

Achievement
Sub-Project on Disease Resistance in Banana
(2004 – 2010)

Mutation Breeding Project
Forum for Nuclear Cooperation in Asia (FNCA)
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Preface

Banana (*Musa* spp.), as dessert fruit and plantains for cooking, is one of the most important crops in tropical and sub-tropical countries. In many Asian and African countries, bananas are important staples next to rice, wheat, and maize. According to FAOSTA, the total production of bananas in the world accounted to 95.6 million tons in 2009. Currently, banana is one of the major fruit crops in the international market. According to FAOSTAT the total world export of banana was 18.0 million tons in 2008. Japan imports about 1 million tons in 2008 making the fourth biggest importer of bananas next to USA, Belgium and Germany. At present, India is the number one producer of banana but the product is consumed domestically to meet the high demand for bananas. For countries such as the Philippines, the third biggest exporter of bananas after Ecuador and Costa Rica, banana generates income and sustains food security for small holder farmers and workers in plantations. Meanwhile, the various production systems, consumption and trading forms, and genetic diversity of cultivated bananas are not well established because the popular banana varieties and cultivars used in each countries and in the international markets are limited. Hence, the limited genetic diversity in bananas makes them prone to pests and diseases. Likewise, it is difficult to find genetic sources for breeding of disease resistant varieties. As most bananas are propagated vegetatively, conventional cross breeding is not possible due to its sterility. With this, mutation breeding is a promising tool to improve banana varieties.

Presently, there are four major diseases threatening the global banana production. These are Fusarium wilt or Panama disease caused by a fungi, *Fusarium oxysporum* f.sp. *cubense* (Foc), black leaf streak diseases or black Sigatoka caused by a fungi, *Mycosphaerella fijiensis*, bacterial wilt caused by *Ralstonia solanacearum* together with recently emerged *Xanthomonas campestris* pv. *musacearum*, and banana bunchy top disease caused by *Banana bunchy top virus* (BBTV). As diseases cause serious and expanding threat to banana, farmers and producers straggled to overcome this problem. The current practice to overcome the problem such as cultural control and chemical application however remain partial in controlling these diseases. We conducted mutation breeding to produce Foc and BBTV resistant lines.

The Mutation Breeding Project focused on the improvement of Fusarium wilt and BBTV resistance in banana as a sub-project developed through the Forum for Nuclear Cooperation in Asia (FNCA), organized by the Ministry of Education, Culture, Sports, Science and Technology of Japan (MEXT), in 2003. The sub-project was established in collaboration with member countries including Malaysia, the Philippines, Vietnam and Bangladesh with partial collaboration of Indonesia. With the termination of the sub-project we are happy to publish a book entitled Achievement of Sub-Project on Disease Resistance in Banana. Primarily, the book aimed at maximizing the mutation breeding technology through gamma-ray irradiation.

We strongly hope that the outcome of this sub-project such as the developed promising lines, technologies and information which we shared and human network built during the project implementation will benefit the banana farmers and researchers in Asia. We also expect that this book will be useful not only for the breeders interested in gamma ray induced mutation breeding but also for the breeders of bananas.

Finally I would like to express my gratitude to the contributing authors for their achievements in this sub-project. Special thanks are due to individuals who cooperated generously in doing experiments inside and outside of the laboratories. We acknowledged Dr. H. Nakai, our Project Leader, Dr. H. Nakagawa, former Project Leader, for their academic support and the assistance given by the staff of FNCA, Japan.

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1. General Background

1.1 Banana production

1.1.1 World

Musa spp., banana and plantain, constitute the fourth most important staple food commodity of the world, after rice, wheat and maize. In Asian and Pacific regions, banana has great socio-economic significance. The region is the major centre of diversity and most of the edible bananas are believed to have originated in South-east Asia and Western Pacific regions. Cheesman (1948) first suggested that cultivated bananas originated from intra and interspecific hybridization between the two wild diploid species *Musa acuminata* Colla. and *Musa balbisiana* Colla., each contributing the A and B genomes respectively. Through complex hybridization events between two species, *Musa acuminata* and *Musa balbisiana*, we have now banana cultivars with acuminata characteristics (AA, AAA, AAAA), balbisiana characteristics (BB, BBB, BBBB) and both acuminata and balbisiana characteristics (AB, AAB, ABB). Likewise, cultivated bananas are referred to by their genome groupings. The crop encompasses a range of diploids, triploids and tetraploids. The identification of *Musa* cultivars has traditionally been based upon various combinations of morphological, phenological and floral criteria. Simmonds and Shepherd (1955) devised a scoring technique based on 15 diagnostic morphological characters to differentiate *M. acuminata* clones from *M. balbisiana* cultivars and their hybrids into 6 genome groups. According to this system, cultivated dessert and East-African highland bananas are classified as AAA whilst plantains are AAB. Though in local consumption, diversified banana cultivars are maintained and used in banana trade, Cavendish subgroup (AAA) is an extremely important. There exist other genome combinations, for example ABB and ABBB. They occur naturally or are produced by artificial hybridization (Stover and Simmonds, 1987).

Banana is now distributed in most of tropical and sub-tropical countries. The world production of banana was about 95 million tons and most of the production was consumed locally (Ganapathi et al., 2002). Meanwhile, two decades ago the world banana and plantain productions are of 41 and 20 million tons respectively (FAO, 1984). This indicates that the world banana production trends increasing significantly as staples and export commodity. Recent FAOSAT (2008~) tells that total banana production in the world was 96 million tons in 49 million ha in 2009. In South-east Asian countries produce 19.0 million tons. Major banana exporting countries are Ecuador (5.2 million tons), Costa Rica (2.0 million tons), the Philippines (1.9 million tons) followed by Colombia and Guatemala. We also notice, however, that in many Asian and Pacific countries, bananas are mainly consumed domestically. India, for example, is not a banana exporting country but the largest banana producer in the world with 26 million tons followed by the Philippines (8.6 millions tons) and China (8.0 millions tons). As banana importing countries, many non-banana producing and developed countries are listed such as USA (4.0 million tons) and Japan (1.0 million tons).

Production and horticultural characteristics of bananas are well documented by Robinson and Saucó (2010). Status of Banana production in each participating country is as follows.

1.1.2 Bangladesh

Banana is one of the most important food and cash crop in Bangladesh and grown around the year in the country as a commercial purpose and homestead area for local consumption. In addition, banana stood first position among the fruits producing in the country and supplies 42% of the total fruit requirements in the country and also its financial return as a crop is higher compared to other fruits and field crops (Haque, 1988b). Banana is the first cultivated crop in South-east Asian region (Sauer, 1952). The first report in banana cultivation came to know from Indian subcontinent region (Reynolds, 1951). The first user of banana is known to South-east Asian countries (Simmonds, 1966). It is also a nutritious fruit crop in the world and grown in many tropical areas where they are used both as a staple food and dietary supplements (Assani *et al.*, 2001).

In Bangladesh, total banana production in year the 1999-2001 was about 0.580 million tons but it increased to 0.654 million tons in the year 2003 (FAO, 2004). In the year 1975 - 1976, the total banana cultivated area was 37,200 ha and it was increased to 49,280 ha in the year 2003 - 2004 and total production was increased 0.5691 to 0.7065 million tons due to increasing cultivated area but yield was decreased during those days from 15.07 t/ha to 14.33 t/ha due to constrains of banana cultivation (BBS, 1980, 2003). The average yield of banana in the country is about 15 t/ha that is far below the average world yield 30.63 t/ha in India (FAO, 2006 - 2007). Through banana cultivation in the country the net income per hectare at about TK. 297,690/= ~USD 4,252.7 (Haque, 2008). The total per capita consumption is about 4.7 kg. This is very much lower than that consumed by Europe especially Belgium (26.7 kg), Sweden (16.7 kg) and Germany (14.5 kg) while USA consumed 13.1 kg and UK at 10.5 kg (Siti Hawa, 1998). Thus the potential for expansion of banana cultivation and increase per hectare yield is needed in the country. Although bananas are important export commodities of some developing countries in Africa, Latin America and the Asia, unfortunately Bangladesh is not exporting country but bananas are consumed by locally. Banana is a very versatile crop. The whole plant or fruit, leaves, stem and other plant parts play a major role in the daily activity or use by the local population where they have multiple uses. While some of the uses form part of the daily activity of the population such as food wrapper, whole plants at ceremonies, landscaping, pharmaceuticals, rope, paper and viable commercial industry.

There are 32 landraces including dessert and cooking banana cultivar in Bangladesh (Haque, 1985a). The popular dessert bananas in the country are Amritasagar (AAA), Sabri (AAB) and Champa (AB) but their yield is not satisfactory. Moreover, the second leading commercial cultivar Sabri (AAB) is highly susceptible to Panama disease (*Fusarium wilt*) and total crop failure due to this disease has been reported. A local seeded cultivar Bichikala (BB) derived from the wild species of *Musa balbisiana* (BB) is resistant to most of the diseases. But the main constrain of the fruit is that it contains huge number of seeds that makes them less popular to the local people. The cooking cultivar is locally called Anajy kala (ABB).



Sabri AAB



Sagar AAA

1.1.3 Malaysia

Bananas (*Musa* spp.) are amongst the most important food crops in the world. Global banana production has been estimated to be about 99 million tons annually, mostly produced by tropical countries (FAO, 2003). Banana is one of the important fruit crops cultivated in Malaysia. It is ranked second in terms of production area and fourth in export revenue based on the balance of trade figures. This crop will remain as an important industry, emphasis given to this crop in addition to the other fruit types listed under the National Agricultural Policy.

In Malaysia, banana is the second most widely cultivated fruit, covering about 26,000 ha with a total production of 530,000 metric tones with more than 15 % of the yearly production and a balance of trade of more than RM30 million (US\$8 million). About 50 % of the banana growing land is cultivated with Pisang Berangan and the Cavendish type. However, banana production in Malaysia has decreased because of an increasing threat of diseases (particularly Fusarium wilt), high labor costs and marketing issues. However, it is still popular grown and contributes about 16 % of the total fruit production areas. Banana remains the second most important fruit crop (after durian) in Malaysia, amounting to about 15% of the total acreage under fruits. Traditionally, it is planted as a cash crop or temporarily intercropped with oil palm, rubber and other perennial crops. There are only a few large banana plantations in Malaysia. The popular dessert cultivars are Mas (AA), Pisang Lemak Manis (AA), Berangan (AAA), Rastali (AAB), Embun (AAA) and Cavendish (AAA); while the popular cooking cultivars are Nangka (AAB), Raja (AAB), Awak (ABB), Abu (ABB), Tanduk (ABB) and Relong (AAB). Most of the bananas produced were consumed locally and about 10% are exported, mainly to Singapore, Brunei, Hong Kong and the Middle East.

Banana cultivation is largely a smallholder enterprise where farms are small, unorganized and farmers often adopt inferior technology. Apparently, this production practice often results in low yield and inferior quality. Poor quality has been a major constraint to export of fresh fruits, including banana. It is therefore necessary to adopt good production practices and inefficient postharvest handling to ensure consistent supply of high quality banana for export. Efforts are being undertaken to more than double the production figures in the next 5 or more years. It is envisaged

that this can be achieved through increasing the production areas, increasing the yield per unit area and enhancing the production technology.

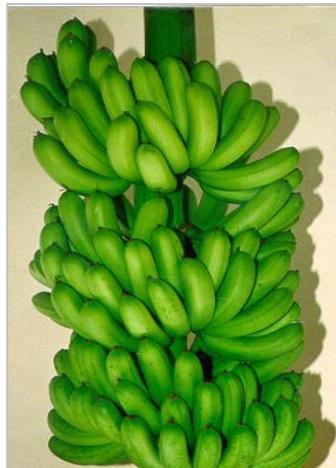
Popular Dessert Bananas



Pisang Mas (AA)



Pisang Lemak Manis (AA)



Pisang Berangan (AAA)



Pisang Rastali (AAB)



Pisang Embun (AAA)



Pisang Cavendish (AAA)

Popular Cooking Bananas (Plantain)



Pisang Nangka (AAB)



Pisang Awak (ABB)



Pisang Abu (ABB)



Pisang Raja (AAB)



Pisang Tanduk (AAB)



Pisang Relong (AAB)

1.1.4 Philippines

Banana is one of the most important fruit crops in the Philippines both for domestic and export markets. The total area for banana production amounts to 446,371 ha with a total production of 9.01 million metric tones valued at about 16 billion pesos (BAS, 2010). %. Banana is grown in all parts of the country with the export banana grown mostly in southern Philippines. More than 75% of the banana producers are small-scale farmers engaged in local or domestic production.

The three major banana cultivars are Saba (BBB) a cooking banana for domestic and export market (banana chips), followed by Cavendish banana (AAA) dessert banana mainly for export, and Lakatan (AA) dessert banana grown for domestic market and as novelty banana for the export market. Of the total banana production of the country, Saba cultivar contributes about 41.5%, Cavendish 17.4% and Lakatan 12.6%. Lakatan is the most popular dessert banana grown for domestic market with a total area of production of about 57,000 hectares and production volume of 0.92 million metric tones (BAS, 2010). The area planted to Lakatan has increased by more than 3,000 has in the last three years (BAS, 2010).

Major banana cultivars grown in the Philippines



Saba (cooking banana)



Cavendish (dessert banana)



Lakatan (dessert banana)

1.1.5 Vietnam

Banana has been grown for thousands of years in Vietnam. It is now one of the most important fruits growing in Vietnam. Its total cultivated area is estimated at 99 340 ha and production is about 1.2 million tons 20% of which are for exportation. The banana cultivation is based on small-scale garden, usually surrounding the household, and on hillside. The common size of banana gardens ranges from 0.2 - 1.0 ha. Bananas are intercropped with other crops like maize, soybean, sweet potatoes or fruit trees. Compared with sole banana gardens, the productivity is lower in mixed banana gardens. The average yield is about 10 - 15 t/ha, depending on the region and the farmer's cultivation level.

The popular banana cultivars in Vietnam are as follows;

Chuoï Tieu (AAA/Gia): This Cavendish group consists of the most popular banana cultivars in Vietnam, which can be divided according to three different plant height; tall (2.8 - 3.5 m), medium (2.0 - 2.5 m) and dwarf (1.5 - 2.0 m). They are grown alongside rivers and highly humid areas. They give high yields of 20 - 25 kg/bunch, with 8 - 14 hands/bunch. Its fruit size is 2.8 - 3.5 cm. Ripened fruits are sweet and aromatic and have yellow skin and flesh. In the North, during the winter, the ripened fruits have a better quality compared with those grown in the South. Its growth duration is 14-15 months. Chuoï Tieu is used for export and local market.

Chuoï Tay (ABB, Xiem): It is planted throughout the country, from the delta to hilly regions. Its pseudostem is 3 - 4 m long. It gives high yield with 18 - 20 kg/bunch, 8 - 12 hands/bunch. The fruit size is 9-11 cm long and 3.0 - 3.5 in diameter. The ripe fruits have dark yellow skin and yellow, sweet and aromatic flesh. Sometimes, there are few seeds in the fruits. Chuoï Tay is tolerant to drought and poor soil. Chuoï Tay is used only for domestic consumption. They also can be eaten as fresh or processed as candies, cake, boil, etc.

Chuoï Ngu (AA, Cau): It is one of the most preferred varieties because of its special characteristics. Pseudostem is 2.2 - 2.6 m long. Normally, its yields are 8 - 10 kg/bunch with 6 - 8 hands/bunch. The fruit size is 7 - 10 cm long and 2.5 - 3 cm in diameter. Ripened fruits have attractive bright yellow and pink, color and a sweet and aromatic flesh. The growth duration is 12 months.

Chuoï Ngu Tien (AA): In the olden days, this variety was used as precious donations to the kings which is why the variety was given the name Tien (donation- Dai Hoang). The fruit's characteristics and growth duration are similar to those of Chuoï Ngu but its fruits have a very attractive form and color and the flesh has better quality. Pseudostem is 1.5 - 2 m in height. These cultivars are grown nowadays in Nam Ha province.

