OF POPULATIONS OF *Fusarium oxysporum* f. sp. *cubense* AND SELECTION OF SOMACLONAL VARIANTS TOLERANT TO BANANA WILT DESEASE

by

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LIST OF ABBREVIATIONS

Foc	*Fusarium oxysporum* f. sp. *cubense*
VCG	Vegetative Compatibility Group
WA	Water agar
CLA	Carnation leaf agar
PDA	Potato dextrose agar
LSI	Leaf Scale Index
DSI	Disease Symptom Index
MS	Murashige and Skoog
BAP	6-bezylaminopurine
v/v	Volume per volume
MM	Minimal media
Ppm	Parts per million
f. sp.	forma specialis

PERCIRIAN POPULASI *FUSARIUM OXYSPORUM F.SP.CUBENSE* DAN PEMILIHAN TUNAS SOMAKLONAL YANG TOLERAN TERHADAP PENYAKIT LAYU FUSARIUM PISANG

ABSTRAK

Sebanyak 40 isolat *Fusarium oxysporum* f. sp. *cubense* (FOC) dari Indonesia, Sumatra, dan Semenanjung Malaysia yang dipencil dan pelbagai variety pisang utama di Asia tenggara telah dikenalpasti dari segi morfologi, ras dan keserasian vegetatifnya. Keputusan analisis keserasian vegetatif (VCG) menunjukkan kehadiran 'clonal lineages' di Semenanjung Malaysia. Sebanyak 12 kelompok keserasian vegetatif telah dikenalpasti. Lima kelompok adalah sama dengan VCG 0121, VCG 0124, VCG 01213, VCG 01216 dan VCG 01213/01216. Tujuh kelompok yang lain tidak dapat dikategorikan menggunakan sistem VCG yang sedia ada. Kemungkinan kelompok-kelompok ini merupakan VCG yang baru. VCG 01213/01216 dikenali sebagai Ras 4 tropikal, merupakan kumpulan yang terbesar, iaitu meliputi 30% daripada isolat dan bertaburan besar. Patogen Ras 4 terdapat dalam kumpulan VCG yang kurang nyata dan tersebar luas di Medan, Indonesia dan Semenanjung Malaysia. Program pemilihan ulangan untuk ketoleranan mengesan terhadap layu Fusarium yang menggunakan teknik cawan atau talam berganda dengan peningkatan kepekatan inokulum adalah tidak berjaya.

CHARACTERIZATION OF POPULATIONS OF *FUSARIUM OXYSPORUM F.SP.CUBENSE* AND SELECTION OF SOMACLONAL VARIANTS TOLERENT TO BANANA WILT DISEASE

ABSTRACT

Forty isolates of *Fusarium oxysporum* f. sp *cubense* (Foc) located in Indonesia (Sumatra) and Peninsular Malaysia were isolated from a diverse banana variety host range which included all significant South East Asian cultivars were characterized by morphology, race and vegetative compatibility (VCG). There appeared no direct pattern of association between race, VCG and morphological characteristics. VCG analysis indicated that there were several distinct clonal lineages present in Peninsular Malaysia. Twelve compatibility groups were identified, five of which were previously characterized as VCG 0121, VCG 0124, VCG 01213, VCG 01216 and VCG 01213/01216. The remaining seven other groups could not be classified by the existing VCG system and may be new VCGs. VCG 01213/01216 commonly known as tropical race 4 made up the largest group, with 30% of the isolates and is especially well distributed geographically. Race 4 pathogens fell within several distinct VCG groups and are extensively disseminated throughout Sumatra, Indonesia and Peninsular Malaysia. A rapid lab based recurrent selection program to detect tolerance to Fusarium wilt by selecting possible somaclonal variants from micropropagated plantlets, challenged with incremental inoculum dosages of Foc using the double cup/tray technique proved unsuccessful.

1.0 INTRODUCTION

1.1 History and Development of the Banana Industry

Edible bananas probably first originated in the Indo-Malaysian region, reaching into northern Australia. Their first arrival into Europe is thought to be some time in the 10th Century A.D. It is believed that further distribution into Central and South America was initiated by Portuguese mariners arriving from West African, some time in the 16th Century.

Bananas and plantains are presently cultivated in over 100 countries world wide. Cultivation is distributed fairly evenly between Africa, Asia, Oceania, Latin America and the Caribbean. The present harvest area is approximately 10 million hectares. Banana production as a carbohydrate staple is mainly by small holders. It is also an important source of income to growers in domestic markets, which account for almost 90% of production and invariably include diverse cultivars, selected for local preference. Banana production in Asia, South America and Africa include plantains (AAB) which are cooked by frying, boiling, roasting and bananas that are specifically grown for beer making. Banana fiber is used for handicrafts while the shoots and the male buds are used as vegetables.

