POTENTIAL MANAGEMENT OF FUSARIUM WILT OF BANANA USING ANTAGONISTIC BACTERIA AND INDUCER CHEMICAL COMPOUNDS (DL-3β-AMINO BUTYRIC ACID AND SALICYLIC ACID)

AESHAH MHANA MOHAMMED

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by

AESHAH MHANA MOHAMMED

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LIST OF SYMBOLS AND ABBREVIATIONS

µg	Microgram
µl	Microliter
µm	Micrometer
β	Beta
ADH	Arginine dihydrolase
API® 20E	Analytical profile index® 20E
ARA	Acetylene Reduction Activity
BABA	DL-3β-aminobutyric acid
BLAST	Basic Local Alignment Search Tool
Bp	Base pairs
CFU	Colony Forming Unit
CIT	Citrate utilization
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide Triphosphates
DR	Disease Reduction
DRB	Deleterious rhizobacteria
DSI	Disease Severity Index
f.sp.	Formae speciales
*Foc*TR4	*Fusarium oxysporum* f. sp. *cubense* tropical race 4
g	Gram
g/l	Gram per Liter
GEL	Gelatinase
GLU	Fermentation or oxidation of glucose
H_2O_2	Hydrogen peroxidase
H_2S	Hydrogen sulfide
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
IND	Indole production
ISR	Induced systemic resistance
JA	Jasmonic acid
KMA	King Agar A
KMB	King Agar B

KOH	Potassium Hydroxide
MgSO4.7H2O	Magnesium sulfate heptahydrate
MRVP	Methyl red and Voges-Proskauer
NaCl	Sodium chloride
NaOCl	Sodium hypochloride
ng	Nanogram
ODC	Ornithine decarboxylase
OF	Oxidation – Fermentation
PCR	Polymerase Chain Reaction
PDA	Potato Dextrose Agar
PGPB	plant growth promoting bacterial
PGPR	plant growth promoting rhizobacteria
PI	Percent inhibition
PSI	Phosphate solubilize index
SAR	Systemic Acquired Resistance
SIM	Sulfur-indole-motility media
SR	Systemic Resistance

POTENSI PENGURUSAN LAYU FUSARIUM PISANG MENGGUNAKAN BAKTERIA ANTAGONISTIK DAN SEBATIAN KIMIA PERANGSANG (DL-3β-ASID AMINOBUTIRIK DAN ASID SALISILIK)

ABSTRAK

Fusarium oxysporum f. sp. *cubense* ras tropika 4 (*Foc*TR4) merupakan penyebab penyakit layu pisang (penyakit Panama), dan dianggap sebagai salah satu ancaman yang paling serius kepada pengeluaran pisang di dunia. Objektif kajian ini adalah untuk memencilkan, mengecam, mencirikan, serta menilai secara *in vitro* aktiviti bakteria peransang pertumbuhan (PGPB) dan meneroka dua sebatian perangsang pertumbuhan, (DL-3β-aminobutyric asid (BABA) dan asid salisilik (SA)) untuk menindas *Foc*TR4. Pemeriksaan secara in vitro telah dijalankan 56 pencilan bakteria dari rizosfera pisang, akar, kulit akar, dan rizom di tiga ladang pisang di Semenanjung Malaysia terhadap *Foc*TR4 virulen. Hasil kajian menunjukkan bahawa rizosfera, akar, dan rizom pisang dengan ketara ($p<0.05$) menunjukkan bilangan tertinggi populasi bakteria endofit. Pencilan yang menunjukkan kesan antagonistik tertinggi terhadap *Foc*TR4 adalah USMPS10, (88.3%), USMPS20 (77.6%), USMPS4 (72.1%), dan USMPS55 (70.4%) manakala USMPS30 (62.1%) dan USMPS12 (59.7%) adalah jauh lebih rendah ($p<0.05$) berbandg kawalan. Berdasarkan pengecaman dan pencirian pencilan bakteria, 80% adalah Gram negatif dan 20% adalah Gram positif. Bakteria yang paling lazim dijumpai pada pokok pisang dan juga majoritinya merupakan pencilan yang berkesan adalah bakteria Gram negatif dari genus Pseudomonas, sementara satu bakteria Gram negatif yang berkesan dikenal pasti sebagai *Serratia*