Chuoï Bom (AAB): It has a high tolerance to drought and is grown 137 popularly in the central highlands. It has a short growth duration (10 - 12 months) and high multiplication rate (8 - 10 suckers/plant). Its yield is 6 - 10 kg/bunch with 6 - 8 hand/bunch. Fruit size is 10 - 15 cm and 2 - 2.5 cm in diameter. Ripened fruits have bright yellow thin skin and yellow pink flesh, and are suitable for processing to make dried banana. These popular banana cultivars are shown in Figure 2 (Nhi 1997). There are some other cultivars such as Chuoï Com (AA), Chuoï Bot (AAB), Chuoï

La (ABB), Chuoi Mat (AB), scattered over the different areas. These can be used as feed, cake draping etc.

1.2 Major constraints of banana production



Banana infected by Fusarium wilt disease

1.2.1 World

Among many constraints in banana production in the world as well as in Asia, two diseases are targeted in this Sub-Project. They are Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc) and Banana bunchy top disease caused by *Banana bunchy top virus* (BBTV).

Fusarium wilt often known as Panama disease is very famous and destructive disease. Since the disease was first reported in Australia in 1876, the symptoms such as wilt and collapse of leaves, as well as discoloration of vascular system, cause serious damage in many banana-producing countries in the world. At the first pandemic of this disease in the 1950s, the production of a susceptible variety, Gros Michel, was almost wiped out in Central America. Cavendish (or varieties in Cavendish subgroup), then introduced as a resistant variety, is now susceptible to a specific race of Foc. Among three races of Foc, which attack bananas, race 4 is most pathogenic and affects many susceptible banana cultivars including Cavendish. The pathogen, Foc, can be disseminated through suckers, soil, water, and by farming practices when farmers use contaminated tools. Chemical control, such as soil fumigation, is promising measure but give strong impact to the environment. As the pathogen persists in the contaminated soil by producing chlamydo spores even in the absence of the host bananas or sometimes by infection of roots of some weeds. As a result, once the field is invaded by Foc, the field cannot be used for banana production up to 30 years. Resistant varieties and/or clones have been produced but not many of them are favorable and marketable in local and international markets.

BBTV, a virus species in the genus Nanovirus, has been reported in many banana-producing countries in Asia, Africa, and Oceania but not in Central and South America. The virus is transmitted in a persistent manner by banana aphid (*Pentalonia nigronervosa*) and through vegetative propagation. Cultural control such as roguing infected plants, control of aphids by pesticides, and replacement by virus-free seedlings if available, is only practical measure taken at present. Thus, once the virus invaded and established in the area, it is almost impossible to manage the disease.



Typical banana bunchy top disease symptom showing yellowing and erected leaves.

Conventionally protection against banana diseases may account for more than 40% of the total production cost and the farmers without good access to chemicals have to cope with yield declines of 30% or more (Sagi et al., 1998). The difficulty in control of these two diseases, Fusarium wilt and banana bunchy top disease, requires the production of resistant varieties. To follow the preferences of farmers, traders, and consumers, mutation breeding is most promising because mutation breeding realizes the rapid production of new resistant varieties with the favorable traits in original ones. Jain and Swennen (2004) reviewed banana improvement technologies including gamma irradiation.

1.2.2 Bangladesh

There are several non-biological and biological constraints affecting banana production in the country and abroad. Out of them a few can cause serious economic losses. Banana is mainly tropical crop and 27°C temperature is optimum for normal growth and development. If temperature raised above 38°C the growth and development stopped and if temperature fall down to below 10°C then crop period extended and reduced the bunch weight (Haque, 2008). Draught, water logging condition and in adequate sun light also cause crop damage and yield loss. At present no non-biological resistant variety developed yet although in Bangladesh the cultivar Bichikala (BB) have some degree of tolerant to insect-pest-diseases and draught and also water logging condition. Biological constraints such as insect-pest-diseases are also cause serious damage and yield loss of banana. The most damaging of the fungal disease is panama disease or Fusarium wilt caused by *Fusarium oxysporum* f. sp. *cubense* (Foc). Infection by this fungus will result in yellowing and wilting of the leaves which eventually turn brown, the leaves will dry up and hang from the plant and eventually the plant dies. Longitudinal sections of the pseudo stem will show sign of vascular discolouration and this discolouration will be more distinct in the corm tissue. Most of our cultivars are more or less susceptible to this disease. Among the cultivars, Sabri (AAB) is highly susceptible to this disease. At present no resistant variety developed against this disease in the country and abroad and chemical control also is not available. Foliage of banana is susceptible to sigatoka leaf spots. *Mycosphaerella fijiensis* causes black sigatoka and *Mycosphaerella musicola* causes yellow sigatoka disease is considered as the most serious disease of banana resulting in yield loss. In Bangladesh, Amritasagar (AAA) is susceptible to this disease. *Ralstonia solanacearum* (*Pseudomonas solanacearum*) causes Moko disease is similar to Fusarium wilt also resulting yield loss, which not very often have seen this disease in the country. Many plant parasitic nematodes were associated with banana plants in the world. The most common nematodes are *Meloidogyne* spp., *Rotylenchulus reniformis*, *Helicotylenchus dihystrera* and *Radopholus similes*. Those are reported to be the most damaging in banana producing countries (Stover and Simmonds, 1987). In Bangladesh *Radopholus similes* is very common and our most of the banana and plantain cultivars are affected by this nematode (Haque, 2008). There are a number of virus diseases of economic importance affecting banana production worldwide. *Banana bunchy top virus* (BBTV) is reported to be affected by some of our cultivar. The virus is transmitted by aphid. Banana leaf and fruit beetle

and also pseudo stem borer causing yield loss and reduced market value. In ensuring high quality banana for the consumers, post-harvest diseases must be controlled to prevent rotting and losses during handling and storage. Bananas after harvest can be infected by anthracnose, crown rot, fruit rot and neck rot (Nik Masdek *et al.* 1998). In Bangladesh more or less we are having those diseases in bananas. At present against those diseases has the precaution measure and chemical control. Low reproductive fertility and slow propagation rate is also one of the constraint of banana production.

1.2.3 Malaysia

In Malaysia, banana production by smallholders involving small farm sizes are not well organized and with low inputs and poor quality planting materials, thus resulting to lower yield and fruit quality. The large scale growers uses better quality planting materials (disease-free tissue cultured plantlets) and higher inputs; and adopt more modern technologies, good agricultural practices and better pest and disease management, thus, higher yield and better quality fruits. In Malaysia, the diseases caused by fungi and nematodes are the major limiting factor in successful quality production of this crop and almost all the commercial cultivars of banana are highly susceptible to certain deadly diseases.

The most serious constraint to the production of banana and plantains is considered to be Fusarium wilt or Panama disease caused by *Fusarium oxysporum* f. sp. *cubense*, a soil-borne disease which affects many important cultivars of banana and plantain. The disease has caused serious crop losses in Malaysia and it is a devastating disease of banana worldwide. Most of the cultivated clones originated as spontaneous variants are highly susceptible. Pisang Berangan is susceptible to *Fusarium oxysporum* f. sp. *cubense* Foc Race 1 and very susceptible to Foc Race 4. Infection occurs through roots and progresses to the pseudostem. Symptoms are internal stem necrosis (reddish or reddish-brown xylem), root and rhizome rot, yellow leaves, plant wilting, and plant death. Plants may die during flowering or during periods of moisture stress. The fungus may survive for a long period of time in the soil.

Field evaluation of disease-tolerant banana plants in soil infested with *Fusarium oxysporum* f. sp. *cubense* (Foc) is highly effective. However, it is slow because disease expression usually takes 4-5 months; and factors affecting disease expression such as inoculum concentration, edaphic conditions, temperature and other variables are difficult to control. An alternative method of pre-screening of seedlings at the nursery stage turned out to be effective and can greatly save cost, labour and time. Only those that showed no symptoms of infection from *Fusarium oxysporium* were further planted and screened in the field.

Black sigatoka or black leaf streak caused by *Pseudocercospora fijiensis* (syn. *Mycosphaerella fijiensis*) is another serious disease of banana in Malaysia. It is globally distributed and epidemic in many locations and is the most important disease of *Musa* worldwide. Common symptoms develop are reddish-brown streaks appear initially on the undersides of the third or fourth youngest leaf; streaks develop into elongated spots with gray or tan centers and dark brown to black margins; lesions may be surrounded by yellow halos; lesions may coalesce to form large, blighted areas of

leaves in parallel with leaf veins, or bands of dark streaks, causing leaves to turn brown and wither. Significant defoliation may occur whereby a banana plant may have only a few or no green (disease-free) leaves upon flowering. Chemicals can be used to control the pests but as the costs, both economically and environmentally, continue to rise, the need for resistant cultivars as the main component of an integrated system for pest management, becomes imperative.

Nematodes are also considered as major causes of diseases of banana in Malaysia. The root-knot nematodes (*Meloidogyne* spp.) and the burrowing nematode (*Radopholus similis*) can significantly weaken root systems, reduce yields, topple plants before harvest, make plants more prone to wind knockdowns, reduce fertilizer uptake and thereby reduce the banana-growing lifespan. Nematodes can be managed by using clean (nematode-free) planting material, heat treatment of planting material, pre-plant soil fumigation, crop rotation, mulching and composting, fallow, chemical nematicides, plant propping, fertilizer use, and varietal resistance.

1.2.4 Philippines

Banana bunchy top virus (BBTV) remains as the most destructive virus disease of banana (*Musa* spp) in the Philippines. It causes stunting and leaf malformation that leads to premature death of infected plant, thus causing yield losses of about 100%. The spread of the disease is greatly aided by an aphid vector, *Pentalonia nigronervosa*. Most dessert banana cultivars including Lakatan are very susceptible to BBTV.

While rapid propagation of disease-free planting materials is a viable disease management option for BBTV, its effectiveness is limited where residual or alternate inoculum sources are present. Regular replanting has to be done as the initially disease-free plants get infected within one or after a few growing seasons. Even in a well managed farm BBTV re-infection in the field ranged from 20-50%.

Another major problem of banana is the nematodes. Nematodes are present in most banana growing areas. The most common species associated with bananas are *Radopholus similis*, *Meloidogyne incognita*, *Pratylenchus* sp. and *Helicotylenchus multicinus*. Of the four species, *R. similis* is considered the most destructive as it causes root rotting leading to toppling down, reduction in bunch weight and death especially when younger banana plants are infected. In the field, when the number of *R. similis* reaches 4,000, a corresponding yield reduction of 60% is observed. On the other hand, *M. incognita* causes root galling, and reduction in bunch weight. Yield loss of 45% is obtained when the *M. incognita* reaches 10,000 larvae. In previous study, Lakatan is susceptible to both nematodes under screenhouse conditions.

1.2.5 Vietnam

In a survey conducted by D.V. Thanh in the north and by Nuong in the south Vietnam in 2000, a total of 19 pathogenic micro-organisms, 4 nematode species and several insects were observed on bananas grown in the field. The most important and popular banana cultivars in Vietnam belonging to genome group AAA/AA such as Chuoi Tieu, Chuoi Bom, Chuoi Ngu, Chuoi Cau and Chuoi

Com are affected by sigatoka and bunchy top diseases. The symptoms of *Banana streak virus* (BSV) are often recorded on Chuoi Cau Lun (AAB). Another important disease is Fusarium wilt caused by *Fusarium oxysporum* f.sp. *cubense* (Foc), which attacks on genome group ABB/BB. Presently, Foc also caused damage in Chuoi Cha Bot (AB/ABB). Major insect pests recorded to infect banana such as corm borer are considered to be very destructive. Banana aphids play an important role in spreading viral diseases. In 1997, Thanh *et al.* recorded that 28 nematode species are parasitic on banana with four important species: *Helicotylenchus* sp., *Pratylenchus coffeae*, *Meloidogyne incognita* and *Radopholus similis*.

2. Mutation Breeding Technology

2.1 Mutation induction for banana improvement

2.1.1 Bangladesh

Mutation breeding is a process by which a gene undergoes a structural change or substitution of one nucleotide for another. A variant emerges, differing genetically and often visibly from its parents and arising rather suddenly or abruptly. Mutation can occur naturally or can be induced by radiation or chemically.

Banana is the world's major food crops and considered as the poor man's fruit in tropical and sub-tropical countries. Edible bananas are mostly sterile polyploids and must be propagated vegetatively. Hence, genetic improvement through cross breeding is not possible. Mutation breeding has been suggested as an excellent alternative approach for banana improvement (Guzman, 1975). Genetic variation is the starting point of any breeding program. Genetic variation may already be present in nature, may be obtained after several years of selection or may be produced through hybridization. For vegetatively propagated crops such as edible banana, it is difficult to obtain genetic variation, because of sterility and polyploidy nature of the crop. Spontaneous somatic mutants have played an essential role in the speciation and domestication of plantain and banana. All bananas and plantains that we grow and eat were selected in prehistory from spontaneous mutations (Buddenhagen, 1987). Radiation can be used to induce mutations at a higher frequency and generate genetic variation from which desired mutants may be selected. Therefore, mutation techniques are important as tools for plantain and banana improvement. Still there is a lot of scope to yield improvement of banana for enhanced food security, nutrition and employment generation. Progress in the development of various biotechnologies has greatly contributed to the application of induced mutations in a wide range of plant species. This is new opportunities to induce mutation in vegetatively propagated crops by the use of *in vitro* techniques. The large scale rapid multiplication of the resulting mutants and characterization using molecular markers is necessary to understand gene structure and gene function. Generally, radiation and especially gamma rays have most often been used to generate desired characters for crop breeding. This technique can be used in many crop species, often those with narrow genetic variation, to improve individual or specific characters in local cultivars that are particularly adapted to specific environmental conditions. Induced mutation techniques are particularly important for banana and plantain where there is limited sexual reproduction that could generate genetic variation. Several researchers suggested that mutagenic agents for induction of resistance to several diseases using regenerating adventitious buds of banana are effective (Panton and Menedez, 1972; Menedez, 1973).

2.1.2 Malaysia

Cultivated bananas are *parthenocarpic*, which makes them sterile and unable to produce viable seeds. Propagation typically involves removing and transplanting part of the underground stem (called a corm). Usually this is done by carefully removing a sucker (a vertical shoot that develops from the base of the banana pseudostem) with some roots intact. It is not necessary to include the corm or root structure to propagate bananas; severed suckers without root material can be propagated in damp sand, although this takes somewhat longer. Within the last 15 years, commercial propagation occurs by means of tissue culture. This method is preferred since it ensures disease-free planting material. When using vegetative parts such as suckers for propagation, there is a risk of transmitting diseases, especially Fusarium wilt, Black Sigatoka and also nematodes.

Most edible bananas are considered to be sterile, therefore genetic improvement through cross-breeding is not possible. Mutation breeding through vegetative propagation has been suggested as an excellent alternative approach for banana improvement (Valez et al., 1972, Siti Hawa et al., 1996). In addition, the heterozygosity of asexual banana clones makes them suitable for mutation induction. The heterozygotic status is expected to be Aa in loci of diploid cultivars while the triploids of A genomic types can exist in either Aaa or Aaa forms. For interspecific hybrids, the heterozygotic constitution could be AaB, Aab, AAb, ABb, aBb or aBB. Mutation induction may uncover a recessive phenotype by mutating, inhibiting or deleting the corresponding dominant allele (Novak et al. 1990).

Mutation is a single cell event and one advantage to utilize mutation breeding is that a reduced breeding time can be achieved. In a conventional cross-breeding program, if a useful trait is to be transferred into an economically important cultivar, this will necessitate the crossing of the economically important cultivar with perhaps a less desirable variety possessing a single advantageous agronomic trait. It will then be necessary to take the F₁ hybrid and backcross it to the superior parent cultivar for at least 7-8 generations, with continued selection of individuals possessing the particular useful trait. In a mutation breeding program, the same breeding objective may be able to induce a mutation of the desired trait in the economically important cultivar. If the trait is successfully obtained in the M₂ generation, purification of the mutant requires only 1 or 2 additional generations. In a mutation breeding program it is important to understand the mode of reproduction of the crops (sexual or asexual, self-pollinated or cross-pollinated). In addition, especially in banana, understanding how the ploidy level (diploid or polyploid) may affect trait expression is also an important consideration.