Although banana is still considered a small holder cash crop, dessert bananas remain the most traded fruit. Only about 14% of total production is exported, which is almost exclusively the Cavendish variety. Banana exports from Asia and Africa together make up less than 15% of world exports, with Taiwan and Philippines dominating non-Latin American exports.

1.2 Fusarial Wilt of Bananas

Fusarial wilt of bananas is one of the most widespread destructive plant diseases recorded in modern agriculture. Large scale plantations were abandoned or destroyed in Central America and the Caribbean in the 1950s, as the then export variety Gros Michel succumbed to this vascular wilt disease. However a wilt resistant and agronomically superior cultivar was selected to replace the susceptible Gros Michel. The Cavendish group of varieties remained unaffected by the disease even when planted in heavily infested Fusarium soils. However, developments in the 1980's have shown that in some parts of the sub-tropics and in the tropical Philippines where commercial Cavendish plantations existed, a new highly virulent pathogenic race (Race 4) had emerged and consequently the fusarial wilt problem has regained prominence. Cavendish plantations were established in the early 1990's in Southern Malaysia and Indonesia (Sumatra and Sulawesi), mainly for export, with the then conventional view that Cavendish cultivars do not succumb to fusarial wilt in the tropics. Yet within three years (in some cases six months), all these plantations had to be forsaken due to the emergence of *Fusarium oxysporum* f. sp. *cubense* (Foc) tropical Race 4 (Lee *et al.*, 1999) Recently new efforts were initiated to establish plantations of Cavendish for export in Malaysia again. The future of these plantations still remain in limbo; although they are far from the first recorded outbreaks of Race 4, it has been proven that only the best managed farms can contain the spread of disease. The question persists as to whether tropical Race 4 is widespread in its endemic distribution in Malaysia or restricted to localized patches.

1.3 Summary of the Aims

Cultural and chemical control of Fusarium wilt had been unsuccessful in containing the spread of the disease, leaving only the planting of tolerant or highly resistant cultivars the viable option. Research by breeders has indicated that host resistance to Race 1 and 2 strains of Foc is largely due to one or two major vertical genes and is not easily overcome (Buddenhagen, 1990), However tolerance to Race 4 appears to be more polygenic, involving minor reinforcing genes. The failure in developing tolerant varieties to Fusarium wilt by conventional breeding techniques has encouraged more novel selection techniques such as the use of irradiation to induce mutations or screening micropropagated somaclonal variants. Developments in these areas have shown varying degrees of success in variety improvement. Nevertheless they are also coupled by constraints of which the vast time factor involved in screening tolerant somaclones being a principal problem. Field tests are likely to take two to nine months to complete, and the results may be questionable due to the inherent variability in pathosystems, climate and edaphic conditions. Greenhouse screening using rhizomes has been difficult and yielded inconsistent results.

The first aim of this study is to evaluate a recurrent selection screening of micropropagated important local variety "Pisang Berangan" plantlets for somaclonal variants that display Fusarium wilt tolerance, using the rapid evaluation screening technique devised by Mohammed *et al.* (1999). Results from this pattern of recurrent selection should establish an adequate platform for statistical tests to detect a change in response to Foc in the population.

Inoculating a pool of micrpropagated plantlets with dosages of Foc then selecting surviving plants that exhibit a low Leaf Scale Index (LSI) (Brake *et al.*, 1995) re-micropropagating and eventually revaluating with increased challenge inoculation concentrations to gain a statically valid result, implying that increased variability and tolerance may arise from the process of continual micropropagation. Secondly, experiments to investigate the morphological and genetic variably of Foc isolates, in particular those found on Penang Island, Peninsular Malaysia and Sumatra, Indonesia using a multi-discipline approach including analysis of the population structure and race evaluation.