marcescens. Sebatian perangsang BABA dengan peningkatan kepekatan (5 Mm, 10 Mm, dan 15 mM) secara *in vitro* menunjukkan tiada kesan yang ketara ke atas pertumbuhan miselium Sebaliknya, kesan rencatan daripada SA telah dijalankan ke atas pertumbuhan miselium *Foc*TR4 dengan peningkatan kepekatan masing-masing pada 10 dan 15mM, namun tidak merencat sepenuhnya. Sebahian daripada *P. aeruginosa* (USMPS10) dan *P. putida* (USMPS4) menyebabkan aktiviti antagonistik yang berkesan, diikuti oleh *S. marcescens* (USMPS55) dan *B. cepacia* (USMPS20). Kawalan biologi yang paling berkesan dari sudut mekanismanya telah dinilai peningkatan keupayaan menghasilkan antibiotik, siderophore, HCN, IAA, fluorescein, pyocyanin, dan sebati metabolit tidak stabil melalui mod tindakan langsung dan tidak langsung oleh bakteria meransang pertumbuhan pokok di berkesan rizosfera. Berdasarkan ujian in vitro, rawatan rendaman akar secara *in vivo* dijalankan di dalam rumah tanaman untuk menilai kesan dua ejen abiotik, iaitu BABA dan SA serta kombinasi 4 PGPB untuk menindas *Foc*TR4. Keputusan menunjukkan rawatan yang paling berkesan adalah pencilan USMPS10 dan kombinasi 4 pencilan PGPB serta BABA pada kepekatan 5Mm yang telah meningkatkan kekuatan pokok dan kandungan klorofil. Sistem belahan akar telah menunjukkan USMPS10, BABA, dan SA mampu memberi permulaan yang signifikan kepada pertahanan menyeluruh terhadap *Foc*TR4. Kesimpulannya, penindasan *Foc*TR4 secara in vitro dan in vivo oleh BABA, SA, dan pencilan rizobakteria efektif dalam pelbagai mod tindakan memberi informasi awal yang berguna mengenai potensi kedua- dua faktor ini terhadap *Foc*TR4.

POTENTIAL MANAGEMENT OF FUSARIUM WILT OF BANANA USING ANTAGONISTIC BACTERIA, AND INDUCER CHEMICAL COMPOUNDS (DL-3β-AMINO BUTYRIC ACID AND SALICYLIC ACID

ABSTRACT

Fusarium oxysporum f.sp. cubense tropical race 4 (*Foc*TR4), is the causal agent of Fusarium wilt of banana (Panama disease), which was the one of the most serious threats to banana production . Therefore, the objectives of this study were to isolate, identify, characterize as well as to screen for in vitro plant growth promoting bacterial (PGPB) activities and to explore two inducer chemical compounds DL-3β-aminobutyric acid (BABA) and salicylic acid (SA) to inhibit FocTR4 growth. In vitro screenings of 56 bacterial isolates of banana rhizospheres, roots, rhizoplanes, and rhizomes from three banana plantations in Peninsular Malaysia were conducted against virulent FocTR4. The results showed that the rhizospheres, roots, and rhizome of the next significantly plants ($p<0.05$) revealed the highest number of bacterial population. Selected isolates displayed the highest antagonistic effects against FocTR4; isolate USMPS10, (88.3%), USMPS20 (77.6%), USMPS4 (72.1%), and USMPS55 (70.4%) while, USMPS30 (62.1%), and USMPS12 (59.7%) showed significantly lower antagonistic effects ($p<0.05$) than the control (100%). Based on identification and characterization of the bacterial isolates, 80% were Gram negative and 20% were Gram positive positive. The most prevalent bacteria in banana plants and the majority of the effective isolates belong to Gram negative bacteria from the genus *Pseudomonas* while, other effective Gram negative isolates was identified as *Serratia marcescens*. In vitro test on BABA showed no significant effect on mycelial growth with increasing