The use of cultured shoot tips for mutagenesis has facilitated mutation induction and the regeneration of potential mutants (Novak and Micke, 1990). An early flowering mutant of Grand Naine, GN-60Gy was induced by Novak (1990) after exposing shoot tips to gamma radiation. Further selection of GN-60Gy in Malaysia has resulted in the release of an early fruiting Cavendish banana called Novaria (Mak Chai et al., 1996).

2.1.3 Philippines

The germplasm of commercially important banana cultivars, both dessert (AA, AAA) and cooking (ABB, BBB) bananas, are not amenable to sexual breeding because they are male and female sterile. Thus, BBTV resistance could not be introgressed into bananas by conventional breeding methods. In addition, there is no known resistance to BBTV in the banana germplasm. In other crops, it has been demonstrated that it is possible to obtain resistance to pests and diseases by variation brought about by irradiation, chemical mutagens, somaclonal variation or by a more direct method of *in vitro* selection in the presence of the stress factor (Roux, 2004).

Mutation and *in vitro* technologies offer opportunities to enhance genetic variability for the improvement of agronomic traits such as disease resistance, earliness in fruiting, yield and quality (Bhagwat and Duncan, 1998; Ho *et al.*, 1994; Mak *et al.*, 1996; Novak *et al.*, 1993; Roux, 2004; Smith *et al.*, 1995). In addition, the availability of tissue culture techniques aids in the induction, selection and multiplication of mutants. Plant regeneration from banana shoot tips (Damasco and Barba, 1984; Damasco *et al.*, 1984) is well established.

2.1.4 Vietnam

The national banana network consists of many research institutions, laboratory and agricultural cooperative conducting different activities in their area of expertise as The Vietnam Agricultural Science Institute (VASI), the National Repository, Multiplication and Distribution Centre, is in charge of conservation of *in-vitro* collection, its propagation and distribution for evaluation, testing and field trials. The Southern Fruit Research Institute (SOFRI) conducts activities such as maintenance of a field collection, disease indexing, production of disease-free planting materials, field trials and postharvest technology. The Fruit and Vegetable Institute established a procedure of banana micro-propagation, provides *in-vitro* plantlets to farmers, and implements trial for *Fusarium* evaluation. The Institute of Agricultural Genetics (AGI) applies mutation technique in plant breeding and molecular technique (RAPD, PCR etc.) in studying banana biodiversity and virus indexing. Fusarium wilt is the second important diseases of banana. It attacked Chuoi Tay (Pissang awak-ABB), Chuoi Ngop (Bluggoe ABB), and huoi Com La (Silk-AAB). Average frequency of infected plants in Chuoi Tay cultivar in summer (24.3%) was higher than in winter (13.2%) in north Vietnam. In the regions which Chuoi Tay was grown much, frequency of infected plant was increased (Table 2). They may cause up to 85% yield loss in banana. So that we focused on application of mutation technique in generate a new mutant local banana for resistance to the disease by Foc.

2.2 Selection of target banana cultivars

2.2.1 Bangladesh

The popular dessert banana in Bangladesh is Sabri (AAB) but the yield is not satisfactory. Moreover, the second leading commercial cultivar Sabri (AAB) is highly susceptible to Panama disease (*Fusarium wilt*) and total crop failure due to this disease has been reported. The cultivar also can not tolerate water logging condition. These are the main reasons to choose this cultivar to overcome those problems. A local seeded cultivar 'Bichikala (BB)' derived from the wild species of *Musa balbisiana* (BB) is resistant to most of the diseases and pests and also has some degree of tolerant to draught and water logging condition. In addition, it is sweeter than that of seedless popular banana cultivar produced in the country and it has much more medicinal value as well. It has also high keeping quality and widely grown in the country in homestead area. But the main constraint of the fruit is that it contains huge number of seeds that makes them less popular to the local people. Therefore, this cultivar is chosen to create genetic variations such as seedless or less seeds in the fruits with improve agronomic traits through the production of doubled haploids. The cultivar Sabri is seedless table fruit and belongs to the genome (AAB). The plant is 2.5 to 3.0 meter tall. It takes 8 to 9 months for flowering after plantation of sucker and another 3 to 4 months needed to ripening the fruit. The total bunch weight is about 9 to 10 kg, in which 7 to 10 hands/bunch and 10 to 12 fingers/hand. Per hectare yield is 14 to 15 tones. The fruit peel became bright yellow when ripen and the peel is very thin. The fruit pulp is off-white in colour. The cultivar Bichikala is a seeded banana eaten as vegetable before ripen and also eaten as a table fruit when ripen and belongs to the genome (BB). The number of seed per fruit is about 150 to 250 and the seeds are soft and brownish in colour. The plant is very strong and tall of about 4.5 to 6.5 meter. It takes 10 to 12 months for flowering after plantation of sucker and another 4 to 5 months needed to ripening the fruit. The total bunch weight is about 15 to 20 kg, in which 10 to 15 hands/ bunch and 9 to 12 fingers/hand. Per hectare yield is about 20 to 25 tones although this cultivar is not producing commercially. The fruit peel became yellowish when ripen and fruit peel is very thick. The fruit pulp is off-white in colour.

2.2.2 Malaysia

The popular dessert cultivars in Malaysia are Mas (AA), Berangan (AAA), Rastali (AAB), Embun (AAA) and Cavendish (AAA). However, Pisang Berangan (AAA) is the most popular banana, having good fruit quality, flavour, colour, pulp texture, size and shelf life. About 50% of the banana growing land is cultivated with Pisang Berangan and the Cavendish type for local consumption and export market to Singapore. However, it is relatively tall and very susceptible to *Fusarium wilt* (*Fusarium oxysporium* f. sp. *cubense*) and freckle disease caused by *Cladosporium musae*. Therefore, Pisang Berangan was selected as the target banana cultivar for mutation induction study to select potential mutants with improved traits such as tolerance or resistance to *Fusarium wilt* disease, short plant stature and early fruiting and high bunch weight.

2.2.3 Philippines

The banana cultivar Lakatan (AA) was used as the initial material for mutation induction. The cv Lakatan is the most popular dessert banana grown for domestic market and as novelty banana for export market. Lakatan is very susceptible to BBTV. The Lakatan industry in some regions of the Philippines was wiped out due BBTV.

2.2.4 Vietnam

In vitro single or multiple shoots of banana are established by culturing isolated shoot tips on MS medium (Murashige and Skoog 1962) supplemented with 30 g/l sucrose and 0.5 - 4 mg/l BA (6-benzylaminopurine). For rooting of shoots, the well grown shoots were separated and transferred to MS medium containing the same concentration of BAP (3.0 mg/l) and NAA (0.2 mg/l). The pH of the medium was adjusted to 5.8 before autoclaving and has maintained at 24 ± 1 °C under 16h cool white, fluorescent lights (4,000 lux). *In vitro* shoot tips 2 - 3 leaf primordia is initial material for gamma treatment. The experiment carried out 5 formulas with five gamma doses at 10, 20, 30, 40 and 50 Gy. The results showed an appropriate gamma dose is 15 or 20 Gy.

2.3 Preparation of plant materials for irradiation

2.3.1 Bangladesh

The suckers of Sabri (AAB) chopped off 3 - 5 cm long and washed thoroughly under running tap water (Fig. 2-3-B-1a to 1c). Explants were prepared by removing the outer layer of leaf-sheaths from the suckers using a clean knife. The pale white tissue blocks of 1.5 x 2.5 cm² in size containing shoot tips were washed under running tap water with 'Tween 20' for 30 minutes. Then they were immersed in 80% v/v alcohol for 30 second to 1 minute followed by sterilization in 50 to 100% clorox with 2 to 3 drops of 'Tween 20' for 20 minutes, and finally rinsed three times with sterile distilled water in the laminar air flow cabinet. The ensheathing leaf-sheaths which surrounded and protected the apex, were removed to obtain shoot tips of about 0.5 cm in length with a 4 to 5 layers of leaf primordia. After surface sterilization, shoot tips of about 0.5 cm in size were treated with 8 doses of gamma rays viz., 10, 20, 30, 40, 50, 60, 70 and 80 Gy from ⁶⁰Co gamma irradiator at a dose rate of 25 Gy/min (Fig. 2-3-B-1d). Number of explants used were 120, 190, 104, 196, 192, 184, 170, 180 and 165 for control. Immediately after irradiation, explants were placed onto MS medium fortified with 5.0mg/l BA + 0.2% Ads (Fig. 2-3-B-1e to 1f). Radiation sensitivity and post-irradiation recovery were assessed by measuring the survival and propagation rate 40 days after irradiation. For bulk irradiation of cv. Sabri, suckers were collected from Narshingdi, one of the major banana growing districts, which is about 50 km away from Dhaka City. All together about 1,200 suckers in several batches (100 - 180 Suckers per batch) were collected from Narshingdi. Three repeated subcultures of growing shoots was carried out at one month interval on the same medium following to M₁V₄ to dissociate chimeras in the regenerated shoots (Fig. 2-3-B-1g) and were also three repeated subcultures done for control plantlets (Fig. 2-3-B-1h).



Fig. 2-3-B-1 *In vitro* mutagenesis for developing Fusarium wilt resistance in commercial cultivar 'Sabri'

- a.** Some collected suckers of Sabri (AAB), **b.** Chopping of suckers,
c. Surface sterilization of chopped suckers with 0.2% Bavestin (fungicide), **d.** Irradiation of suckers,
e. Slicing of shoot tips, **f.** Inoculation of shoot tip explants on culture medium,
g. Multiple shoot formation (M_1V_4) from irradiated shoot tip explants,
h. Multiple shoot formation from un-irradiated (control) shoot tip explants, **i.** Rooting of M_1V_4 shoots,
j&k. Hardening of M_1V_4 plants, l&m. Screening of M_1V_4 plants against Fusarium wilt under greenhouse condition

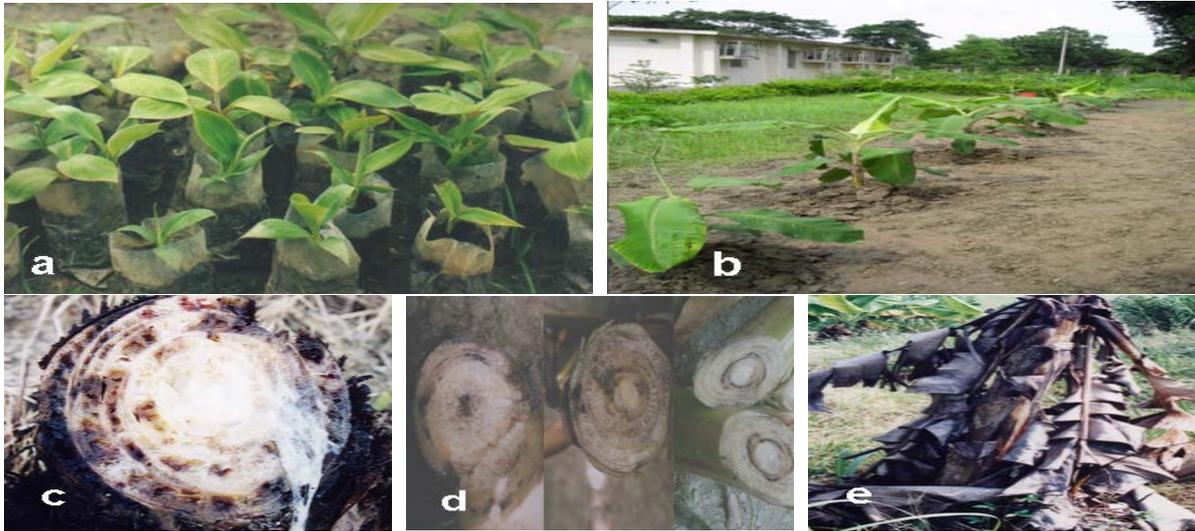


Fig. 2-3-B-2 *Fusarium* infestation in commercial cultivar ‘Sabri (AAB)’

- a.** Irradiated plantlets in poly bags with hot spot soil, **b.** Irradiated plants in hot spot affected field,
c & d. Vascular dis-colouration and pseudo stem rotting, **e.** Death plant

2.3.2 Malaysia

Suckers of cultivar Berangan were obtained from our collaborator, United Plantations Berhad and they were cleaned and surface sterilized using sodium hypochlorite for 30 min. They were excised into small pieces of meristem tissues about 1 - 2cm in size and explanted onto MS media containing 5 mg/l BAP. Adventitious buds begin to appear after a period of 1 - 2 months in culture and later *in vitro* shoots start to produce. Newly formed *in vitro* shoots were transferred onto fresh MS media and multiplication was routinely carried out every 3 - 4 weeks after culture. Rooting of *in vitro* shoots was established in MS rooting media supplemented with 1 % activated charcoal.

Preparation of plant materials for irradiation



Original suckers cleaned with tap water



Surface sterilized with Chlorox



Cutting under laminar flow cabinet



Meristem cultures for irradiation



Adventitious buds formation after 1 month



Shoots formation after 2 months

2.3.3 Philippines

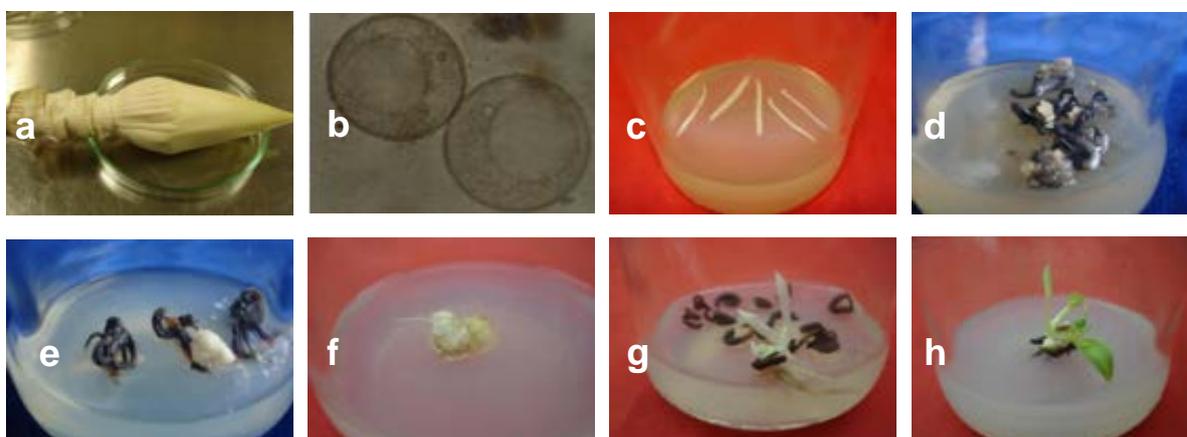
Disease free suckers of cv Lakatan collected from the field were established *in vitro* following the standard banana tissue culture procedure (Damasco and Barba, 1984). Suckers were cleaned and surface sterilized in pure bleach for 45 min. Shoot explants (1 cm x 1 cm) were excised, cut into 4 sections and inoculated onto MS medium + 5 mg/l BAP. Shoots were regularly sub-cultured every 4 to 6 weeks onto shoot multiplication medium (SMM) containing MS basal medium + 3 mg/l BAP. Shoots were transferred onto MS basal medium + 0.1 mg/l activated charcoal for rooting and plantlet development. All irradiated shoots cultures and selected mutant lines were micropropagated using the SMM.