2.0 LITERATURE REVIEW

2.1 Banana Cultivation and Biology

2.1.1 Botany of Bananas

Banana and plantain (*Musa* spp.) are large herbaceous perennial herbs consisting of around 30 species, which have arisen for thousands of years through natural and human selection. Belonging to the family Musaceae, which contain two genera (*Musa* and *Ensete*). *Musa* is normally divided into four sections of which Eumusca contains nearly all of the edible bananas derived from *Musa acuminate, M. balbisiana* and *M. schizocarpa.*

The pseudostem of the banana consists of leaf sheaths, which arise from its corm. Suckers spring up from around the main plant and are attached to the mother plant by vascular strands. The oldest sword sucker, often called the "follower" is used to replace the mother plant after fruit harvesting and is permitted to grow to provide the ratoon crop. This process is continued for an infinite number of successions. The long oblong leafs, which vary in pigmentation and size from cultivar to cultivar are arranged spirally and unroll as the plants mature. The inflorescence appears as a terminal spike shooting out from the heart of the pseudostem, which bends over with the weight. Along the floral stalk, creamy white flowers are clustered. The male flowers and their bracts are shed, while the young fruits develop from the female flowers without fertilization (Simmonds, 1966; Stover and Simmonds, 1987).

2.1.2 Conventional Banana Breeding

Due to the genetic nature of seedless bananas and as it is vegetatively propagated, conventional breeding by hybridization is complex and difficult. Nearly all banana breeding is based on maintaining the intact genome of a triploid and the genes of a diploid male parent. Cavendish varieties are agronomically preferred but female seed sterility prevents them from being used in banana breeding. Popular techniques generally use the triploid Gros Michel or Highgate as the female parents, both of which are agronomically inferior as well as being extremely susceptible to Fusarial wilt, which is endemic in all major banana growing regions. Crossing with pollen from a superior diploid produces only a very small number of tetraploids seeds. Other problems which are linked to the F1 tetraploids include the possibility of production of abundant seeds as tetraploids undergo meiosis and thus produce pollen, and the constant need for recurrent selection (Stover, 1986). Other popular notions have included crossing improved diploids to obtain a tetraploid. Valkili (1967) used colchicine to induce tetraploidy, but this had lead to increased homozygostiy.

2.1.3 Banana Agronomic History in Malaysia

Banana is ranked as an important fruit crop in Malaysia and is widely cultivated in all of the States (Abdullah *et al.*, 1990). Malaysian consumption is around 18.4kg/Ca/yr (FAO, 2000). Fruit hectarage is estimated at 40 000ha. Of this area almost half is cultivated with Pisang Berangan and the Cavendish types, the remainder planted with local cultivars: Pisang Mas, Rastali, Awak, and Nangka (see Table 2.1). Traditionally banana cultivation has been largely by small subsistence farmers or by small holders as a cash crop, often intercropped. However since the 1980's small quantities of Pisang Mas have been exported, and now bananas have developed into a more important commodity. In the early 1990's monocropping has became a more popular method of cultivation but inevitably has lead to increased problems e.g. availability of land, cost of labor and the outbreak of disease (Jamaluddin *et al.*, 1999). The industry is now moving away from non-progressive farming, with the establishment of large plantations (>100ha). Cavendish types and Pisang Berangan have been planted for both domestic and export markets (Ho *et al.*, 1999). Export production is around 560,000 metric tonnes (FAO 2000). Diploid consumption and production throughout Malaysia is based on the dessert cultivar "Pisang Mas". However, most Malaysian banana plantation production is based on dessert triploid cultivars, namely "Pisang Berangan," "Pisang Rastali" or more recently, some varieties of the Cavendish group. Genetically triploids are agronomically superior plants as they yield higher, are more vigorous but sterile thus lack undesirable seeds.

Table 2.1: Popular and important cultivars for banana cultivation in Malaysia (Abdullah *et al.*, 1990)

Cultivar	Genotype	Comment
"Pisang Mas"	*AA*	Important export cultivar
"Pisang Rastali"	*AAB*	Widely distributed.
"Pisang Embun"	*AAA*	Popular in Indonesia
"Pisang Berangan"	*AAA*	Very highly priced.
"Pisang Awak"	*ABB*	Hardy cultivar
"Pisang Nangka"	*AAB*	Very widely distributed
"Pisang Masak Hijau"	*AAA*	Widely distributed
"Pisang Raja"	*AAB*	Highly priced

2.1.3 Banana Cultural Significance in Malaysia

Other than its economical importance Bananas have cultural significance in Malaysia where a large variety of fruits can be readily found for sale. It remains an important source of income in many rural areas, and its nutritional value makes it an essential part of the Malaysian diet. Banana plants can be often seen attached to the entrance of Indian temples, where they have important religious significance.