concentrations from 5 to15 mM. In contrast, the inhibitory effect of SA on mycelium growth of FocTR4 showed significant effects ($p<0.05$) with increasing concentrations at 10 and 15mM respectively, but it was not inhibited completely. The bacteria extracts of *P. aeruginosa* (USMPS10) and *P. putida* (USMPS4) resulted in an efficient antagonistic activity followed by *S. marcescens* (USMPS55) and *B. cepacia* (USMPS20), respectively. The most effective biocontrol isolates were further assessed for their ability to produce biocontrol mechanisms (antimicrobial antibiotic, siderophore, HCN, IAA, fluorescein, pyocyanin, and volatile metabolites) against *Foc*TR4. Based on in vitro results, in vivo root dipping treatments were carried out in plant house to evaluate the effects of antagonistic as well as, the two abiotic agents, BABA and SA to suppress *Foc*TR4. Results indicated that the most effective isolate was by *P. aeruginosa* (USMPS10) and combinations of the four PGPB (*P. aeruginosa*, *P. putida, S. marcescens*, and *B. cepacia*), as well as 5 mM BABA which showed promising biocontrol efficacy and improved plant vigor and chlorophyll content. The split root system has demonstrated that *P. aeruginosa* (USMPS10), BABA, and SA were capable of rendering significant ($p<0.05$) induction of systemic resistance against *Foc*TR4. Hence, *in vitro* and *in vivo* inhibition of *Foc*TR4 by BABA, SA and the effective rhizobacterial isolates in the presence of various modes of action provide useful preliminary information on the potential of these two factors against *Foc*TR4.

CHAPTER 1

INTRODUCTION

Banana is the most important food crop in the world after rice, wheat and maize. In many developing countries such as Rwanda, Uganda, sub-Saharan Africa, Latin America, and Malaysia, banana production plays a major role in nutrition and economy (Ploetz, 2005). In Malaysia, banana is second most important fruit crops after durian (Durio zibethinus). The major producing states are Johor, Sabah, and Sarawak which occupy about 31,300 hectares. However, banana production in Malaysia has significantly decreased because of the increasing threat of diseases, causing substantial yield losses (Mohammed *et al.*, 1999). Banana is vulnerable to many diseases caused by fungi, bacteria, viruses and nematodes (Jones, 2000). Fusarium wilt or Panama disease caused by *Fusarium oxysporum* f. sp. *cubense* (*Foc*) was first reported in Australia in 1876 (Ploetz and Pegg, 2000). The fungus remains dormant in agricultural soils until stimulated by a susceptible host species (Nelson, 1981), thus germinates and infect the roots and colonizes vascular vessels to cause lethal wilt in banana plants. The pathogen primarily spread by movement of diseased plant materials and infected soil particles as well as disseminated by seeds (Geetha, *et al.*, 2005). The diseases is active under a wide range of environmental conditions and survive in the soil as chlamydospores; making it very difficult to eliminate from the soil by conventional control measures (Marois, 1990; Ploetz, 2006a; Ploetz, 2006b; Jaroszuk-Scisel *et al.*, 2008).