2.4 Special culture methods

2.4.1 Bangladesh

Anther culture of diploid banana

A local diploid seeded cultivar Bichikala (BB) derived from the diploid wild species *Musa balbisiana* (BB) was used in this study. The male flower bud containing all developmental stage of pollen was used as donor material for anther isolation. The mature male flowers along with the bracts were removed from upper part of the male flower bud to get immature male flower having fully developed anthers with appropriate developmental stage of pollen. Male flower bud (Fig. 2-4-B-1a) was then surface sterilized by swabbing with cotton soaked in 70% ethanol. The bract was then separated from immature flowers and cluster of male flowers with fully developed anthers were isolated and transferred into petri dish under laminar air flow cabinet. The surrounding tepals and ovary were removed. One anther from each cluster of male flower was examined under microscope to identify the developmental stage of pollen i.e., uni-nucleate stage (Fig. 2-4-B-1b). Five anthers (Fig. 2-4-B-1c) containing uni-nucleate pollen were placed in each jar containing agar-gelled (8% w/v) MS (Murashige and Skoog, 1962) or N6 (Chu *et al.*, 1975) based medium. MS medium contained basic macro- and micro-nutrients, morel vitamins (Morel and Wetmore, 1951) and supplemented either with 2.5 mg l⁻¹ 2,4-D and 1.0 mg l⁻¹ kinetin (designated as MS1) or with 1 mg l⁻¹ BA, 0.4 mg l⁻¹ IAA and 500 mg l⁻¹ casein hydrolysate (CH) (designated as MS2) while N6 medium comprised basic salts and Morel vitamins, 1 mg l⁻¹ BA, 0.4 mg l⁻¹ IAA and 500 mg l⁻¹ CH. Sucrose at a concentration of 2.5% was used as carbon source in all cases. The pH of the medium was adjusted to 5.7 before autoclaving. Cultures were kept at 26 ± 1°C in darkness and maintained on the same medium without subculture until callus is produced. Calli thus obtained (Fig. 2-4-B-1d & 1e) were transferred to fresh medium composed of MS basic salts, Morel vitamins, 0.5 mg l⁻¹ BA and 0.4 mg l⁻¹ IAA and kept under fluorescent illumination (at 1,500 lux intensity) with 16/8 h light/dark cycle for regeneration of plants. Cytological study of the root tips was carried out for ploidy determination of the regenerated plants.



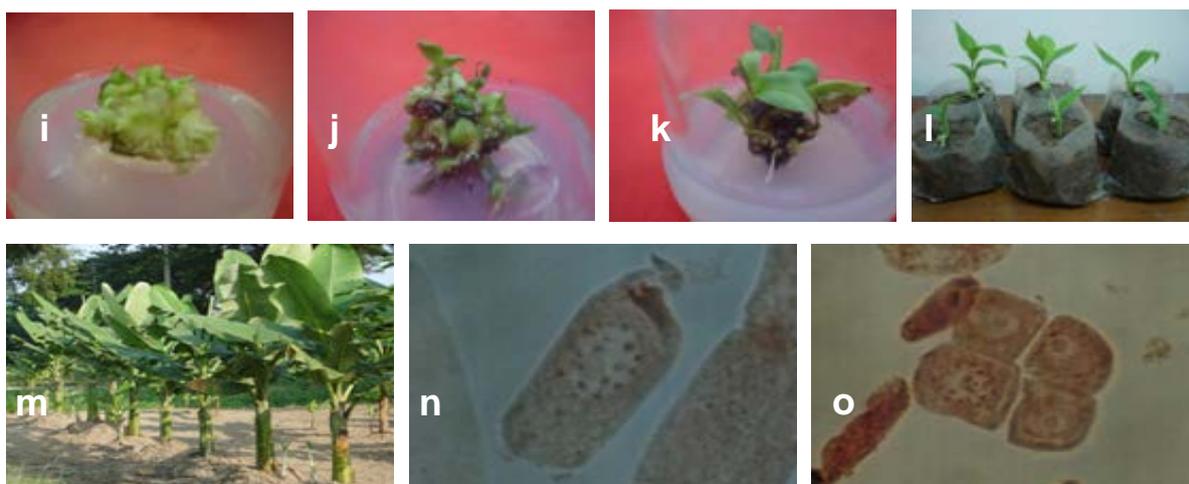


Fig. 2-4-B-1. Anther culture in *Musa balbisiana* cultivar 'Bichikala'

- a.** Male flower bud, **b.** Uni-nucleate pollens, **c.** Anthers on the culture medium,
d. Starchy callus on 2,4-D and kinetin supplemented MS medium,
e&f. White and compact callus on BA, IAA supplemented MS medium,
g&h. Shoot regeneration on N6 based callus induction medium, **i.** Callus on regeneration medium, **j&k.**
 Regeneration of shoots from calli/embryoids, **l.** Acclimatization of plantlets,
m. Anther-derived plants in the field, **n&o.** Root tip cytology for chromosome counting

2.4.2 Malaysia

Application of somatic embryogenesis for plant regeneration can be used as plant materials for mutation induction. Somatic embryos were successfully induced from male inflorescence of *Musa acuminata* var. Mas (AA). Suspension cultures were successfully initiated and translucent spheres and torpedo shaped embryos were obtained upon transfer to embryo development and regeneration medium. Complete plant regeneration from embryogenic cell suspensions were also obtained.

I. Explant source & sterilization

Inflorescence male buds of *Musa acuminata* cv. Mas (AA) were used as explant materials. First dissection was carried out in the general lab where the male bud was shortened to 6 - 8cm in length by removing the enveloping bracts. For second dissection the male bud was then transferred to a laminar air flow. Explants were sterilized in 70% ethanol for about 15 minutes and rinsed 3 times with sterile distilled water. The male bud was further shortened to 1 - 1.5cm in length for culture and immature male flower clusters position 1 - 15 were removed under stereo-microscope until the meristem is exposed.

II. Initiation of embryogenic callus

Immature male flowers were cultured on initiation medium, M1 (Escalant *et al.* 1994; Cote *et al.*, 1996) to initiate embryogenic callus supplemented with combinations of different concentrations of 2,4-D over 2 subcultures for every 3 months.

III. Suspension culture

Friable embryogenic callus was transferred to two different suspension medium that is M2a (Cote *et al.*, 1996) and M2b (Dhed'a *et al.*, 1991) to optimize the best medium for initiation of suspension culture for *Musa acuminata* cv. Mas (AA). Growth of cell suspensions was measured for the selected M2 media.

IV. Development of somatic embryos

Embryos were developed on M3 (Cote *et al.*, 1996) media.

V. Regeneration of somatic embryo

Regeneration of somatic embryos was performed on M4 (Cote *et al.*, 1996) medium with 3 different concentrations of BAP (0.05mg/l, 0.1mg/l and 0.2mg/l) and MS media without any plant growth regulators (MS0).

VI. Culture conditions

Cell suspensions were maintained at 70 r.p.m continuously on a gravitory shaker.

For plant regeneration, cultures were initially placed in the dark until shoot bud appeared and subsequently transferred to light conditions.

Somatic embryogenesis for plant regeneration



Male flower



Male bud as explant



Somatic embryos



Shoot regeneration



Multiplication of *in vitro* shoots



Rooted plantlets

2.4.3 Vietnam

I. Effect of gamma ray on subsequent survival of banana shoot tips cultured *in vitro*

Under the effect of radiation ray, which should be reduce the growth and morphogenetic performance to 5 - 45 % of the control. Considerable phenotypic variation was observed among the

shoots regenerated from shoot tips. In early stages of shoot development the irradiation affected emergence and expansion of the youngest leaves. The vegetative growth was influenced by a higher dose of irradiation (above 20 Gy). Here we calculated for the percentages of surviving after mutagenic treatment of shoot tips observed at 7, 14, 21, 28 days. Surviving percentage as described in Table 2-4-V-1.

Table 2-4-V-1. Surviving percentage of *in vitro* cultured shoot tips of dwarf banana

Dose of gamma irradiation (Gy)	Survival days (%)				
	1 day	7 days	14 days	21 days	28 days
0	100.0	100.0	100.0	100.0	100.0
10	100.0	95.0	84.2	82.2	79.0
20	100.0	93.8	80.4	78.6	76.4
30	100.0	87.4	63.6	57.0	52.0
40	100.0	82.2	57.0	42.6	35.6
50	100.0	77.0	34.6	26.2	22.6

Table 2-4-V-2 and Fig. 2-4-V-1 showed that: Gamma ray has direct influence to surviving of Tay banana shoot tips, if irradiation dose is increased, surviving percentage should be decrease and decreases at 30 Gy. In seven first days, surviving percentage decreases smoothly and increases significantly following 14 days of cultivation in all formulas. At that time, gamma ray made many shoots turned black in color and died. At irradiation dose 10 and 20 Gy, there weren't differences in surviving percentage. In 50 Gy, living shoot tips had phenotypic changed with yellow leaf and slow growth. There were a little of living shoot tips.

II. Effect of gamma irradiation dose on propagated coefficient of *in vitro* cultured

After 28 days of gamma treatment, banana shoot tips were transferred on medium MS supplemented with 0.5 ppm Naphthalene acetic acid (NAA), 4 ppm Benzylaminopurine (BAP). We reported on propagated coefficient (after 5 times subculture). As shown by the results given in Table 2-4-V-2. Gamma ray has direct influence to propagated coefficient of banana shoot tips; the shoot propagated coefficient is lower than control. In the 3rd of subculture, this coefficient was constant and shoots formation grown well in the cultured medium.

Table 2-4-V-2. Effect of irradiation dose on surviving percentage (5 times subculture)

Dose of gamma irradiation (Gy)	Propagated coefficient (times)				
	1 st	2 nd	3 rd	4 th	5 th
0	3.22	3.27	3.83	3.72	3.72
10	1.30	1.80	2.53	2.92	3.34
20	1.19	1.57	2.12	2.51	2.81
30	1.10	1.52	1.87	2.14	2.66
40	1.08	1.29	1.32	1.38	2.03
50	1.05	1.15	1.10	1.05	1.91

III. Effect of gamma ray on percentage root initiation of banana shoots

Beside propagated coefficient, quality of roots and percentage root initiation is also target to evaluate effect of gamma ray on banana shoot tips. In this period, shoots were transferred on half-strength MS medium, supplemented with 0.05 ppm NAA, 0.02 ppm IBA. The number of roots was counted at the last internal after 7, 14, 21, 28 days.

Table 2-4-V-3. Effect of gamma irradiation dose on percentage root initiation of plantlets

Dose of gamma irradiation (Gy)	Percentage root initiation (%)				
	7 days	14 days	21 days	28 days	Quality of root
0	47.78	66.67	78.89	93.33	+++
10	14.44	58.89	72.22	88.89	+++
20	12.22	52.22	66.67	86.67	++
30	4.44	37.78	53.33	72.22	+
40	3.33	36.67	50.00	70.00	+
50	2,56.	36.10	43.00	62.00	

+: weak root; ++: average root; +++: strong root

Table 2-4-V-3 showed that the gamma ray had direct influence to appearing roots of banana shoots. Percentage root initiation decreased after mutagenic treatment and root was slow growth and weak (except 10 Gy irradiation dose). The percentage root initiation decreased smoothly in 14 first days, the lowest level in 40 Gy (36%)

IV. Effect of gamma ray on appearance variation of banana shoots

Adventitious origin of shoot buds from the superficial cells of the rhizome cultured *in vitro* reveals the possibility that entire shoot can be raised from a single somatic cell. This system is particularly important for practical mutation breeding because a high frequency of solid plant mutants can be obtained after explanting irradiation, these results described in Table 2-4-V-4.



Fig. 2-4-V-1. a. Dwarf plants, b. Thin variation, c. Color variation

Table 2-4-V-4. Effect of irradiation dose on mutation frequency

Dose of gamma irradiation (Gy)	Variation							
	Stump	Long-shoots	Dwarf-shoots	Stem color	Formed leaves	Leaf-like stem	Leaf-blade like stem	Total
10	1.7	0.1	0.1	1.5	1.5	0.1	2.5	7.5
20	2.3	0.1	0.1	3.5	3.5	0.5	4.5	14.5
30	2.3	0.1	0.2	3.5	3.6	0.7	5.3	15.7
40	1.5	0.2	0.1	1.3	2.7	0.2	2.5	8.5
50	1.1	0.3	0.1	1.0	2.1	0.1	2.2	8.1

2.5 Determination of irradiation condition

2.5.1 Bangladesh

Radiosensitivity study of cv. Sabri (AAB)

Radiation sensitivity and post-irradiation recovery were assessed by measuring the survival and propagation rate 40 days after irradiation (Fig. 2-5-B-1). From the result it was found that LD₅₀ for Banana cv. Sabri laid in between the gamma irradiation dose of 30 and 40 Gy. So, 35 Gy have been selected as an optimum dose to be used in the experiments for induction of *Fusarium* resistant mutants and getting highest percentage of variants in cv. Sabri.

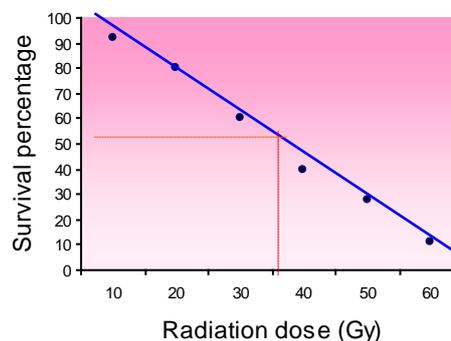


Fig. 2-5-B-1 Radiosensitivity test curve of shoot tips from Banana cv. Sabri

2.5.2 Malaysia

Radiosensitivity test (dose response) for cultivar Berangan was carried out by irradiating meristem tips with a series of gamma ray doses of 0,10, 20, 30, 40, 50, 60, 70, 80, 90, 100Gy. For each dose, 100 meristem explants were irradiated and divided into 5 replicates for statistical analysis. Data for radiosensitivity test was determined by 3 parameters, such as:

1. Plant height (shoot height)
2. % survival of irradiated explants
3. Multiplication rate

Based on % survival of irradiated explants, LD₅₀ and LD₁₀₀ were obtained for Berangan:

$$LD_{50} = 50Gy$$

$$LD_{100} = 80Gy$$

In a mutation breeding experiment, LD₅₀ gives an indication value for the response of different types of explants of the same species to radiation so that, a researcher can select the right dose (s) for the main field experiment. Usually, selecting the most effective dose (s) for the main experiment, it is advisable to select a few doses (more than one) which are 20 - 30% lower than the LD₅₀ value. Since induction of mutations by radiation for desired mutants is by chance, therefore it is safer to choose more than one dose and the doses that can cause less damage and give higher multiplication rate.

Therefore, based on the percentage survival of irradiated meristems which are 20 - 30% lower than LD₅₀, selected effective doses for the main experiment were 20, 30, 40Gy. Explants from meristem tissues of Berangan were irradiated with gamma rays at 20, 30 and 40 Gy. Irradiated explants were subcultured until M₁V₅ at 4 weeks interval to eliminate chimerism. Irradiated plantlets at different subculture stages were maintained on MS multiplication medium containing 5 mg/l BAP for further regeneration and were incubated in the incubation room at 24 °C with 16 hours photoperiod. Rooting of the irradiated plantlets was carried out on MS rooting medium containing 2 mg/l IAA. Rooted

plantlets were transplanted to soil-mix in polybags and then transferred to the nursery for hardening process and screened for Fusarium wilt disease using artificial inoculation.

2.5.3 Philippines

The radio-sensitivity response of banana shoot explants was determined by irradiating shoot explants at 0, 5, 10, 20, 25, 30, 40, 60, 80 and 100 Gy. After irradiation, shoots were immediately transferred to shoot multiplication medium (SMM) and multiplied for five subculture cycles (M_1V_1 to M_1V_5). The LD_{50} was determined by the 50% reduction in shoot proliferation rate expressed as % reduction based on the control or unirradiated treatment (Fig 2-5-P-1).

Proliferating shoot cultures were subjected to bulk irradiation the using LD_{50} 20 and 25 Gy. Irradiated shoots were multiplied for five cycles (M_1V_5), rooted, potted out and established in the nursery. Regenerated shoots were rooted, potted out and established in the greenhouse.

Shoot cultures irradiated at low dosage (5 Gy) showed higher multiplication rate while cultures irradiated at higher dosage (40 - 60 Gy) showed more than 80% reduction in the multiplication rate. For cv. Lakatan, the LD_{50} was established at 20 - 25 Gy. For mutation induction, bulk irradiation of shoot tips was done using the LD_{50} . All plants regenerated from irradiation treatments were evaluated for BBTV resistance.