2.2 Fusarium Wilt of Bananas

2.2.1 Origin of Foc

Fusarium wilt of bananas is thought to have co-evolved with its host bananas in Southeast Asia. Edible bananas are principally derived from two wild seeded species in the EuMusa series, *Musa accuminata* Colla and *Musa balbisiana* Colla. People have selected pathenocarpic bananas for obvious

reasons and consequently transferred, unsuspectingly diseased suckers to propagate them. It is possible that susceptibility to Foc is present within the gene pool of wild bananas, long before triploid bananas were selected and disseminated. One interesting notion is that Foc has arisen as a saprophyte and formed an endophytic association in the xylem of wild diploid *Musa* species before developing pathogenic potential on its host on several occasions, subsequently giving rise to the separate VCGs. It has maintained its presence in the soil as chlamydospores and as parasites on the roots of non-host weed species, capable of saprophytic survival on organic matter. Man has consequently introduced the pathogen to domestic clones (Pedrosa, 1995).

2.2.2 The Pathogen *in vivo*

Foc is present wherever susceptible bananas are grown with only a few exceptions, which are mainly on South Pacific islands (Stover, 1987). When cultured the species is known for its morphological variability, but the formae speciales of Foc can only be distinguished by differential pathogenicity.

Foc has no known sexual stage; it produces three types of asexual spores: microconidia, macroconidia and chlamydospores. Macroconidia are oval or kidney shaped, they occur on short microconidiophores in the aerial mycelium. Macroconidia are thin-walled with a definite foot cell and a pointed apical cell. *In planta* microconidia are produced in xylem elements and facilitate spreading of the pathogen within the host's vascular system. Although less frequently macroconidia may also be found in xylem. During progressive stages of the disease, it is possible to find macroconidia in leaves and petioles, while microconidia are found in parenchyma tissue. Chlamydospores are thick-

walled asexual spores produced in hyphae or conidia through the condensation of their contents (Nelson, 1990). Their formation within the host signals final stages of the disease cycle. They are essentially the survival structures for the pathogen in the absence of the host, and have the ability to remain dormant for several years. Germination of the spores will occur when stimulated by the presence of host root exudates.

2.2.3 Foc Cultural Characteristics

Foc is a soil-borne fungus and is indistinguishable morphologically from non-pathogenic, saprophytic strains of *F. oxysporum*. Non-pathogenic strains of *F. oxysporum* can colonize roots of non hosts in competition with pathogenic strains (Gordon & Okamoto, 1990). Pathogenic strains are able to move past the cortical layer of their susceptible host and invade the vascular system, causing wilt and invariably death.

F. oxysporum is known for its great morphological variability when cultured. Inconsistencies within pigmentation of the stroma, variability of septation, presence or absence of sclerotia and sporodochia are commonly observed from culture to culture. Cultural mutation is a common problem encounted when working with vascular wilt isolates of Foc. Maintenance of virulence and morphology of the organism in culture is extremely important. Frequent subculturing especially on nutrient rich media will lead to sectoring (Nelson, 1990). Early work by Waite & Stover (1960) classified the distinguishing features into four major morphological cultivars on PDA after three weeks incubation under standardized conditions; they noticed the deterioration of many of the cultures. Most isolates produced macroconidia in

sporodochia on carbohydrate rich media, with the fungus often mutating to produce either an abundant aerial mycelium, and featureless with sparse macroconidia or to slimy pionnote type with adpressed aerial mycelium, highly pigmented and production of abundant macroconidia (Nelson, 1990). The pathogen is highly variable and can only be distinguished from saprophytic strains by pathogenicity tests and vegetative compatibility tests.

2.2.4 Disease Cycle

Studies have shown that uptake of *Fusarium* spores requires rupture of roots (Wardlaw, 1930). However pathogenic strains are able to penetrate into healthy roots. Transpiration pull then ensures spores are carried further. Although initial rate of hyphae movement is quite low, secondary sporulation is very rapid, thereby enabling the disease to progress.

Rapid prevention of secondary spore distribution plays an important part in disease tolerance of the host (Beckman *et al.*, 1962). Infected plants will produce occluding gels to immobilize spores, restricting further spread and the production of tyloses that isolate off infected vessels. It is also likely that bananas have secondary metabolite production due to a stress response which may destroy the pathogen The defense systems of vascular plants is largely effective under optimal conditions for host growth. Under conditions that favor disease development (27°C) there is a delay in onset of host defense mechanisms and production of weaker gel structures, whereby resistance is compromised and systemic colonization can occur (Beckman *et al.*, 1962). There are apparently no structural difference between the xylem elements in

resistant and susceptible cultivars, thus host response time seems to be of critical importance.