The strategies to control Fusarium wilt including breeding of *Fusarium*-resistant banana hybrids, chemical, and biological controls. Although breeding strategies (using resistant plants) is the most effective method to control Fusarium wilt (Nelson, 1981 and Tushemereirwe *et al.*, 2003), no single method is fully effective on its own. The management of Fusarium wilt depends on the integration of different control strategies. These strategies concentrated on lowering the amount of inoculum in the field, while enhancing plant vigour and disease tolerance (Erwin, 1981). The use of cultural control measures like crop rotation has provided some control over the years against many diseases (Baker, 1981). However, propagules of many causal agents of vascular wilt diseases stay viable in the soil for extensive periods. Hence, chemical treatments such as soil fumigation and foliar spray are important in managing plant disease. However, many of these compounds proved to be quite toxic to the environment and it can lead to the suppression of other beneficial (Lindbeck *et al.*, 2009). Biological control offers a potential alternative to the use of resistant banana varieties against *Foc*. Several reports have demonstrated the successful use of biological control agents against Fusarium wilt (Larkin and Fravel, 2002; Weller *et al.*, 2002). Most of these biocontrol agents have been isolated from naturally suppressive soils to control Fusarium wilt (Larkin *et al.*, 1993; Larkin *et al.*, 1996). In such soils, the disease incidence remains low, despite the presence of a susceptible host and the pathogen (Alabouvette *et al.*, 1993).

The biological control agents that contribute to disease suppression include non pathogenic *F. oxysporum*, *Bacillus* spp., *Trichoderma* spp. and *Pseudomonas* spp. (Schallmey *et al*., 2004; Thangavelu *et al*., 2004; Sun *et al*., 2011). *Pseudomonas* spp. have frequently been linked to plant disease suppression (Bolwerk *et al.*, 2003). The

mechanisms of disease suppression by *Pseudomonas* spp. are through antibiosis, competing for iron, nutrients and, production of antifungal compounds (Van Loon *et al.*, 2006; Haas and Défago 2005; Glick *et al*. 2007a), as well as, induced systemic resistance (Van Loon *et al.*, 2006). Several studies have investigated the ability of *Pseudomonas* to suppress the infection of *Foc* Race 1 and Race 4 for banana tested under greenhouse condition (Thangavelu *et al.,* 2001; Rajappan *et al.,* 2002).

Plant responses to pathogens are multi-defence reactions, which try to limit and eventually stop the invading pathogen that includes production of antimicrobial pathogenesis-related proteins, and low molecular weight phytoalexins (Heath, 2000; Dangl and Jones, 2001). Hence the induction of resistance to pathogen is a promising approach for controlling plant diseases. Induced resistance is the general term by which all types of elicited responses that lead to enhance protection against diseases including both locally and systemically induced resistance (Hammerschmidt *et al*., 2001). One of the classic forms of induced resistance is systemic acquired resistance (SAR) controlled by a signaling pathway that depends on endogenous accumulation of salicylic acid (SA) (Durrant and Dong, 2004).

Salicylic acid is a phenolic acid that generally not abundant in most plants, is an important defence compound because it mediates (SAR), a resistance mechanism whereby SA is used as a signalling molecule to relay information on pathogen attack to other parts of the plant (Vermerris and Nicholson, 2006). Salicylic acid (SA) and DL-3β-aminobutyric acid (BABA) have been reported as plant immune inducers against many bacterial, fungal and viral pathogens. These chemical inducers can produce high

concentration of pathogen related (PR) proteins in plants (Klessig and Malamy, 1994; Yun *et al.,* 1999; Heil and Bostock, 2002).

DL-3-β aminobutyric acid (BABA) is a non-protein chemical inducer, which has been reported to activate disease resistance in various crops (Siegrist *et al.,* 2000; Silue *et al.,* 2002). BABA has been reported to have an effect against soil-borne fungi (Oka *et al.,* 1999). However, it did not significantly lower the incidence of Fusarium wilt in the greenhouse. Induced resistance by BABA involves the SA pathway or another pathway due to much evidence that showed interactions exist between the different defence signalling pathways (Pieterse *et al.,* 2000; Silué *et al.,* 2002). This cross talk between the pathways provides a great regulatory potential for activating multiple resistance mechanisms (Pieterse *et al.,* 2001). These strategies to control Fusarium wilt are concentrating on lowering the amount of inoculum in the field, while enhancing plant vigour and disease tolerance (Erwin, 1981).