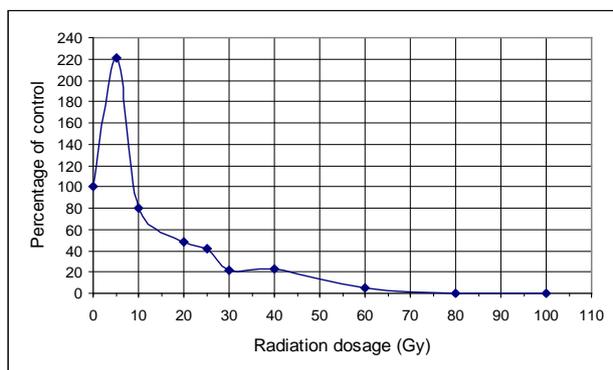


Fig. 2-5-P-1. Effect of increasing dosage of gamma radiation on the multiplication rate of Lakatan (AA) shoot cultures after five subculture cycles. Data are expressed as percentage of control (non irradiated shoot cultures)

2.6 Preparation of pathogens for artificial inoculation

2.6.1 Malaysia

I. *Fusarium oxysporum* (Foc race 4) cultures.

The cultures (4 petri dishes) were kindly provided by Prof. Vikineswary from University of Malaya (Fig. 2-6-M-1). These cultures were then subcultured onto fresh PDA medium supplemented with 1% yeast extract for further multiplication. Subculturing was done by taking one agar slab (0.5 x 0.5cm) containing the fungal mycelium and transferred onto PDA medium (Fig. 2-6-M-2). The cultures were incubated under light at 23 - 25°C in growth chamber. The growth of Foc mycelium will take about 4 - 5 days (Fig. 2-6-M-3).



Fig. 2-6-M-1



Fig. 2-6-M-2



Fig. 2-6-M-3

II. Preparation of *Fusarium* suspension

With a sterile bacterial loop, the surface of the *Fusarium* culture (1 petri dish) was scraped (Fig. 2-6-M-4) and transferred into a sterile test tube containing 10 ml sterile distilled water (Fig. 2-6-M-5). The suspensions were vortex for a few seconds to disperse the mycelium (Fig. 2-6-M-6). Then, another 10 ml of sterile distilled water was added into the suspension and vortex for another few seconds. One petri dish of *Fusarium* culture = 20 ml solution = 20 plants.



Fig. 2-6-M-4



Fig. 2-6-M-5



Figure 2-6-M-6

III. Observation of *Fusarium* mycelium

A drop of the Foc solution was placed onto a glass slide and stained with Evans Blue (Fig. 2-6-M-7). Observation was done under the light microscope (Fig. 2-6-M-8). Foc mycelium observed under light microscope at 40X magnification (Fig. 2-6-M-9).



Fig. 2-6-M-7



Fig. 2-6-M-8



Fig. 2-6-M-9

2.6.2 Philippines

I. Greenhouse Screening for BBTV resistance

Irradiated plants materials regenerated from radio-sensitivity study (5 - 60 Gy) and bulk irradiation (20/25 Gy) were screened for BBTV resistance in the greenhouse using aphid inoculation of the virus. Plantlets about three months from potting and with 4 - 5 fully expanded leaves were subjected to artificial inoculation of the virus using the aphid vector, *Pentalonia nigronervosa*.

The procedure for greenhouse screening consisted of the following steps (Fig. 2-6-P-1):

1. Aphids rearing in healthy *Caladium* or *Diffenbachia* plants
2. Aphids starvation for 30 min
3. Acquisition feeding on infected BBTV infected banana plants for 24h
4. Aphid inoculation to banana plants (24h; 10 aphids/plant)
5. Observation for BBTV symptoms 1 - 9 months after inoculation
6. BBTV indexing by symptomatology, ELISA and PCR
7. Planting the seedling resistant plants without BBTV infection in the field for further evaluation for BBTV resistance and agronomic traits

Plants were kept in the greenhouse and observed for BBTV symptom expression six to nine months after inoculation. The presence or absence of BBTV infection was assessed using symptomatology, ELISA and PCR based techniques. Mutant plants without BBTV symptoms were selected nine months after virus inoculation.

Selected resistant mutant plant were selected, micropropagated and evaluated in the field under high disease pressure or farmer's field until the resistant mutant lines are stable (3 generations of selections)

II. Nematode screening (greenhouse experiment)

Tissue cultured plants from selected G1 mutant lines and check cultivars were evaluated for resistance to nematode *R. similis*. Tissue cultured plantlets were planted in sterilized coir dust: garden soil (1:1 v/v) potting mix. One month after planting, the seedlings (10/line) were inoculated with *R. similis* (1,000 larvae/plant). Evaluation for root damage/dead roots, root health assessment, root necrosis and nematode reproduction (number of juveniles) was done two months after

inoculation. The % dead root was calculated from the number of dead roots divided by the total number of roots multiplied by 100. Root health assessment refers to the assessment of secondary and tertiary roots: 1-0 to 5% of roots healthy, 2-6 to 50% of roots healthy, 3-51 to 95% of roots dead, 4- 96 to 100% of roots dead.

**BBTV SCREENING: GREENHOUSE (aphid inoculation)
FIELD (natural disease infection)**

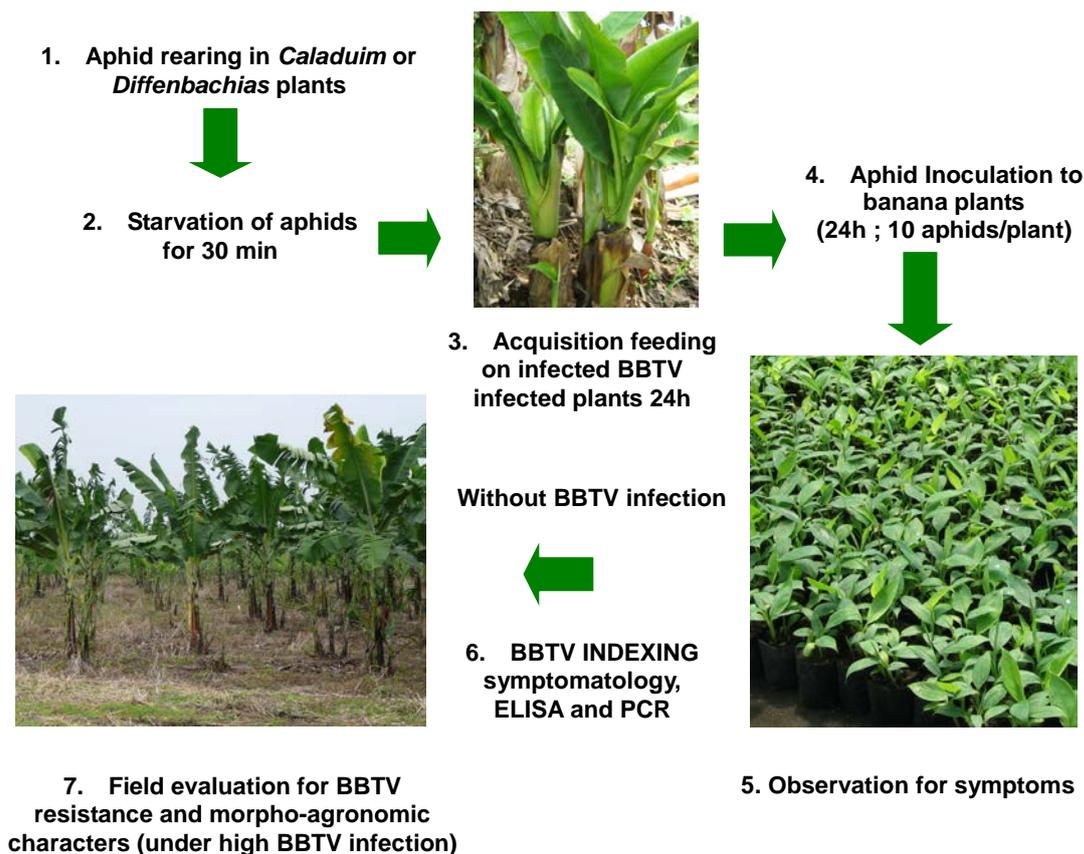


Fig. 2-6-P-1 BBTV disease screening procedure using artificial inoculation of the virus using aphid vector *Pentalonia nigronervosa* (greenhouse) followed by field evaluation under natural disease infection.

2.6.3 Vietnam

I. Effect of artificial inoculation methods on *Fusarium oxysporum* f.sp *cubence* of banana

We used fourth methods with density 10^5 spore/ml. The results showed in Table 2-6-V-1.

Table 2-6-V-1. Effect of artificial inoculation methods on banana plantlets

Method	Wilt rate after 14 days (%)			
	Numbers of repeat			
	I	II	III	Average
wounded	56.6	66.6	60.0	61.0
inf. injection	40.0	43.3	46.6	43.3
root immerge	53.3	46.6	56.6	52.1
cultured hydroponic	76.6	83.3	73.3	77.7

The experiment used four methods to carry out the Foc artificial inoculation on plants but cultured hydroponic is the best method (Fig. 2-6-V-1.a).

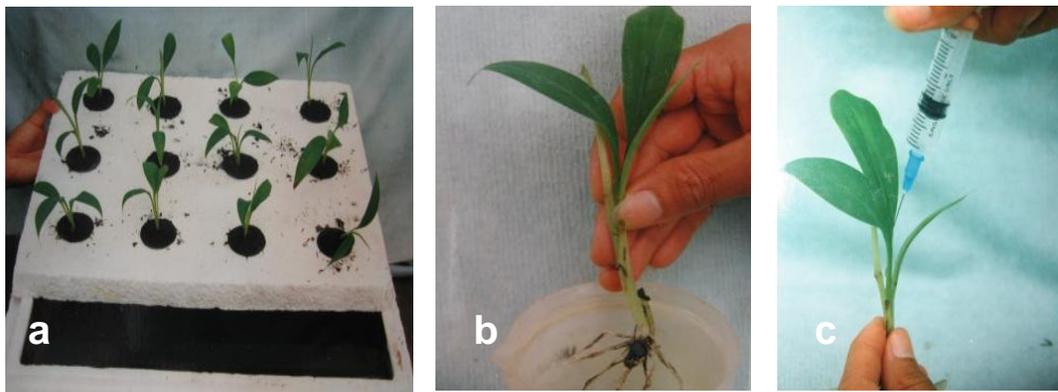


Fig. 2-6-V-1. a. cultured hydroponic, b. root immerge, c. inf. injection

II. Select banana lines anti-Foc by artificial disease inoculation on small banana

The results of selection somatogenic variation lines anti-Foc (*Fusarium oxysporum f.sp cubense*)

After 5, 6, 7, 8, 9, 10 times subcultured, shoots were transferred on rooted medium and plants root reached 2 - 3 cm in length and transplanted on the pots in green house. Each formula, we selected 500 plantlets at random. The results showed in Table 2-6-V-2.

Table 2-6-V-2. Synesis rate of bananas in green house

Experiments	Targets	
	No of infected bananas	Synesis rate (%)
Control	285	57.0
I	240	48.0
II	301	60.2
III	189	37.8
IV	241	48.2
V	235	47.0
VI	201	40.2

Selected lines resistant to Foc after gamma treatment (⁶⁰Co)

The banana plantlets were transferred on pots in green house and were carried out artificial pathogen. We selected 100 plants and observed after 1 month, take image of infected individual. After continual 3 times, we calculated plants that weren't infected, the results showed in Table 2-6-V-3.

Table 2-6-V-3. Survival rate after artificial inoculation

Dose (Gy)	Number of diseased inoculation		
	The first	The second	The third
0	48/100	22/100	11/100
10	41/100	27/100	15/100
20	35/100	18/100	10/100
30	45/100	29/100	12/100
40	41/100	27/100	14/100

The experiment showed good results in all formulas and infection rate decreased with times of taking artificial inoculation.

Selection of lines enhance resistant to Foc pathogen in green house

The results showed in Table 2-6-V-4.

Table 2-6-V-4. Selection of lines resistant to Foc (green house exp)

Order	Plant lines	No of infected plants	Synesis rate (%)	Levels
1	Control	12	60	MS*
2	I-3	11	55	MS*
3	I-22	11	55	MS*
4	I-35	13	65	MS*
5	I-37	9	45	MR
6	I-41	11	55	MS*
7	I-42	13	65	MS*
8	I-48	15	75	MS*
9	I-53	17	85	S
10	I-57	9	45	MR
11	I-62	15	75	MS*
12	I-68	15	75	MS*
13	I-75	13	65	MS*
14	I-83	13	65	MS*
15	I-84	11	55	MS*
16	I-96	11	55	MS*
17	I-9	14	70	MS*
18	I-25	10	80	S
19	I-36	4	20	R
20	I-37	2	10	R
21	I-45	11	55	MS*
22	I-47	3	15	R
23	I-58	4	20	R
24	I-73	4	20	R
25	I-87	9	45	MR
26	I-92	11	55	MS*
27	I-7	13	65	MS*
28	I-25	14	70	MS*
29	I-35	6	30	MR
30	I-47	2	10	R
31	I-51	2	10	R
32	I-62	7	35	MR
33	I-68	3	15	R

34	I-73	5	25	R
35	I-77	3	15	R
36	I-82	5	25	R
37	I-88	7	35	MR
38	I-95	1	5	R
39	I-6	13	65	MS*
40	I-24	9	45	MR
41	I-34	11	55	MS*
42	I-38	16	80	S
43	I-46	8	40	MR
44	I-53	4	20	R
45	I-58	3	15	R
46	I-67	4	20	R
47	I-75	6	30	MR
48	I-78	8	40	MR
49	I-79	7	35	MR
50	I-85	6	30	MR
51	I-93	4	20	R
52	I-94	4	20	R

S: sensitive; **MS:** moderate sensitive; **MR:** moderate resistance; **R:** resistance

The banana plants weren't infected by *Fusarium oxysporum* f.sp. *cubense*, that were transferred on *in vitro* medium and propagated

2.7 Inoculation of pathogens and observation of symptom development in the laboratory and/or nursery

2.7.1 Malaysia

Four effective methods for screening of *Fusarium* wilt disease in banana had been established using artificial inoculation with *Fusarium oxysporum* f. *cubense* (Foc Race 4). These methods can be applied for pre-screening of irradiated *in vitro* plantlets or seedlings to select for resistant/tolerant lines before they can be transferred to the field for final selection of potential mutant lines.

I. Methods of screening:

1. *In vitro* screening - dipping of roots of *in vitro* plantlets in *Fusarium* spore suspension
2. Double-tray technique - inoculation of tissue culture plantlets with *Fusarium* spore suspension in the nursery
3. Planting of 2 months old hardened plants in soil medium inoculated with *Fusarium* mycelium
4. Field screening - planting of treated plants in *Fusarium* 'hot spot' area.

II. Establishment of screening methods for *Fusarium* wilt disease:

1. Dipping method:

This is done by soaking of rooted *in vitro* plantlets (4 - 5 inches tall) in *Fusarium oxysporum* suspension (10^6 spores/ml) for 1 - 2 hrs and later transferred to sterile sand media in the greenhouse. Inoculated plants were observed for disease symptoms such as yellowing of the leaves and for confirmation, the lower part of the plant is cut across which shows purple discoloration of the pseudostem.



Rooted *in vitro* plantlets as explants for dipping method

2. Double tray method:

This technique consists of 2 separate plastic tray or container:

First tray contains sterile sand media whereby rooted plantlets were hardened for 3 - 4 weeks. After the plants were hardened, *Fusarium oxysporum* spore suspension (10^6 spores/ml) is poured into the tray containing plantlets. The treated plants were watered daily and excess water containing the spores is collected in the second tray placed below the first tray. This technique is most suitable since excess spores are contained and



Double tray method

will not escape to the drain or ground which might contaminate the area. Similar symptom for yellowing of the leaves will be recorded and discoloration of the pseudostem gives confirmation for infected plant.

3. Nursery screening method:

Rooted *in vitro* plantlets were hardened in the greenhouse using individual polybags containing sterile sand media for 8 weeks. Later the hardened plants were transferred to fibre-glass trough

(1x2m) containing mixture of coir dust which had been inoculated with *Fusarium oxysporum* spore suspension (10^6 spores/ml) for 2 weeks. Evaluation for disease infection is done 4 - 6 weeks after planting. Similar symptom for yellowing of the leaves will be recorded and discoloration of the pseudostem gives confirmation for infected plant.