2.2.5 Disease Expression

Symptom manifestation does vary somewhat from each banana variety (Stover, 1962). Different strains are alleged also to produce different symptoms and are also affected by a variety of other factors, such as bacteria and nematodes when present in the host. However there is a general disease expression. The progression of disease symptoms begins with older leaves and then infection continues with the younger leaves. Initially the older leaves show a slight off-green or yellow discoloration, two weeks before the more distinctive yellowing on the leaf margins advancing toward the midrib giving a obvious streaking effect (Su *et al.*, 1986). The most affected leaves often stand out conspicuously (Wardlaw, 1972). The leaf often then turns completely yellow/dark brown, occasionally the whole outer leaf sheath separates from the pseudostem when the petiole buckles. All of the leaves will eventually collapse and die leaving the pseudostem still erect but dead, which will eventually be blown over by the wind or succumb to rotting (Stover, 1972).

The pseudostem of a heavily diseased plant also shows typical symptoms when cut longitudinally. The vascular tissue shows distinctive discoloration. Reddish or brown vascular strands appear which develop to be more pigmented and numerous as the disease progresses. As the rhizome is not immediately killed, infection can pass from the parent plant to the young sucker through diseased vascular strands. However usually suckers less than

5ft tall or below 4 months old do not show visual symptoms of the disease, but often do not yield marketable bunches.

Parenchyma tissue below the soil line around the effected vascular tissue may also show quite distinctive symptoms, caused by secondary saprophytes, which discolors the tissue to a dark brown/black (Su *et al.*, 1986; Liew, 1997).

The fruit is not discolored or infected by the pathogen which distinguishes this disease from bacterial wilt (Moko disease) which has remarkably similar foliage symptoms, caused by *Ralstonia solanacearum* (*Pseudomonas solanacearum* E. F. Smith)(Stover *et al.*, 1987).

Table 2.2: Common Malaysian cultivars susceptibility to the different pathogenic races of Foc (Liew, 1997)

Local Name/Banana Cultivar	Genotype	Disease Reaction Race 1	Disease Reaction Race 4
"Pisang Mas"	*AA*	Tolerant	Susceptible
"Pisang Lemak Manis"	*AA*	Susceptible	Susceptible
"Pisang Jari Buaya"	*AA*	Resistant	Very Susceptible
"Pisang Berangan"	*AAA*	Susceptible	Very Susceptible
"Pisang Embun" ("Gros Michel")	*AAA*	Very Susceptible	Very Susceptible
"Pisang Udang"	*AAA*	Susceptible	Susceptible
Cavendish Group ("Valery" etc.	*AAA*	Resistant	Susceptible
GCTCV 215-1 (Taiwan Cavendish Varient)	*AAA*	Resistant	Tolerant
"Pisang Serendah" ("Dwarf Parfitt")	*AAA*	Resistant	Tolerant
"Pisang Rastali" (most clones)	*AAB*	Very Susceptible	Very Susceptible
"Pisang Rastal"l (selected)	*AAB*	Tolerant	Tolerant
"Pisang Seribu"	*AAB*	Susceptible	Susceptible
"Pisang Raja"	*AAB*	Susceptible	Susceptible
"Pisang Relong"	*AAB*	Susceptible	Susceptible
"Pisang Nangka"	*ABB*	Susceptible	Susceptible
"Pisang Awak"	*ABB*	Tolerant	Susceptible
"Pisang Tanduk/Helang"	*ABB*	Susceptible	Susceptible
"Pisang Abu Keling" ("Bluggoe")	*ABB*	Tolerant	Tolerant
"Pisang Abu Nipah" ("Saba")	*ABBB*	Susceptible	Susceptible
FHIA 1 ("Goldfinger")	*AAAB*	Resistant	Resistant (?)

2.2.6 Genetic Diversity within the Pathogen

Within the banana forma specialis (f. sp.), there exists another specialized subgroup called pathogenic races, differentiated by host susceptibility to the fungal isolate. Four races are presently recognized three of which are primary pathogens of bananas (Stover, 1986). Race 1, found in all major banana growing regions, is pathogenic on many common cultivars and was the causal agent in the destruction of the Gros Michel cultivar principally in South America. Race 2 is a pathogen of Bluggoe (ABB), other banana cooking varieties and some bred tetraploids, and is presently found only in Central and South America, (Ploetz, 1990). Race 3 affects species of *Heliconia*, a close relative of banana, but has a minor or no effect on bananas. Race 4 which was reported from Taiwan (Sun *et al.*, 1978) has a wider virulence spectrum and attacks Cavendish clones which are resistant to Race 1, in addition to all other commercial cultivars. Little is known about the mechanisms in race development of Fusarium, and other Fungi Imperfecti. New races are thought to arise through parasexual recombination, within or between preexisting races (Elisa & Schneider, 1991). Several VCGs in Foc are known to contain different races. It may also be possible that saprophytic strains had evolved to become pathogenic.