In this regard, a little progress have been done in the effective management to control banana wilt disease caused by *Foc*TR4 which is a threat to the multi-billion dollar global banana trade especially in Malaysia. Therefore the objectives of present study were:

i) To isolate and identify potential bacterial as biological control agents against Fusarium wilt disease (*Foc*TR4) of banana,

ii) To evaluate the effects of bacterial isolates and inhibition of Fusarium wilt incidence on growth promotion on Berangan seedlings under plant house conditions,

iii) To assess the effectiveness of two inducer compounds, namely DL-3β-aminobutyric acid (BABA) and salicylic acid (SA) to inhibit Fusarium wilt incidence *in vitro* and *in vivo* on Berangan seedlings under plant house conditions.

CHAPTER 2

LITERATURE REVIEW

2.1 Banana Plant

Banana belong to the genus *Musa*, for both, dessert (*Musa sapientum*) and plantains (*Musa paradisiaca*) varieties and originated from the wild diploid species of *Musa acuminata* and *Musa balbisiana* (Daniells *et al*., 2001). Banana plant is a monocotyledonous giant herb that consists of a sympodial rhizome from which both the root system and pseudostem, consist of tightly clasping leaf sheaths and arise (Jones, 2000). Flowers are produced when the apical meristem stops producing leaves and forms an inflorescence. Once flowering has been completed, the pseudostem dies, and new plants develop from suckers that arise freely from the underground rhizomes (Jones, 2000).

Banana plant propagation depends on the use of vegetative materials such as suckers or rhizome pieces (Heslop-Harrison and Schwarzacher, 2007). Therefore, *in vitro* propagation of bananas was developed for mass production of uniform and disease-free planting materials (Israeli *et al.*, 1995). Commercial production of micropropagated bananas can now be found in many countries, and a variety of *in vitro* techniques can also be applied for the genetic improvement of banana (Israeli *et al.*, 1995). Bananas are cultivated in many subtropical and tropical regions of the world, including Asia, Africa, Central and South America, the Caribbean, and Oceania (Dale, 1999). In Malaysia, it is the second most important fruit crops after durian, covering about 26,000 ha (Rozeita, 2012). Bananas are the fourth most important staple food

crop in the world. The fruit can be produced all year round and provides a stable income to farmers in resource poor areas (Jones, 2000).

Bananas are divided into two main groups, dessert bananas and cooking bananas (Mohapatra *et al*., 2010). Dessert bananas form 43% of the world's production of bananas, and are eaten raw when ripe. Cooking bananas, which account for the remaining 57%, are a staple food that need to be fried, baked, boiled or roasted before they can be eaten (Cane, *et al.*, 2005). Bananas are becoming increasingly more important due to their use in industries such as beer brewing, as well as their fibrous material that can be used in paper and textile productions (Zainuddin, 2002). Commercially important cultivars in Malaysia include Pisang Mas (Sucrie), Berangan (Lakatan), Rastali (Silk), Embun (Gros Michel), and Cavendish (acuminata Colla) (Nik Masdek *et al*., 1998).

Like any other crops, banana is susceptible to many diseases and pests that are threatening the worldwide production of bananas (Stover, 1996). Among these disease,

Fusarium wilt is responsible for significant economic losses throughout the world and affects many important cultivars of banana (Ploetz *et al.*, 2003).

Fusarium wilt is caused by *Fusarium oxysporum* f.sp. *cubense* (*Foc*), a soil-borne fungus. Fusarium wilt causes considerable economic losses and affects many important banana cultivars (Jeger *et al.*, 1995).Banana production in Malaysia has significantly decreased because of the increasing threat by this disease, causing substantial yield losses (Mohammed *et al*., 1999).