Nursery screening method



Tolerant/Resistant plants



Susceptible plants

4. Field screening:

Those inoculated plants that survived from the above screening methods are transferred to hot spot. Resistant plants are multiplied and transferred back to hot spot for at least 2 - 3 generations. Similar symptom for yellowing of the leaves will be recorded and discoloration of the pseudostem gives confirmation for infected plant.



Field screening of irradiated seedlings and leaf-yellowing, typical symptom for Fusarium wilt disease starts to develop after 4 months.

2.7.2 Philippines

I. Greenhouse screening BBTv resistance

A total of 6,012 plants regenerated from gamma irradiation treatments (radio-sensitivity study using 5 - 60 Gy and bulk irradiation at 20/25 Gy) were evaluated for resistance to BBTv using artificial inoculation of the virus by aphid transmission. Of the 6,012 irradiated plants screened in the greenhouse, 114 plants were selected without symptoms of BBTv nine months after virus inoculation *(Table 2-7-P-1)

Table 2-7-P-1. Total number irradiated plants screened in the greenhouse for BBTV resistance using aphid inoculation of the virus

Radiation Treatment	Total no. of plants screened for BBTV resistance.	No. of plants without BBTV symptoms	
		6 mos. after inoculation	9 mos. after inoculation
Radio Sensitivity (5-60 GY)	1,847	78	72
TC Control	161	0	0
Bulk irradiation (20/25)	4,165	79	42
TC control	388	0	0
TOTAL Irradiated plants	6,012	157	114
TOTAL TC Control	549	0	0
BBTV indexing ELISA PCR	- (negative) in 33/45 plants sampled (73%) - (negative) in 18/25 plants sampled (72%)		

II. Evaluation for nematode resistance under greenhouse condition

Based on the results on root damage, almost all roots of mutant lines 2-45, 4-45, 10-45 were healthier than the TC control (non-irradiated) (Table 2-7-P-2). In case of mutant line 5-45, although the % of dead roots was 0, most of the secondary and tertiary roots were dead (3.0). For % root necrosis, mutant line 4-45 had the highest value (90%) followed by the TC control Lakatan (73.6%); The values obtained by these two entries are even higher than the susceptible check Grand Naine. For number of juveniles, mutant line 4-45 had the lowest (20) followed by PR 2-45 (298).

Based on the results, mutant lines 2-45, 4-45, and 10-45 were resistant to *R. similis* under greenhouse condition. Due to lack of funds, field evaluation for nematode resistance was not conducted.

Table 2-7-P-2. Root damage assessment and nematode reproduction of irradiated and unirradiated banana cv Lakatan inoculated with *Radopholus similis*

Mutant line	% Dead Roots*	Root Health Assessment**	%Root necrosis	***Number of Juveniles
2-45	7.1	2.2	14.2	298
4-45	0	2.0	6.5	20
5-45	0	3.0	90.0	7,269
10-45	0	2.0	8.0	2,020
Unirradiated Lakatan (TC control)	41.5	3.0	73.6	2,317
Yangambi Km 5 (R check)	0	1.0	15.0	58
Grand Naine (S check)	28.3	2.8	60.0	2,959

*%Dead roots= number of dead roots divided by total number of roots multiplied by 100.

**Root Health Assessment refers to the assessment of the secondary and tertiary root: 1.0-5% of roots healthy, 2-6 to 50% of roots healthy, 3- 51 to 95% of roots dead, 4- 96 to 100% of roots dead.

+++ Number of juveniles taken from one gram of root samples.

2.8 Inoculation of pathogens and observation of symptom development in hot spots

2.8.1 Malaysia

I. Field screening of irradiated plants in farmer's field

Rooted *in vitro* plantlets were first treated with *Fusarium oxysporum*, Foc race 4 (10^6 spores/ml) suspension cultures using double-tray method. Plants that survived and which showed no symptoms of infection (yellowing of the leaves and discoloration of pseudostems) were selected for further screening in the field. A total of 1,115 treated plants (mutant lines) from doses of 20, 30 and 40Gy including 183 control plants were planted in farmer's field in May 2007 to screen for Fusarium wilt. Numbers of seedlings used for the field screening are;

1. Control: 183 seedlings
2. 20 Gy: 458 seedlings
3. 30 Gy: 343 seedlings
4. 40 Gy: 314 seedlings

II. Screening for virus using ELISA Test

Both tissue-cultured treated plants and control were screened for the presence of the virus. About 10% of the total population of the treated and control tissue culture plants were sent to Crop Protection & Plant Quarantine Services Division, Department of Agriculture, Malaysia for laboratory test against the following viruses:

1. *Banana bunchy top virus* (BBTV)
2. *Banana streak virus* (BSV)
3. *Cucumber mosaic virus* (CMV)
4. *Banana bract mosaic virus* (BBrMV)

III. Scoring for disease symptoms and evaluation of agronomic characters

Disease scoring for Fusarium wilt was done after 4-months planting period whereby each individual treated plants and control plants were analyzed for symptoms of yellowing of the leaves, discoloration of pseudostems and other factors that affects growth of the plants. For the control plants, out of 183 plants tested, 66 plants (36%) showed symptoms of *Fusarium* infection. On the other hand, for treated plants, the following observations were made:

IV. Disease scoring: susceptibility to Fusarium wilt

1. Control: 66 plants (36%)
2. 20 Gy : 10 plants (4%)
3. 30 Gy : None (0%)
4. 40 Gy : None (0%)

The percentages of Fusarium wilt infection in treated plants were observed to be very low. This might be because of the resistance or this could probably due to many of the plants might have escape

infection due to environmental factors. Therefore, it is suggested that second or even third field screening should be carried out in order to select for mutant plants which are stable and resistance to Fusarium wilt disease. Besides screening for Fusarium wilt, desired agronomic characters such as early flowering and short stature plant were selected as below:

V. Selection for agronomic characters:

1. 20 Gy: 458 seedlings
Early fruiting: 52 plants (11%)
Dwarf: 5 plants (1%)
2. 30 Gy: 343 seedlings
Early fruiting: 19 plants (5%)
Dwarf: 21 plants (6%)
3. 40 Gy: 314 seedlings
Early fruiting: None (0%)
Dwarf: 75 (24%)

Three potential mutants tolerance to Fusarium wilt disease having improved agronomic characters such as high yield, early flowering and short stature were selected from a total population of 1,115 treated plants as follows:

- 20 Gy: 2 mutants of high yield and early flowering
- 40 Gy: 1 mutant of high yield and short stature.

VI. Stability test for selected mutants

At present, a total of 1,000 plants from 20 and 40 Gy populations which had been micropropagated, were further field tested in the second location in farmer's plot for stability against Fusarium wilt disease.

2.8.2 Philippines

I. First field evaluation BBTV resistance under high disease pressure

The selected seedling resistant M₁ mutant plants (114) were planted in the field under high BBTV infection. After 21 months of exposure to natural BBTV infection, 64 irradiated M₁ mutant plants were selected (Table 2-8-P-1; Fig. 2-8-P-1 and 2). Suckers of selected mutants were collected and micropropagated for further field evaluation.

Table 2-8-P-1. Total number of M₁ BBTV resistant mutant plants selected after 30 months of evaluation (greenhouse artificial inoculation of the virus followed by and natural field disease infection).

Radiation treatment	Total no. of irradiated plants evaluated	No. of selected M ₁ mutant BBTV resistant plants (M ₁ plants without BBTV symptoms)	
		After greenhouse evaluation	After field evaluation
Radio sensitivity experiment (5 - 60 Gy)	1,847	72	38
Bulk irradiation 20/25 Gy	4,165	42	26
TOTAL irradiated plants	6,012	114	64
% M₁ resistant plants			1.06%

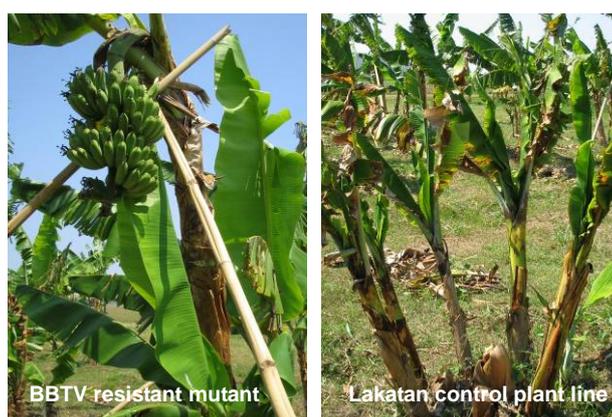


Fig. 2-8-P-1. BBTV resistant mutant line and Lakatan control plant after field evaluation



Fig. 2-8-P-2 Remaining Generation 1 suckers of selected resistant plants without BBTV symptoms at harvest. Cut plants were all BBTV infected.

II. Second field evaluation (generation 1 and 2 plants) for BBTV resistance

Thirty-two selected mutant lines (out of 64 mutant lines from initial selections) were planted in experimental field under high BBTV infection. Plants were planted in a randomized complete block design with two blocks and three replications with five plants per replication or a total of 30 plants per mutant line. Each block was surrounded with BBTV infected plants as sources of inoculum. All infected experimental plants were also kept in the field to provide additional sources of inoculum. The incidences of BBTV infection were taken every three months until harvest. Agronomic data were taken at harvest.

Out of 32 mutant lines evaluated 10 mutant lines with % BBTV infection less than control plants 17 months after field planting were selected (Table 2-8-P-2).

Suckers from the 33 line/mat selections from the 10 mutant lines were collected indexed and micropropagated and further evaluated for stability of resistance of generation 3 plants.

Table 2-8-P-2. The % BBTV disease incidence in selected BBTV resistant mutant lines 3 to 17 MAP.

Mutant line	% BBTV incidence (G1 and G2 plants)* month after planting (MAP)					% BBTV-free fruiting (harvested) plants* month after planting (MAP)		
	3	7	11	15	17	12	14	17
25-28	0	0	6.9	17.2	31.0	6.7	53.3	96.0
23-30	0	0	3.3	13.3	33.3	3.3	43.3	70.0
2-45	0	3.4	14.3	21.4	50.0	0.0	26.7	46.7
13-30	0	0	3.8	7.7	50.0	3.3	46.7	66.7
22-28	0	6.7	6.7	16.7	50.0	0.0	60.0	76.7
23-28	3.4	14.3	21.4	46.4	57.1	10.0	40.0	76.7
7-29	6.9	7.1	21.4	28.6	57.1	6.7	46.7	70.0
23-45	3.6	3.6	3.7	14.8	66.7	0.0	56.7	80.0
9-28	6.7	18.5	37	59.2	66.7	43.3	50.0	63.3
4-28	0	3.7	3.7	26.9	69.2	3.0	50.0	76.7
TC Control	1.4	14.1	18.3	29.6	71.8	0.0	14.1	32.4

*Combined G1 and G2 plants

III. Selection of Promising Generation 3 BBTV Resistant Mutant Lines for Multi-location Field Evaluation and Characterization of Disease Resistance Mechanism

10 lines were selected based on BBTVD incidence in the field, % BBTV-free fruiting plants and the results obtained from the studies on mechanism of resistance. The ten selected resistant lines namely lines 13-30-2, 7-29-1, 22-28-2, 23-28-7, 6-30-2, 9-28-2, 9-28-3, 9-29-1, 23-30-2, and 28-30-2 showed low disease incidence in the field, and/or obtained low disease incidence amid the presence of insect vector compared to LKD control plants. The selected lines were composed of nine mutant lines and one somaclonal variant (6-30-2). Past evaluations regarding stability of resistance in succeeding generations were also considered in the process of selection.

IV. Studies on the mechanism of resistance in selected G3 mutant lines.

A. Aphid preference (Insect vector-host relationship)

The aphid preference on 10 selected mutant lines was determined using the free choice or no choice feeding of the aphid. Five lines were found significantly less preferred by the aphids namely: 6-30-2, 9-28-2, 9-28-3, 23-28-7 and 28-30-2 (Table 2-8-P-3). Two lines (9-28-2 and 9-28-3) were consistently less preferred by the aphids in both free choice and no choice feeding.

Table 2-8-P-3. Relationship between aphid preference (aphid colony count) and BBTV incidence in selected mutant lines

Mutant lines	Aphid colony count at the peak of colonization (3 rd week of feeding)	
	Free choice feeding of the vector	No choice feeding of the vector1
28-30- 2	40**	62*
13-30 -2	22	65*
9-28 3	12*	36*
22-28 -2	NT	65*
9-28-2	12*	49*
6-30 -2	27	59*
7-29-1	14*	110
9-29 -1	1*	83
23-28-7	NT	16*
23-30-2	40**	76*
LKD control	23	127

*Significantly different at P= 0.05%

** Significantly different at P=0.05%

NT- not tested due to lack of planting materials

B. Reaction of mutant lines to artificial inoculation of the virus

The reaction of 10 selected mutant lines to BBTV was determined through artificial (aphid) inoculation of the virus. Five lines consistently showed low BBTD incidence (<50%) in repeated artificial inoculation trials namely: 23-30-2, 28-30-2, 23-28-7, 13-30-2 and 9-28-2 (Table 2-7-P-4). Expression of the virus ranged from bunchy top growth of the shoot, chlorosis of the youngest fully expanded leaf and streaks on petiole and pseudostem (Fig. 2-8-P-3).

Seven lines showed the % BBTD incidence lower than the LKD control plants. Line 28-30-2 showed the lowest BBTD incidence (19.6%) followed by lines 13-30-2 (25.0%), 9-29-1 (32.8%) and 22-28-2 (38.4%). Likewise, lines 28-30-2 and 13-30-1 had the longest incubation period of up to 6 weeks. Other lines including the LKD control had 4 - 5 weeks incubation period. The symptoms observed on infected plants ranged from bunchy top, rosette growth, and marginal chlorosis.

Based on ELISA (OD_{405nm}) reading (Table 2-8-P-6), all mutant lines except 9-29-1 had min OD reading less than that of the LKD control plants. On the other hand, for max OD reading only lines 13-30-2 and 7-29-1 had max OD reading less than that of the control plants. The results showed that some mutant lines had low virus titer, suggesting some degree of resistance to virus.