2.2.7 Fusarium Wilt Race 4 in the Tropics

The tropical region of the world lies between the tropic of Cancer at 23°27'N and the tropic of Capricorn at 23°27'S. It has been recognized that in the Philippines, Jamaica, Guadeloupe, Indonesia and Malaysia, Cavendish

cultivars have succumbed to Fusarium wilt Race 4. In Jamaica, Cavendish cultivars that were diseased were originally believed to be heavily stressed due to poor growing conditions, namely water logged soils (Risbeth, 1957). In the Philippines, where most isolates belong to the vegetative compatibility group (VCG):0122, the disease while severe is not considered widespread, and has occurred only on localized patches. In recent years the presence of race 4 pathogens have both been confirmed in Peninsular Malaysia and Indonesia (Lee et al., 1999). Malaysia's first recorded race 4 outbreak occurred in 1992, when Kulim Montel Farm began cultivating Cavendish cultivars (395 ha) on Nam Heng Estate. The assumption was that Cavendish cultivars, in this case Grande Naine was resistant to Foc in the tropics. A national institute also gave the assurance that Foc Race 4 was not present in the country. Fusarium wilt (VCG: 01213/01216) was first detected six months after planting and after four years the incidence was at 32% (Lee *et al.*, 1999). The high rate of incidence was supposedly aggravated by the irrigation system. The reaction of some common Malaysian cultivars to Foc Race 1 and Race 4 are summarized in Table 2.2.

2.2.8 Cultural Characterization *in vitro*

Many attempts had been made to characterize groups of Foc, according to their cultural morphology when grown under uniform conditions in laboratories. Foc cultures often have many different features and are highly variable with respect to pigmentation (Burgess *et al.*, 1989) including odor, colony color and sporulation. More general aid to identification includes substrate color on potato dextrose agar (PDA), usually tinted rose, white or

peach (Synder and Hansen, 1940). Ovoid macroconidia (5-12×2.2-3.5µ) are normally abundant, borne on simple phialides or branched conidiophores, usually non-septate. Macroconidia have 3-4 septa (27-66×3.5 µ), are falcate or almost straight, thin-walled and tapered at each end. Chlamydospores are often formed in older cultures but their production may be slow especially in pathogenic strains (Burgess *et al.*, 1994).

2.3 Vegetative compatibility in *Fusarium oxysporum* f. sp. *cubense*

2.3.1 Importance of VCGs within Foc

Primarily before Vegetative Compatibility Group (VCG) testing the only means to distinguish different pathogenic strains of Fusarium was by pathogenicity testing, which is often tedious and expensive. This type testing does not demonstrate whether isolates are genetically similar. Puhalla (1985) introduced a novel complementation test using non-nitrate utilizing mutants which is indicative of genetical relatedness. For asexual genetic exchange to occur, a heterokaryon must be formed. The ability of two separate mutants that differ in their genetic loci to anastomose and form a heterokaryon, indicates a VCG. However, if the hyphae of the two strains do not fuse, or if the cells form a barrage zone between the two mycelia, the strains are considered vegetatively incompatible. The term heterokaryosis is applied to the condition where two or more genetically different haploid nuclei occur together in the same cytoplasm. Laboratory VCG tests use auxotropic non-nitrate utilizing mutants for forcing markers to determine whether or not strains are able to form heterokaryons. Isolates belonging to the same VCG are vegetatively

compatible with one another, and can be presumed to have identical alleles at the *vic* loci. Forma speciales and races in general are limited to only a few VCGs and do not overlap (Leslie, 1990). VCGs serve as a way to subdivide fungal populations. Asexually reproducing fungal populations tend to restrict the number of VCGs as differences at the *vic* loci will limit genetic exchange. This makes it extremely unlikely that two vegetative compatible isolates would not be related by colonial descent, indicating that the two VCG compatible individuals should also be identical in other genes, including those that are responsible for pathogenicity. It is believed that gene sets that determine VCG compatibility and pathogenicity became fixed together in evolution, thus suggesting that isolates belonging to the same VCG have the same host range and similar virulence (Katan *et al.*, 1988). The distribution of Foc VCGs in the major banana growing countries are summarized in Table 2.3.