2.2 Fusarium wilt of banana (Panama Disease)

2.2.1 Fusarium oxysporum f. sp. cubense

Of many special forms or sub-species of *F. oxysporum*, only *Foc* is specifically responsible for banana wilts disease (Ploetz, 2005). It is a soil-inhabiting filamentous fungus that belongs to the genus *Fusarium* (Stover, 1990). The fungus is characterized by micro and macroconidia (one or two celled, oval to kidney shaped), produced on branched and unbranced monophialides. However, macroconidia are four to eight celled and crescent shaped with a foot-shaped basal cell (Ploetz, 2005).

Four well recognized *Foc* pathogens have been separated based on host susceptibility. Race 1 is known to wipe out Gros Michel (AAA) cultivars in Central America and cause epidemics in 1950s, also attacks Okra as well as AAB desert cultivars Silk and Pome varieties (Molina *et al.*, 2008). Race 2 affects cooking bananas such as Bluggoe (ABB) and Race 3 that is capable of affected Cavendish as well as other varieties of banana affected by Race 1 and race 2. Race 3 specifically affects *Heliconia* spp., a close relative of the banana, but not considered to be a banana pathogen (Daly and Walduck, 2006).

In addition, Race 4 can be divided into two types; namely sub-tropical andtropical strains. The tropical Race 4 is more virulent and has the capability of causing disease in Cavendish under growing conditions, while the subtropical Race 4 causes disease in plant growing in sub-optimally condition such as water stress or grows in cool temperature and poor soil (Daly and Walduck, 2006; Groenewald *et al.*, 2006).

In subtropical regions such as South Africa and Australia, an isolate diagnosed

as *Foc*R4 (known as VCG1020) infected Cavendish (AAA). Whereas, in other tropical regions such as Cosat Rica, the same VCG isolate unable to affect Cavendish, thus was referred as *Foc* Race 1. Consequently, the same genotype of the isolate can be classified as different Races (Pegg *et al.*, 1996). The term *Foc* Race does not imply defined genetic relationship with the host. Therefore, *Foc* Races are groups of strains which have been observed to be pathogen to particular host cultivar (Gerlach *et al*., 2000).

There have been various reports regarding the VCGs the affected banana in Malaysia. Masdek *et al.* (2003) and Nasdir (2003) reported that in subtropics only Cavendish cultivars were affected. The arising of *Foc* tropical Race 4 (GCV 01213-01216) has caused important losses in Malaysia and Indonesia plantation of which more than 8 million plants on traditional plantations and more than 5,000 ha of commercial Cavendish plantations has been affected with annual losses over 75 million USD and has affected family income of thousands of workers and farmers (Leslie and Martin, 2016).

2.2.2 The global history and distribution of Fusarium wilt of banana

Diseases are a major constraint to banana production worldwide and a number of diseases affected banana (Jones, 2000), of which one of the most important disease is Fusarium wilt. The disease was first recorded in Australia in 1874 (Bancroft, 1876) and in 1890, the disease became epidemic in Panama. In the period of 1890-1960, approximately 40000 hectares of the susceptible banana cultivar Gros Michel (grown for export) were destroyed or abandoned in Central and South America and the Caribbean because of *Foc* Race 1. Export industries were forced to replace the

susceptible Michel variety with Cavendish cultivars, which continue to show resistance to *Foc* Race 1 in these areas (Stover, 1990).

Cavendish cultivars remain the banana varieties of international trade. However, these cultivars are not resistant to all strains of *Foc*. The subtropical *Foc* Race 4 strain causes losses to Cavendish cultivars in the subtropical regions of the Canary Islands, South Africa, Australia and Taiwan (Ploetz *et al.*, 2003). More importantly, in the tropical commercial and subsistence production regions of the Philippines, Indonesia, Taiwan, Malaysia, and in the southern provinces of China, a new strain of *Foc* designated as tropical Race 4 has caused widespread devastation (INIBAP, 2006). Tropical *Foc* Race 4 affects banana cultivars that comprise 80% of the world's banana production, including the important Cavendish and plantain subgroups (Ploetz, 2005). The tropical *Foc* Race 4 could cause significant damage to the major world export production areas. As it stands, the tropical Race 4 poses a very real threat to the multi-billion dollar global banana trade including Malaysia, and the food security of millions of subsistence farmers (Ploetz, 2005).