Table 2-8-P-4. Reaction of the ten lines to artificial inoculation of BBTV under greenhouse condition

Mat/Line selection	% BBTD incidence	Incubation period (WAI)	Symptoms	ELISA (OD _{405nm}) Range	
				Min	Max
28-30- 2	19.6	4-6	bunchy top,	0.189	1.276
13-30-2	25.0	5-6	rosette	0.1735	0.956
9-28 3	50.0	4-5	bunchy top, marginal chlorosis	0.1885	1.605
22-28 -2	38.4	5	rosette	0.1875	1.57
9-28-2	50.0	4-5	bunchy top	0.1785	1.267
6-30 -2	55.6	4-5	bunchy top	0.1835	2.2915
7-29-1	57.5	4-5	bunchy top, rosette	0.1815	1.128
9-29-1	32.8	4-5	bunchy top, rosette	0.203	1.545
23-28-7	45.0	4-5	bunchy top	0.1705	1.336
23-30-2	70.7	4-5	bunchy top, rosette	0.1785	1.68
LKD control	58.3	4-5	bunchy top	0.194	1.287

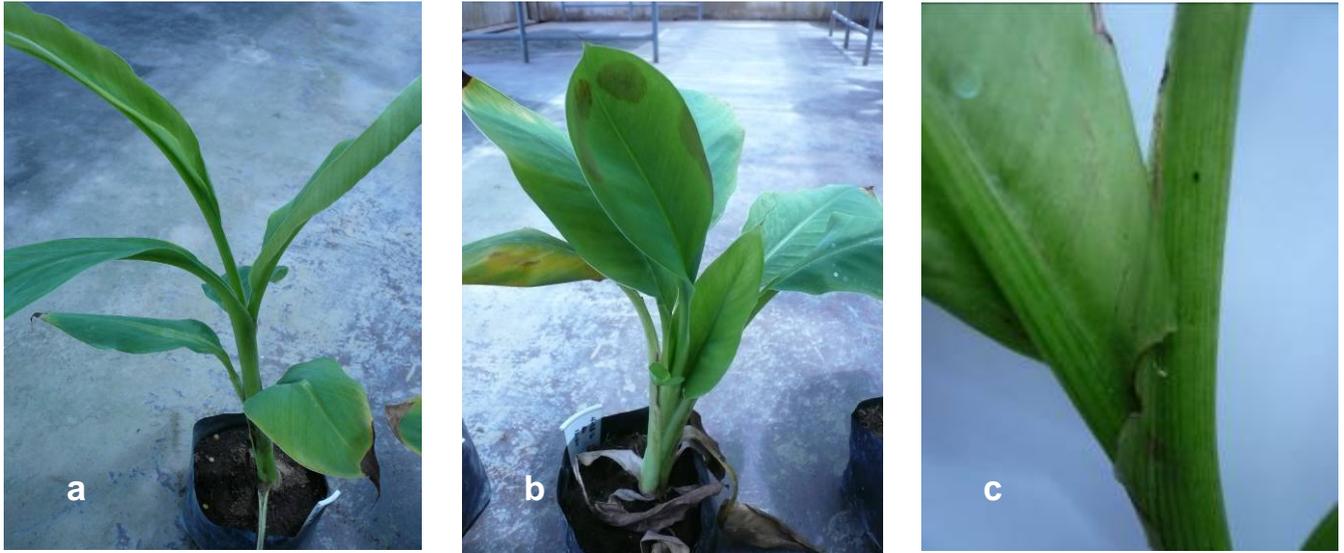


Fig. 2-8-P-3 Symptoms of BBTD observed under greenhouse condition:

- a. Chlorosis on the youngest full expanded leaf,
- b. Bunchy top growth of the shoot,
- c. Clear streaks on the leaf petiole and in the pseudostem

V. Multi-location field evaluation of selected mutant lines

Due to limited funds, only the top five promising mutant lines namely 9-28-2, 9-28-3, 13-30-2, 22-28-2 and 28-30-2 were planted in multi-location trials.

Low incidences of the banana bunchy top disease were observed in all trial sites despite the presence of infected plants and vectors in the vicinity of trial sites. The highest percentage incidence of the disease was recorded at 11.6% (in the case of the Bay, Laguna trial site). In Cavite, which is considered a hot spot for BBTV infection (Table 2-8-P-5 and 6), the diseases did not spread intensively within the trials sites and the infection occurred at random. From planting until 19 months, there was very little spread of the disease with time within the experimental area. The aphid vectors within the experimental site were observed in lines 13-30-2, 22-28-2 and 28-30-2.

The low incidence of BBTD in trial site could be due higher number of resistant plants planted in the area; approx. 70% of the total plants showed varying degrees of resistance. Furthermore, the random arrangements of test plants with different resistance mechanisms in the field, in some way prevented the rapid spread of the vector and virus within the experimental site. Regular rouging of infected plants within the trial sites prevented secondary infection. Further studies on the use and deployment of resistant lines as part of the BBTD management strategies are needed.

Table 2-8-P-5. Percentage incidence of BBTD in five mutant lines of banana cv. Lakatan selected for multi-location trial planted in Indang, Cavite (CAV 1).

Mutant line	Mat/Line selection	No. of plants	% Disease incidence (MAP)									BBTV- free fruiting plants (%)	
			6	8	10	11	12	14	16	18	19		
9.28	9.28.2	60	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.6	38	
9.28	9.28.3	60	0	0	0	0	1.6	1.6	1.6	1.6	1.6	23	
13.30	13.30.2	60	1.6	1.6	1.6	1.6	3.3	3.3	3.3	3.3	3.3	35	
22.28	22.28.2	60	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	3.3	26	
28.30	28.30.2	60	1.6	1.6	1.6	1.6	3.3	3.3	3.3	3.3	3.3	35	
Control	LK	60	0	0	0	0	0	0	0	0	0	28	
Border	LK	110	1.8	2.7	2.7	2.7	2.7	2.7	2.7	2.7	2.7	20	
Selected Resistant Lines		300										2.7	
LK (Control + Border)		170										1.8	
Whole experimental area		470										2.3	

Table 2-8-P-6. Percentage incidence of BBTD in five mutant lines of banana cv. Lakatan selected for multi-location trial planted in Indang, Cavite (CAV 2).

Mutant line	Mat/Line selection	No. of plants	% Disease incidence (MAP)									BBTV free fruiting plants* (%)	
			6	8	10	11	12	14	16	18	19		
9.28	9.28.2	60	0	0	1.6	1.6	3.3	3.3	3.3	3.3	3.3	67	
9.28	9.28.3	60	1.6	3.3	3.3	3.3	5	5	5	5	5	42	
13.30	13.30.2	60	3.3	5	5	5	5	5	5	5	5	47	
22.28	22.28.2	60	0	0	0	0	0	0	0	0	0	53	
28.30	28.30.2	60	0	3.3	3.3	3.3	3.3	5	5	5	5	58	
Control	LKD	60	0	0	1.6	1.6	1.6	3.3	3.3	3.3	3.3	28	
Border	LKD	80	1.25	1.25	2.5	2.5	2.5	2.5	2.5	2.5	2.5	30	
Selected Resistant Lines		300										3.7	53
LKD (Control + Border)		140										2.9	
Whole experimental area		440										3.4	

*Observations were made on mother plants

VI. Agronomic and Yield Performance of Five Selected Lines in Multi-location Trial

The BBTV resistant mutant lines were comparable with LK control in terms of plant height, girth, total number of fruits per bunch, number of hands per bunch and number of fingers per hand (Table 13;). Number of days to flowering was significantly early for lines 13-30-2 (26 says earlier), 22-28-2 (21 days earlier) and 28-30-2 (17 days earlier). Mutant lines had a mean of 6 - 7 hands/ bunch and 17-18 fingers/hand. The crop stand in farmers field is shown in **Fig. 2-8-P-6**.

Table 2-8-P-7. Agronomic and yield parameters taken from BBTV resistant mutant lines (mother plants, mean from multi-location trials)

Mutant line	Mat/Line selection	Plant height* (cm)	Girth (cm)**	No. of days to flowering	No. of fruits per bunch	No. hands/ bunch	No. of fingers per hand
9.28	9.28.2	342.6	56.1	408	123	6.6.	17
9.28	9.28.3	340.8	54.8	408	128	6.5	17
13.30	13.30.2	334.5	54.1	397*	119	6.3	17
22.28	22.28.2	347.0	55.5	402*	130	6.6	18
28.30	28.30.2	342.3	55.3	406*	130	6.5	18
LK control	LK	319.6	52.3	423	108	6.0	19
t- test P=0.05		NS	NS	SD	NS	NS	NS

*Measurements taken at flowering

** Measurements taken 1m from the base of the pseudostem at flowering



Fig. 2-8-P-4 Crop stand and bunch yield of BBTV resistant mutant line

3. General Discussion

3.1 For application of mutant lines in the future

3.1.1 Bangladesh

I. *In vitro* mutagenesis of cv. Sabri

Mutation techniques in conjunction with *in vitro* culture (e.g. irradiation of shoot tips and subsequent regeneration of plants) have been suggested suitable alternative for the improvement of banana including disease resistance (Novak *et al.*, 1990). A total of 1,120 irradiated shoot tip explants in seven batches were cultured on regeneration media MS + 5.0 mg l⁻¹ BA + 0.2% Ads (Table 3-1-B-1). An average of about 4 M₁V₄ plantlets ((Fig. 2-3-B-1g) were regenerated from each irradiated shoot tip explant after three repeated subculturing in the same medium whereas in case of control (un-irradiated) it was 10-12 plantlets/explant ((Fig. 2-3-B-1h). Repeated vegetative propagation is needed to dissociate chimeras, but the minimum number of cycles required is unknown (Roux, 2004). Altogether 4334 M₁V₄ plantlets were harvested from irradiated shoot tip explants and transferred to the hormone free MS media for root induction (Fig. 2-3-B-1i). After rooting the plants were transplanted to the poly-bags filled with sterilized soil mix and kept in the hardening room with controlled light, temperature and humidity for 4 - 6 weeks (Fig. 2-3-B-1j & 1k). During hardening, about 85% plantlets were survived. After proper acclimatization, out of survived 3,744 numbers of M₁V₄ plantlets, about 2,664 numbers of M₁V₄ plantlets were transferred to the earthen pots containing *Fusarium* infected soil which was collected from the hot spots and kept under greenhouse condition for screening ((Fig. 2-3-B-1l & 1m) and found heavily infested (Fig. 3-1-B-1a) and died after 2 to 3 months. The rest of about 1000 plantlets were planted in the hot spot affected field (Fig. 3-1-B-1b) and also found to showed symptoms of *Fusarium* and died (Fig. 3-1-B-1c,d & e) after 6 to 8 months. No *Fusarium* tolerance line was obtained during the study of 4 years.

Table 3-1-B-1.

Response of irradiated and un-irradiated shoot tip explants of cv. Sabri to MS medium supplemented with 5.0 mg/l BA and 0.2% Ads on multiple shoot regeneration after three subcultures in the same medium.

Batch No.	No. of shoot tip explants inoculated		No. of plants regenerated		No. of plants transferred to the Greenhouse/Field for screening	
	Control	Irradiated	Control	Irradiated	Control	Irradiated
1	10	160	105	608	40	505
2	10	160	111	656	40	551
3	10	160	109	576	40	478
4	10	160	115	624	40	537
5	10	160	122	706	40	593
6	10	160	140	660	40	560
7	10	160	90	504	40	520
Total	70	1120	792	4334	280	3744

II. Anther culture of diploid banana

The number of anthers inoculated and response of anthers to callus induction in different culture media is presented in Table 2. A total of 698 anthers were cultured in MS1 medium and five (0.7%) of them responded to produce calli after 7 - 9 weeks of culture in this medium. These calli were creamy and starchy and failed to regenerate any plants when transferred to regeneration media. On the other hand, a total of 2,640 and 2,010 anthers were plated onto MS2 medium and N6 media respectively. Anthers in both the media responded to produce calli. The days required to callus induction ranged from 4 - 8 months. The frequency of callus formation accounted to 11 (0.4%) for MS2 and 5 (0.2%) for N6 media (Table 3-1-B-2). These calli were white, compact (Fig. 2-4-B-2f) and was found to morphogenic response when transferred into fresh MS medium containing Morel vitamins, 0.5 mg l⁻¹ BA and 0.4 mg l⁻¹ IAA. In some cases, embryoids were produced in this medium. Plant regeneration occurred when these calli/embryoids were sub-cultured on to the same media but with 0.1 mg l⁻¹ IAA instead of 0.4 mg l⁻¹ IAA (Fig. 2-4-B-2i, j & k). In one instance, regeneration of shoot in callus induction medium was noticed on N6 based medium (Fig. 2-4-B-2g&h). Regenerated shoots were rooted on growth regulator free MS medium. A total of 49 plantlets were regenerated, out of which 32 survived in the potted condition (Fig. 2-4-B-2l) during acclimatization and finally transplanted to the field for further evaluation (Fig. 2-4-B-2m). Cytological study of the root tips was carried out on 6 anther-derived plants. It was very difficult to separate and clearly distinguish each and every chromosome for counting under normal compound microscope due to its very tiny size. But it seemed that all of them possessed diploid number of chromosomes (doubled haploids, 2n =22, Fig. 2-4-B-2n & o). The growth of the doubled haploid plants was very slow and stunted and after 18 months their average height becomes only around 2.0 - 2.5 meter and started flowering after 8 to 12 months and still flowering after 18 months old. But the control diploid plants reached up to 4.5 - 5.5 meter in height and will set fruit within a year.

Table 3-1-B-2. Effects of media composition on number of anthers responded to callus induction and plant regeneration in *Musa balbisiana* (BB) variety Bichikala

Type of callus inducing media	No. of anthers inoculated	No. of anthers responded (%)	No. of green plants regenerated from calli on MS + 0.5 mg l ⁻¹ BA + 0.1 mg l ⁻¹ IAA medium
MS (Morel Vit.) + 2.5 mg l ⁻¹ 2,4-D + 1.0 mg l ⁻¹ kinetin	698	05 (0.7)	0
MS (Morel Vit.) + 1.0 mg l ⁻¹ BA + 0.4 mg l ⁻¹ IAA + 500 mg ⁻¹ CH	2,640	11 (0.4)	36
N6 (Morel Vit.) + 1.0 mg l ⁻¹ BA + 0.4 mg l ⁻¹ IAA + 500 mg ⁻¹ CH	2,010	05 (0.2)	13

From the results it was observed that anthers containing pollen in the uni-nucleate stage responded to produce calli. In majority of the crops, pollen in the uni-nucleate stage is considered as the optimum stage of development for culture of microspores (Alemano and Guiderdoni, 1994). The production of diploid banana plants could be a consequence of spontaneous chromosome doubling in haploid cells. The other possibility is the regeneration of diploid anther tissues. But in monocots, participation of somatic tissues of anther in organogenesis has been rarely reported (Assani *et al.*, 2003). As described in (Assani *et al.*, 2003), 41 plants were found haploid out of 147 plantlets obtained from four genotypes of diploid banana (BB) varieties. This reference also described that the frequency of haploid plant production is depended on genotype. In barley, up to 90% of the anther derived plants have been found diploid (Lyne *et al.*, 1986). The absence of within family segregation results in doubled haploids being a valuable source of cultivar production (Kasha and Reinbergs, 1975).

3.1.2 Malaysia

Mutations are the ultimate source of genetic variation. They provide the raw materials which other factors of evolution act and therefore all new species ultimately arose from mutation. Mutations were defined as heritable changes in the genetic material not caused by recombination or segregation. In all breeding procedures, major steps to be followed are as follows:

1. Choice of starting materials
2. Choice of mutagen (radiation or chemical)
3. Choice of suitable explants for mutagenesis
4. Radiosensitivity test
5. Selection of effective doses for main experiment
6. Desired traits for improvement
7. Methods of screening
8. Selection of the genotypes required
9. Maintenance and propagation of new cultivars

For banana breeding program, the identification of an efficient screening approach is essential in a mutation breeding approach since a high mutation induction rate, followed by a poor screening approach could result inefficiency and ultimately project failure. In general, selection of a trait, such as resistance to diseases, it is recommended to screen more than 1,000 plants (sometimes more than 10,000 plants) in the field or in a greenhouse to achieve the ultimate objectives. Following selection of potential genotype with desired traits, it is important to verify if the selected trait is heritable. In order to assess the heritability of a newly identified mutation, it will be necessary to conduct multi-location trials for a minimum period of 2-3 generations.

Banana is the second most commonly grown fruit crop in Malaysia. Overall banana production has decreased due to the increasing threat of Fusarium wilt disease, high labour costs and marketing

issues. This program was initiated to improve banana cultivars by induced mutations and biotechnology, especially to produce mutant varieties with improved traits such as tolerance to *Fusarium* wilt disease, high yield, early maturity, and short stature plants. Banana shoot-tip cultures were most suitable for micropropagation for large-scale plant production and most commercial companies have adopted this method for mass propagation of *in vitro* plantlets. However, the cost of production of *in vitro* plants could be reduced by low-cost micropropagation.

To date, somatic embryogenesis had been successfully employed for plant regeneration and could be an alternative method for mutation induction in banana improvement. Since mutation is a single cell event, therefore the use of somatic embryos for mutation induction could lead to the production of higher percentages of mutation and formation of solid mutants. In comparison, the use of meristem cultures had been proven to produce chimerism, whereby irradiated cultures had to be sub-cultured for a few generations to minimize chimerism. Somatic embryos obtained could be used for large scale propagation of plants, target materials for genetic engineering and the production of synthetic seed. Cell suspension could be established from the embryos for mass propagation.