In essence, gene associations should be static as meiotic recombination does not occur. This can be confirmed as Foc isolates within the same VCG generally will produce an identical DNA fingerprint pattern (Bentley *et al.*, 1999). VCG diversity had been examined in over 30 formae specialis of *F. oxysporum* and a systemic numbering system had been developed (Krister *et al.*, 1998) Heterokayosis along with parasexuality have been alleged to occur frequently in nature and are believed to be an important source of variation in fungi which only undergo asexual reproduction (Buxton, 1960; Parameter, 1963). However more recent studies had brought reports of frequent occurrence of parasexuality into question.

Table 2.3: Recognized vegetative compatibility groups located in common banana and plantain growing regions. (Bentley *et al.*, 1999; Kangire *et al.*, 1999; Singburauom *et al.*, 1999; Ong, 1996)

Country	VCG group	Number of VCGs
Australia	0120, 0124, 0124/5, 0125, 0128, 0129,01211, 01213/16, 01220	8
Brazil	0124	1
Canary Islands	0120	1
Costa Rica	0120, 01215	2
Thunderous	0120, 0124, 0124/5, 0126	4
India	0124, 0124/5, 0125	2
Indonesia	0120, 0120/15, 0121, 0124/5, 0126, 01213, 01213/16, 01215, 01216, 01218, 01219	11
Irian Jaya	0124/5, 0126, 01213/16	3
Jamaica	0120, 0124, 0125	3
Kenya	0124, 01212	2
Malawi	0124, 0124/5, 01214	3
Malaysia	0120, 0121, 0123, 0124/5, 0125, 01213, 01213/01216, 01216, 01217, 01218, 01222	11
Mexico	0124/5	1
Nicaragua	0124	1
Philippines	0122, 0123, 0126	3
South Africa	0120	1
Sri Lanka	0124	1
Taiwan	0120,0121, 0123, 01213	2
Tanzania	0124, 01212	2
Thailand	0123, 0124, 0124/5, 0125, 01218, 01220, 01221	6
Uganda	0125	1
U.S.A.	0124, 0124/5, 01210	3
Viet Nam	0124/5	1
Zaire	0125	1

2.2.3 VCGs in Malaysia

Malaysia is home to a large a number of VCGs (Table 2.4) which had been isolated throughout the country. VCGs that so far have been identified include 0120, 0121, 0123, 01213, 01214, 01215, 01216, 01217 and 01213/16 (Pegg *et al.*, 1993; Ong 1996; Bentley *et al.*, 1999). South East Asia has the greatest diversity of Foc, which supports the theory of co-evolution, as it is also thought to be the region where edible bananas first arose (Bentley *et al.*, 1999). There are currently at least 24 different VCGs for Foc (Ploetz & Correl 1988; Bentley *et al.*, 1998)

Table 2.4: Number of vegetative compatibility groups located in Asian Countries (Liew, 1997, Bentley *et al.*, 1999) *

Country	Distribution of VCGs in Foc
Australia	8
Indonesia	11
Malaysia	17
Philippines	3
Taiwan	4
Thailand	13

* Including VCGs that cannot be identified using the existing testers.

2.4 Disease management strategies

2.4.1 Planting of Resistant Clones

Planting of resistant clones is widely accepted as the only cost-effective means of sustaining large perennial plantations with a monocropping production system. Field evaluations had been the most reliable method for disease resistance screening despite the man-power demands and high costs (Pegg *et al.*, 1996). However, early screening of locally cultivated seedlings with Foc produced inconsistent results which did not correspond with field evaluations of fully grown plants (Pegg *et al.*, 1996). Mohammed *et al.*, (1999) revised a revolutionary screening technique, which was originally devised for testing pathogen virulence (Liew, 1996). Results obtained from screening of newly developed cultivars "Gold Finger", "Intan", "Mutiara" and "Novaria" were similar to those reproduced in field tests. The double tray technique had been shown to be a relatively simple, low cost and practical laboratory based system that yielded rapid and reproducible results.