2.2.3 Disease symptom of Fusarium wilt

The *Foc* pathogen infects the xylem vessels by penetrating the root tips through wounds or injuries (De Ascensao and Dubery, 2000), then invades the xylem by producing microconidia, and blocks the transport nutrients to the rest of the plant by plugging the vascular tissue, resulting in discoloration and wilting (Ploetz, 2000). The obvious symptoms of *Foc* in the field are typical of vascular wilt diseases. Early symptoms are the infected plants show premature yellowing of the older leaves. The yellowing progresses from the older leaves to the younger leaves. The yellowing of the

older leaves start along the margins of the leaf and continue to the midrib until the leaves are completely brown and die forming skirt of dead leaves surrounds the pseudostem. Frequently, the pseudostem splits longitudinally only above the soil and the outer leaf sheaths separate from the pseudostem and collapse, thus the infected plants become thinner than the uninfected ones (Hwang and Ko, 2004).

Internally, disease symptoms become obvious in the xylem of the root, vessels of the roots as it spread into a rhizome and finally colonizing all the way up to pseudostem. The fungus starts growing through the tissues where turns reddish brown to maroon colour become visible when the plant is cut longitudinally. Inside the cross section of an infected plant, the change in the colour appears in circular shape around the centre of the rhizome. Disease progresses into the pseudostem and the lines of discolouration are considered as proof of infection. Moreover, the infection may move up to the top of the pseudo-stem (Daly and Walduck, 2006). However, infection has not been shown to progress into the fruit (Davis, 2005).

2.2.4 Disease cycle of Fusarium wilt

In response to the chemical composites released from banana roots, *Foc* spores germinate and begin to develop near to the roots of the banana tree. The disease attacks at the finer and secondary roots then moves forward into the bigger primary roots via the xylem vessels before entering the rhizome. The primary roots and the rhizome do not show any symptoms of infection (Figure. 2.1). Once *Foc* is inside the host plant, it secretes the toxin fusaric acid (toxic substance), which kills the plant tissue in advance of hyphal penetration (Ploetz, 2000).

Furthermore, movement of the spores along side the sap flow is obstructed, momentarily as soon as they become stuck at the end walls. Soon after, the spores sprout and the hyphae progress through the holes into attached vessels where further spores are created accordingly. The production of gels and tyloses (a defense mechanism) by the the plant blocks off the infection, thus, avoiding the infection from effectively travelling to and inflowing the rhizome (Van der molen *et al*., 1987; Beckman, 1987). Other than that, numerous infections arise throughout the lifecycle of a plant and constantly one or more will lead to its widespread invasion. The virulence of tropical Race 4 on "Cavendish" suggests that the defense mechanisms activated by the plant against this strain are not as effective as for "sub-tropical" Race 4. This strain only leads to unembellished losses in plants under stress (Figure 2.1) (Daly and Walduck, 2006).