The present research program was initiated with the objective of improving the important dessert bananas in Malaysia, particularly Pisang Berangan. This includes production of resistance or tolerance to *Fusarium* wilt or Panama disease, short plant stature, early fruiting, and high bunch weight. Banana cultivars are vegetatively propagated clones and are generally triploids and sterile. Tissue culture techniques have been exploited for (a) propagation of selected lines or natural variants; (b) generation of somaclones; (c) production of meristem pieces for *in vitro* mutagenesis and polyploidy induction; and (d) zygotic embryo culture to generate seed progenies for genetic and molecular studies, e) Pre-screening for diseases using artificial inoculation using the pathogens and e) Screening and selection of desired genotypes with improved traits in hot spot, f) Multi-location trials for stability of selected genotypes, g) Molecular characterization of selected mutants using marker-assisted selection, h) Use of micropropagation techniques for multiplication and maintenance of potential mutants, 1) Registration of potential mutants for release and commercialization.

3.1.3 Philippines

Ten promising BBTV resistant mutant lines (13-30-2, 7-29-1, 22-28-2, 23-28-7, 6-30-2, 9-28-2, 9-28-3, 9-29-1, 23-30-2, and 28-30-2) were selected after three generations of continuous field evaluation under high BBTV infection.

The reactions of the selected mutant lines to the aphid vector were determined. Mutant lines 13-30-2, 9-28-2, 9-28-3, 6-30-2 and 23-28-28-7 were significantly less preferred by the aphids while lines 7-29-1 and 9-29-1 and Lakatan control plants were equally preferred by the vector. The reactions of selected mutant lines to artificial inoculation of the virus using viruliferous aphids were likewise evaluated. Seven lines consistently showed low BBTV incidence compared with the Lakatan control plants. Line 28-30-2 showed the lowest BBTV incidence (19.6%) followed by lines 13-30-2 (25.0%), 9-29-1 (32.8%) and 22-28-2 (38.4%). Likewise, lines 28-30-2 and 13-30-1 had

the longest incubation period of up to 6 weeks. Other lines including the Lakatan control had 4-5 weeks incubation period. The symptoms observed on infected plants ranged from bunchy top, rosette growth, and marginal chlorosis.

Results of the vector-virus-host relationship study using the free choice or no choice feeding of the vector showed that BBTV resistance in some mutant lines (28-30-2, 13-30-2, 22-28-2 and 23-28-7) was due to less preference of the vector to the host plant. On the other hand, for lines 6-30-2, 13-30-2, 23-28-7, 28-30-2 which showed low BBTVD incidence (<50%) despite the high aphid colony count the underlying mechanism could be resistance of the host to the virus.

The five mutant lines planted in multi-locations showed low incidences of the BBTV from planting to harvest. The highest incidence of the disease in the whole experimental area was recorded at 11% in Bay, Laguna trial site. The random mixture of mutant lines with varying resistance mechanisms could have prevented the rapid spread of the vector and virus in farmer's field. The farmer's practice of regular rouging of infected plants at least once a month within the trial sites prevented secondary infection. There was no intensive spread of the disease and the infection occurred at random.

Further studies on the use and deployment of resistant mutant lines as a component of BBTV management strategy are needed. The use of mutant multi line (mixture of mutant plants with different resistance mechanisms) as an approach for BBTV control needs further testing. Mass propagation and dissemination of BBTV-resistant mutant lines will make a tremendous impact on the banana industry. The use of BBTV- resistant mutant lines of bananas cv. Lakatan will offer the most effective control measure against the devastating BBTV disease.

3.1.4 Vietnam

The data showed that yield parameters of mutant lines were significantly different and higher than control line. The II-47 mutant line had a number of fingers is 18.65; otherwise, the control is 17.29. The yield estimate reached 32.75 tons/ha, meanwhile, control yield reached 30.56 tons/ha. The mutant banana lines is being assigned by The Fruit and Vegetable Institute for their maintenance.

3.2 Conclusion and Suggestion

3.2.1 Bangladesh

In vitro regeneration protocol of *Fusarium* susceptible banana cv. Sabri (AAB) was established on MS medium supplemented with 5.0 mg/l BA + 2% Ads. *In vitro* radiosensitivity (LD₅₀) was determined at 35 Gy. About 2664 plantlets were transferred to the poly bags containing *Fusarium* infected soil and found heavily infested of plantlets and died after 2 to 3 months. About 1,000 Plants were transferred to the field with hot spot condition also showed symptoms of *Fusarium* and died within 6 to 8 months. Hence, further study needed to induce scalp and establish embryogenic cell suspensions for irradiation in order to develop disease resistance banana genotypes.

From the overall results of doubled haploid production from anther culture, it may be concluded that MS based medium is superior to N6 based medium in anther culture of banana. However, the frequency of anther response is very low. So it is needed to improve the efficiency of anther culture in banana by manipulating medium composition and/ or cultural conditions. The ultimate aim of this work is to regenerate plants from mutagenised gametic cells using anther culture of banana. So efforts will be made to regenerate plants from irradiated anthers.

3.2.2 Malaysia

In this project, meristems from shoot tips were used as explants or starting materials for mutation induction. Radiosensitivity test using a series of gamma ray doses of 0-100 Gy showed that LD50 for Pisang Berangan was 50Gy and selection for optimum doses based on shoot regeneration were 20, 30 and 40Gy. Four artificial disease screening techniques had been developed which can be used effectively for nursery and field screening.

1. Dipping method: 1-2 hrs soaking in spore suspension (10⁶ spores/ml)
2. Double tray method: hardened plants for 4 weeks are planted in sterile sand media in tray and *Fusarium* spores are poured in the first tray.
3. Nursery screening method: Plants are hardened for 4-8 weeks in individual polybags and transfer in tray with coir dust containing *Fusarium* for 2 weeks. Evaluation is done 4-6 weeks after planting.
4. Field screening: Those that survived from the above screening method are transferred to hot spot. Resistant plants are multiplied and transferred back to hot spot for three generations.

From a total population of 1,115 irradiated plants which had been screened in the field, at present 3 potential mutant lines of Pisang Berangan tolerance to *Fusarium* wilt disease had been selected with improved traits such as high yield, early fruiting and short stature.

1. High yield - 20 Gy (high bunch weight, more than 30 kg/bunch)
2. Early flowering (9 months) - 20 Gy
3. Shorter tree - 40Gy

Other significant achievements

1. Collaboration with University of Malaya, private nursery (Syarikat Jalur Lipur), farmers and commercial company (United Plantations Sdn. Bhd.)
2. Supplying of tissue culture materials to nurseries and growers
3. Signing Memorandum of Agreement with commercial grower, Selamat Indah Sdn. Bhd. for the technology transfer of tissue culture technique for micropropagation of Pisang Berangan and commercialization of selected mutants

Suggest to carry out the following studies:

1. Molecular characterization of mutant lines.
2. Proposal for new related projects, such as study is to understand molecular responses demonstrated during the host-pathogen (*Fusarium oxysporum* and nematode) interaction, which presents an important step towards developing transgenic banana with survivability against Fusarium wilt and nematode infestations.

3.2.3 Philippines

Ten promising BBTV resistant mutant lines (13-30-2, 7-29-1, 22-28-2, 23-28-7, 6-30-2, 9-28-2, 9-28-3, 9-29-1, 23-30-2, and 28-30-2) were selected after three generations of continuous field evaluation under high BBTV infection. The reactions of the selected mutant lines to the aphid vector and BBTV and the possible underlying mechanism of resistance were studied. Resistance in some lines could be due to less preference of the vector to the mutant lines, while in other lines, it could be resistance of the host to the virus.

The low incidences of the banana bunchy top disease observed in multi-location trial sites despite the presence of virus and vector suggest that random mixture of several mutant lines with varying resistance mechanisms could offer a more sustainable control measure.

Mass propagation and dissemination of BBTV-resistant mutant lines will make a tremendous impact on the Philippine banana industry. The use of BBTV-resistant mutant lines (as mutant multi line) of bananas cv. Lakatan will offer the most effective control measure against the devastating BBTV disease. Prior to full commercialization of these mutant lines, there is need for a multi-location and demonstration trial in several banana growing areas for yield and agronomic performance and as means of technology demonstration and promotion to farmers, growers and interested entrepreneurs. Registration of mutant lines will also be made upon completion of the required data. A new project proposal to conduct multi-location trials and commercialization of these mutant lines was submitted to the PCARRD for funding. The mutant lines are maintained and micropropagated while waiting for support for commercialization.

3.2.4 Vietnam

Throughout all experiments, we had some conclusions: regeneration medium: MS + 0.5 ppm NAA + 3 ppm BAP, rapid multiplication medium: MS + 0.5 ppm NAA + 4 ppm BAP, root medium: MS + 0.5 ppm NAA + 0.2 ppm IBA. Gamma treatment had some variations: change in high, the color

of bud, leaf, and frequency variation from 1.8% to 12.5% at *in vitro* formula and from 7.5% to 15.7% at gamma mutagenic treatment formulas. We carried out artificial inoculation on banana in greenhouse. Spore density; 10^5 spores/ml, with 4 methods to taking inoculate in which, cultured hydroponic reveals high infected sensitive. We have obtained 17 banana lines with enhanced resistance to Foc disease in greenhouse (infected rates <25%).

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Appendix i. List of Presentation & Publications

6.1 Malaysia

Rusli Ibrahim, Norazlina Noordin and Edrina Azlan (2006). In Vitro Mutagenesis using Gamma Rays: Improvement of Banana for Fusarium wilt Resistance and High Fruit Quality through Mutation Induction: Presented at Forum for Nuclear Cooperation in Asia (FNCA) Sub-Project Meeting on Disease Resistance in Banana, Philippine Nuclear Research Institute (PNRI) and University of Philippines Los Banos (UPLB), Philippines 25-28 July, 2006

Rusli Ibrahim, Norazlina Noordin and Edrina Azlan (2006). Progress Report on In Vitro Mutagenesis using Gamma Rays: Improvement of Banana for Fusarium wilt Resistance and High Fruit Quality through Mutation Induction: Presented at FNCA Mutation Breeding Workshop, Takasaki, Japan, September 11 - 15, 2006

Rusli Ibrahim, Norazlina Noordin and Edrina Azlan (2007). Preliminary screening for Fusarium wilt resistance disease: Improvement of Banana for Fusarium wilt Resistance and High Fruit Quality through Mutation Induction: Presented at FNCA Mutation Breeding Workshop, Korea Atomic Energy Research Institute (KAERI), Daejeon City, Korea, November 19 - 23, 2007.

Rusli Ibrahim and Norazlina Noordin (2008). Country Report: Improvement of Banana for Fusarium wilt Resistance and High Fruit Quality through Mutation Induction. Presented at FNCA Mutation Breeding Sub-Project Meeting on Disease Resistance in Banana, Kuala Lumpur, Malaysia, 30 June -3 July 2008

Rusli Ibrahim, Norazlina Noordin and Azhar Mohamad (2008). Final Report on Sub-Project on Disease Resistance in Banana: Improvement of Banana for Fusarium wilt Resistance and High Fruit Quality through Mutation Induction. Presented at FNCA 2008 Workshop on Mutation Breeding, Dalat City, Viet Nam, October 27 - 31, 2008.

Rusli Ibrahim, Norazlina Noordin and Siti Maryam Mohd Nahar (2009). Field Trial and Selection for Fusarium wilt Tolerance Mutants: Improvement of Banana for Fusarium wilt Resistance and High Fruit Quality through Mutation Induction: Presented at FNCA 2009 Workshop on Mutation Breeding, Hangzhou, China, September 21 - 24, 2009

Rusli Ibrahim, Norazlina Noordin and Siti Maryam Mohd Nahar (2010). Final Report on Multi-Location yield Trial: Improvement of Banana for Fusarium wilt Resistance and High Fruit Quality through Mutation Induction. Presented at FNCA Collaboration Workshop on Mutation Breeding and Biofertilizer Project, Manila, The Philippines, November 8 - 12, 2010

Ibrahim, R. B., Noordin, N. B., Mijen, A. L., Mohamad, A. B., Harun, A. R. B., Hussein, S. B. (2010). Novel production systems for banana and pineapple through in vitro mutagenesis and biotechnology. Paper presented at the 28th International Horticultural Congress, Lisbon, Portugal, 22 - 27 August 2010.

6.2 The Philippines

Damasco, Olivia P., Teodora O. Dizon, Fe M. Dela Cuevas, Ma Luz J. Sison, Felipe S. dela Cruz, Charlemagne Alexander Lim. 2010. Multi-line approach in controlling the spread of banana bunchy top disease of the banana cultivar lakatan. Poster presented 41st PMCP Anniversary and Annual Scientific Conference. Waterfront Insular Hotel, Davao City, March 9-12, 2010.

Damasco, Olivia P. Fe M. Dela Cueva, Ma. Luz J. Sison, Teodora O. Dizon, Felipe S. Dela Cruz, Jr., and Charlemagne Alexander P. Lim. 2010. Development Of Disease Resistance In Banana (*Musa Sp.*) Cv. Lakatan By Mutation Breeding: Multi-location Field Evaluation and Characterization of *Banana Bunchy Top Virus* (BBTV)-Resistant Mutant Lines. Paper presented 11th Forum for Nuclear Cooperation in Asia (FNCA) Coordinators Meeting. March 11-12, 2010, Tokyo, Japan

Damasco, Olivia P., Teodora O. Dizon, Felipe S. Dela Cruz, Jr, Fe M. Dela Cueva, Ma. Luz J. Sison and Ryan Rodrigo P. Tayobong. 2008. Development of disease resistance in banana (*musa sp.*) by mutation breeding: screening for disease resistance (*banana bunchy top virus* (BBTV) and nematode) in gamma ray irradiated plants of banana (*musa sp.*) cv. Lakatan. Country report presented Forum for Nuclear Cooperation in Asia (FNCA) Meeting on Disease Resistance in Banana The Melia, Kuala Lumpur, Malaysia, June 30 – July 3, 2008.

Damasco, Olivia P., Teodora O. Dizon, Judith B. Estrella, Leila S. Caymo, Efrelyto Jay M. Guittap, Felipe S. Dela Cruz, Jr. and Evelyn Mae T. Mendoza. 2007. Selection and characterization of gamma ray induced bunchy top virus resistant mutants in banana cv. Lakatan. *Plant Mutation Reports* 1(3):22-30.

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- Damasco, Olivia P., Judith B. Estrella, Leila S. Caymo, Teodora O. Dizon, Ruel C. Rabara, Felipe S. Dela Cruz, Jr. and Evelyn Mae T. Mendoza. 2004. Development of disease resistance in bananas. Proceedings Forum for Nuclear Cooperation in Asia Mutation Breeding Workshop. Yogyakarta, Indonesia, August 30-September 3, 2004. Paper presented at the Forum for Nuclear Cooperation in Asia Mutation Breeding Workshop. Yogyakarta, Indonesia, August 30-September 3, 2004.

Appendix ii. History of FNCA Mutation Breeding Meetings

1. FNCA 2003 Mutation Breeding Project

Program Formulation Meeting for “Disease Resistance in Banana”

September 29 - 30, 2003, Manila, the Philippines

2. FNCA 2004 Workshop on Mutation Breeding Project

August 30 - September 3, 2004, Yogyakarta, Indonesia

3. FNCA 2005 Workshop on Mutation Breeding Project

December 5 - 9, 2005, Kuala Lumpur, Malaysia

4. FNCA 2006 Mutation Breeding Project

Sub-Project Meeting on Disease Resistance in Banana

July 25 - 28, 2006, Quezon, Philippines

5. FNCA 2006 Workshop on Mutation Breeding Project

September 11 - 15, 2006, Takasaki, Japan

6. FNCA 2007 Workshop on Mutation Breeding Project

November 19 - 23, 2007, Daejeon, Korea

7. FNCA 2008 Mutation Breeding Project

Sub-Project Meeting on Disease Resistance in Banana

June 30 - July 3, 2008, Kuala Lumpur, Malaysia

8. FNCA 2008 Workshop on Mutation Breeding Project

October 27 - 31, 2008, Dalat, Vietnam

9. FNCA 2009 Workshop on Mutation Breeding Project

September 21 - 24, 2009, Hangzhou, China

10. FNCA 2010 Workshops on Mutation Breeding Project and Biofertilizer Project

November 8 - 11, 2010, Manila, the Philippines

Appendix iii. List of Contributors

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