CHAPTER ONE

CHAPTER ONE

INTRODUCTION

1.1 Importance of Bananas and Plantains

Banana is the most important fruit crop in the world (Navarro *et al.* 1997; Kaemmer, *et al.*, 1997). It forms the primary food source for millions of people in many parts of the world and rank next only to rice and wheat (INIBAP 1992). Banana fruits provide one of the major commodities in international trade, but are far more important as a starchy staple in local food economics (Stover and Simmonds, 1987).

Banana and plantains (*Musa* spp.) are giant perennial herbs belonging to the genus *Musa* and the family *Musaceae*. They form an integral component of the farming systems in the humid agroecological zones of the tropics (Crouch *et al.* 1998). The center of origin of these plants is Asia and a wide range of varieties exists that have been domesticated, both as dessert and cooking bananas, to feed large local populations. In the Philippines and Taiwan, banana cultivation is of significant importance to the national economy and the welfare of the individual growers.

Malaysia is one of the most important centers of origin and diversity for wild and cultivated bananas such as diploid AA and the natural hybrids of AAA, AAB and ABB (Siti Hawa, 1998). Four wild *Musa* species have been reported in Malaysia, namely: *Musa acuminata, Musa balbisiana, Musa gracilis* and *Musa violascens* (Simmonds, 1962).

Banana is one of the most popular fruits in this country and is available throughout the year. Besides being consumed fresh, the banana is also used daily for culinary purposes. Annual world production of banana (including cooking types) is estimated to be 85.0 million tones (FAO, 1999) of which only 10% are exported, giving a world trade value of about $ 2.5 billion a year. The humid forest and mid-altitude zones of Sub- Saharan Africa accounts for 35% of the world banana and plantain production which provides more than 25% of the dietary carbohydrates for approximately 70 million people in the region (FAO, 1990; Vuylsteke *et al.* 1992; Muller *et al.* 1997). Other main production areas are in Latin America and the Caribbean (35%), Asia and the Pacific (29%), East Africa (25%) and West and Central Africa (12%). By types, plantains and cooking bananas make up the highest production (39.5%) followed by the Cavendish (30.0%), highland bananas (18.0%) and other dessert bananas (12.5%) of which Pisang Mas (Sucrier) and Pisang Rastali (Silk) are included (Siti Hawa, 1996).

In Malaysia about 29,000 ha are planted with different cultivars, by small–scale and commercial scale farmers with a production of 237,000 metric tones in 1998 (Abdullah, *et al.* 1998). Banana is the second most important fruit crop in Malaysia ranking next only to durian (Table 1.1), accounting for 20% of the total hectarge planted to fruits (Doon, 1995).

Table 1.1: Fruit hectareage in Peninsular Malaysia in 1997*

Fruit types	Hectareage (ha)
Durian	112,206
Banana	25,635
Rambutan	18,402
Duku	16,609
Dokong	16,266
Pineapple	11,067
Cempedak	8,377
Mango	8,336
Mangosteen	7,950
Duku Langsat	6,306
Others	27,085
Total	258,239

*(*Source: Crop Hectareage Statistics, Peninsular Malaysia 1997, Dept. of Agriculture.)*

The economically important dessert bananas in Malaysia, are Pisang Mas (AA), Pisang Berangan (AAA), Pisang Rastali (AAB) and the Cavendish cv. Montel (AAA). The popular cooking types with ABB genome are Pisang Nangka, Pisang Tandok, Pisang Raja and Pisang Awak. Most of the production is for local consumption and about 37,813 metric tones are exported, mainly to Singapore. Popular clones planted in Malaysia on a commercial scale are mainly Cavendish bananas and Pisang Berangan cv. Intan (AAA) (Mak, *et al.* 1995). Of the total cultivated area, almost half are cultivated with Pisang Berangan and the Cavendish types for both local consumption and export (Siti Hawa, 1998).

African consumption in some areas is considerably higher than the world average (IITA, 1993). IDRC (1997) reported that the average percapita consumption in Africa is about 250 kg a year. The estimation of banana consumption in Malaysia was 153,660 metric tonnes in 1998. In the years 1999 and 2000, with the estimated of population growth of 2.6% per year the banana consumption was expected to reach 159,778.8 metric tonnes and 166,376.6 metric tonnes respectively.

The processing of bananas and plantains includes the production of medicinal alcohol (ethanol); banana juice; crisp or chips; jams and jellies; banana figs; powder; flour; puree; and starch (Thompson, 1995). Also, processing for storage consists of canning, drying, freezing or fermentation in case of vinegar and alcoholic beverages.

Constraints in banana production include the availability of suitable land. However, in recent years banana cultivation has shown a decreasing trend from 40,000 ha in 1993 (Tan *et al.* 1993) to about 30,000 ha. in 1996 (Siti Hawa 1998), probably due to high cost of agricultural input, such as labour, fuel, fertilizers, agro-chemicals, packing materials and transportation, in addition to marketing issues. Other major constraints on banana production are pests and diseases, the important ones being fungal diseases such as Sigatoka leaf spots and *Fusarium* wilt (FOC) race 4.