2.4.2 Banana and Plantain Micropropagation

Micropropagation of bananas is now a routine technique and the potential was realized in 1974 by Berg and Bustamante, when heat treatment was deemed as an inadequate way of sterilizing suckers. Micropropagation has greatly improved the *Musa* germplasm and has enhanced handling for the purposes of clonal propagation, uniform production and has also played an important role in breeding (Rowe & Rosdale, 1996; Vuylsteke *et al.*, 1993). Micropropagated planting material generally performs very well and are often

superior to conventional planting material (Smith & Drew, 1990a; Vuylsteke, 1998). Micropropagated plantlets establish more rapidly, are more vigorous have higher yields and uniform production cycle, provided they are properly maintained agronomically (Drew & Smith, 1990a; Vuylsteke & Ortiz, 1997).

In Malaysia private sector laboratories offer a variety of popular clones. Plantations recognize the benefits of using the materials especially clean planting materials that reduce spread of pests and diseases. One of the most serious problem in using this technique is the regularity of micropropagated off-types that generally range from 0-30%, depending on the genotype (Israeli, *et al.*, 1995; Smith, 1988; Vuylsteke, 1989; Vuylsteke, *et al.*, 1991; Stover, 1987). The problem has serious consequences, frequently producing agronomically inferior plants (Vuylsteke *et al.*, 1988) and often off-types cannot be detected in early stages of development (Persley & De Lange, 1986) thus micropropagation for uniformity has come under serious criticism. Tests had also shown that micropropagated plants were more susceptible to disease epidemics (Smith *et al.*, 1999), although some field trials had found no conclusive evidence for this (Sabadell & Hernandez, 1999)

2.3.3 Flooding

Flood fallow had been shown to be an effective method for controlling Fusarium wilt on bananas, and yielded promising results in South America (Stover, 1962), although speculation remains on is effectiveness as it has the likelihood of distorting the soils natural suppressiveness over continual periods (Stover, 1990a). Crop rotation is employed on a regular basis in Taiwan where farmers grow bananas after two crops of vegetables then one of rice; this is so

that the fields are flooded for up to five months of the year. Tests had shown rotation with paddy rice for one and three years reduced disease incidence by 40% to 12.7% and 3.6% respectively (Su *et al.*, 1986). Taiwanese farmers currently practice this to reclaim soils previously infested with Foc. However, reinfestation is still likely to occur when susceptible bananas varieties are replanted.

2.3.4 Intercropping

Some Malaysian farms have begun to intercrop banana with young oil palm, Encouraging results were reported after a two year crop when fusarium wilt infection was only 4.9% and considered negligible. The results in this case are subjective as plantations lack irrigation which is considered an important means of spore dissemination (Lee *et al.*, 1999).

2.3.5 Soil Amendments

Banana wilt occurs in a wide range of soil types, although some soils are know to be more susceptible. Wilt appears to be more severe in acid and lightly textured soils. Healthy plantations are more likely to be found on medium to heavy loam, with good aeration, and high pH range 7.6-8.5 (Risbeth, 1957). Cavendish plantations which succumbed to Fusarial wilt in localized patches, thought to be race 1 in Grand Canaries in the 1970s (Stover & Mao, 1972), were subjected to below optimum growing conditions, i.e. poor soils with low permeability and insufficient drainage. It is well known that Fusarium wilt becomes more prevalent in Malaysia in years with heavy rainfall (Ward, 1930).

Appropriate fertilization can also play an important role in affecting the severity of wilt. Fusarial wilt incidence in tomatoes (Walker & Foster, 1946) had been shown to increase by applications of nitrogen based fertilizers; this is also true with banana fusarium wilt, as one field trial using ammonium sulphate had to abandoned due to the increase in wilt incidence (Rishbeth, 1957).

2.3.6 Exclusion and Quarantine.

Exclusion has been proven to be highly effective in stopping the spread of Fusarial wilt on bananas to unaffected areas by restricting the movement of diseased planting material from infested areas (Stover, 1990b). Australia has imposed strict government controls on the inter-state transportation of banana materials, since their recent discoveries in the late 90's of Fusarium wilt tropical race 4 near Darwin. Race 4 has not been reported to be present in South and Central America.

2.3.7 Chemical Control

Fumigation of soil with methyl bromide followed by a non banana crop has proved effective provided the disease incidence is still low (Stover, 1990a). This is only likely to be a temporary solution and is expensive (Ploetz *et al.*, 1990). Other studies have had varying successes; benomyl is known to reduce germination of spores in *F. oxysporum* (Decallone & Meyer, 1972). Mercury-based compounds had been shown to be extremely effective (Meredith, 1943), but are also highly toxic. Unfortunately *F. oxysporum* has been often reported to be one of the first organisms to recolonize soils treated with broad-spectrum biocides, with achieved migration rates of up to a centimeter a day (Marois *et*