In Malaysia *Fusarium* wilt (Liew, 1997), caused by the soil inhibiting fungus *Fusarium oxysporum* f. sp. *cubense* (FOC) race 4, was found to be the main important factor limiting large scale commercial production of banana. There are no chemical or cultural practices, and all clones cultivated, Pisang Berangan cv. Intan, and Pisang Mas and Pisang Rastali are highly susceptible to *Fusarium* wilt disease (Mak *et al.*, 1995).

Bananas and plantains are of great nutritional significance, being the key staple food for nearly 400 million people in many countries of the tropics. The energy value of bananas range between 80-120 K cal/ 100g (Chandler, 1995). Bananas are rich sources of carbohydrates (CH): 22% of the edible portion contributes about 95% of the total energy of the food. They are rich in minerals such as phosphorous, calcium and potassium, Vitamin C (dessert banana), Vitamin A (plantains) and Vitamin B_6, and dietary fiber (INIBAP, 1987; Stover and Simmonds, 1987; Forsyth, 1980). However, protein provides a low 3% of total energy.

Generally, sugar content and acid content of ripe bananas is 27% and 1.2-4.0 %, respectively (Zainun, 1998). The trace level of sodium in the banana makes it a good candidate for low sodium diets, while there is a case to be made for the protection effect of high potassium against excessive sodium intake.

1.2 Genetic Improvement of Banana

Musa breeders face many problems intrinsic to this crop, mainly its low fertility due to triploidy. The cultivated bananas are mostly triploid (2n = 3x = 33) and exhibit a marked degree of sterility. Sterility is a main problem of banana breeding, which is very complex depending on meiotic irregularities (Novak, 1992). The cultivated bananas are vegetatively propagated plants with fruit developing through parthenocarpy. The trisomic pattern of genetic inheritance, low seed fertility, and slow field propagation, long time span from one generation to the next (almost two years from seed to seed), and a large area requirement for field testing are the most conspicuous problems in breeding (Crouch *et al.* 1998).

The absence of improved hybrids has been attributed to difficulties associated with breeding the banana crop at the genetic and practical levels. Therefore, genetic improvement has been difficult due to the lack of useful genetic variability and low levels of female fertility. Hence the potential for biotechnology techniques to complement conventional *Musa* breeding has been investigated (Wiame, *et al.* 2000; Vuylsteke, *et al.* 1998; Ortiz and Vuylsteke, 1996; INIBAP, 1993; Novak, 1992; Vuylsteke, 1989; Dale, 1991). These techniques involved *in vitro* multiplication, mutation breeding and genetic manipulation.

In vitro multiplication by meristem culture is now well established in *Musa* spp. This technique offers great opportunity for mutation induction to generate genetic variability suitable for selection to improve sterile *Musa* clones. In addition, somaclonal variation has been used as a secondary source of variability for the genetic improvement of *Musa* (Jain, *et al.* 1998; Novak, *et al.* 1993; Remotti, 1998; Vuylsteke, 1998 and Hwang *et al,* 1994).

1.3 The objectives of the study

The objectives of this study includes:

(1) To generate genetic variability in Pisang Berangan (AAA) through somaclonal variation induction by tissue culture techniques and *invitro* mutation induction by gamma irradiation. The study consists of different aspects on micropropagation, mutation induction, field evaluation and characterization, changes due to mutagenic effects and screening for disease resistance.

Mutation is the ultimate source of genetic variation that provides the raw material upon which other factors of evolution act. The process of occurrence of mutation (mutagenesis) followed by recombination of genes and chromosomes, and by natural selection, is the fundamental force in evolution (Van Harten, 1998). Mutation induction is a valuable complementary tool to conventional breeding.

Somaclonal variation provides additional source of variation for the vegetatively propagated bananas. Ahloowalia (1998) explained that meiotic studies showed that this variation is associated with chromosomal variation, which involved numerical changes in the whole genome. Such changes are usually observed among plants, which have been treated with high doses of gamma rays.

(2) To study mutagenic effects at morphological, cytological and molecular levels.

Variability generated by gamma irradiation and through somaclonal variation, induced through tissue culture are evaluated for changes in morphology, molecular variation (by

RAPD analysis), nuclear DNA content and chromosome changes by Flow Cytometric analysis. At the same time, the feasibility of using Gibberellic acid (GA_3) to detect dwarf off-types was also attempted.

(3) To screen for resistance or tolerance to *Fusarium* wilt disease.

Mutants generated through gamma irradiation and somaclonal variation are subjected to challenge with the pathogen *Fusarium oxysporum* f. sp. *cubense* in the *Fusarium* "Hot-spot" in the field or using "double –tray" method for screening plants at nursery stage.