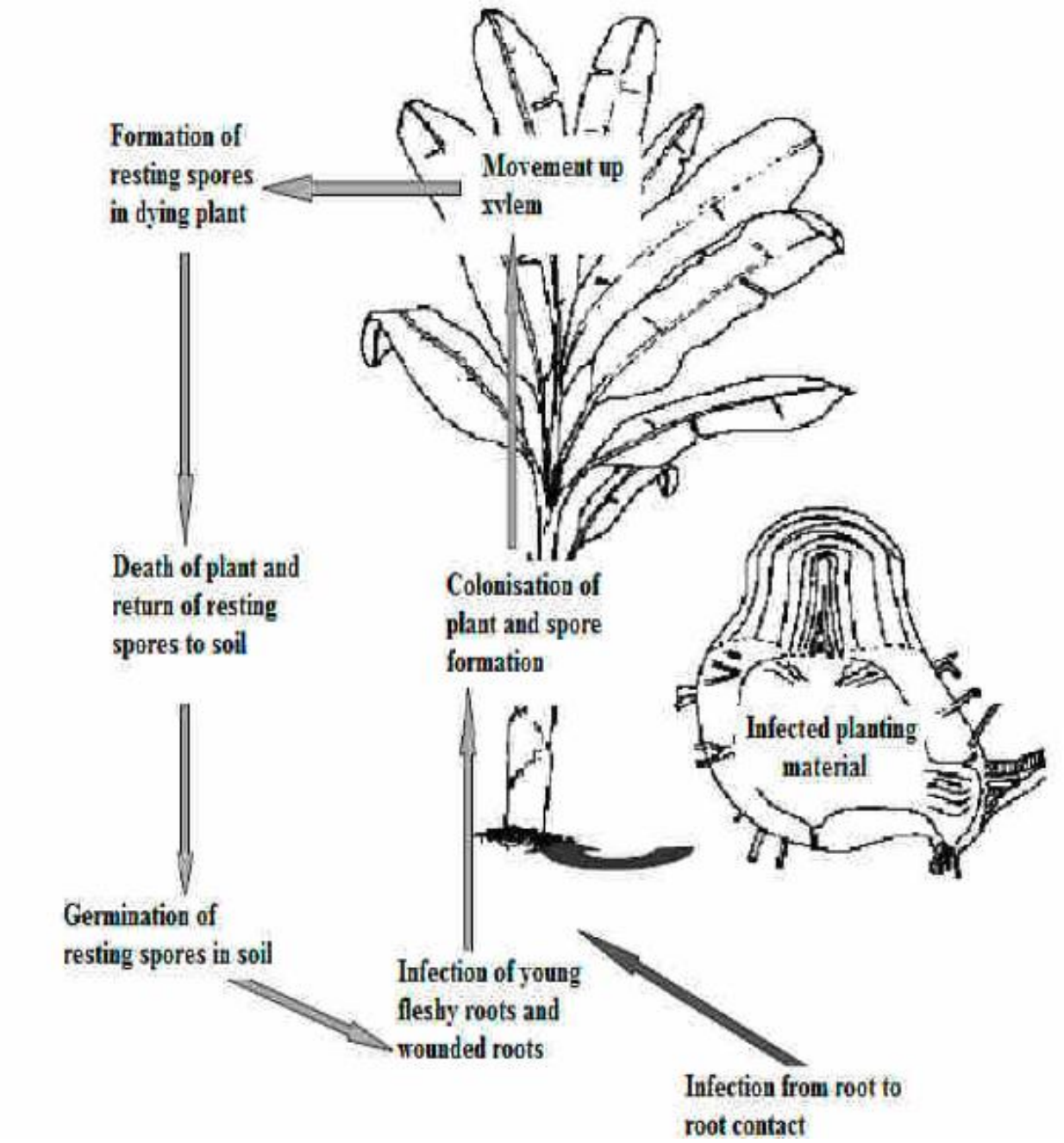

Figure 2.1 Disease cycle of *Fusarium oxysporum* in a banana plant (Daly and Walduck, 2006)

2.3 Control management of Fusarium wilt of banana

Various attempts have been made to control banana wilts, caused by *Foc*. Nevertheless, no long-term control measures are available other than the organic amendments (Stover, 1962), fungicides (Gullino *et al.*, 2000), crop rotation (Martin, 2003), soil fumigation (Fourie *et al*., 2009), and flood-fallowing (Zhang, 2013), which are some of the control strategies practiced so far.

Fusarium wilt may be controlled by the use of chemical, biological and cultural methods, or by introducing resistant varieties. Although the use of resistant planting material is the most effective means of reducing disease, a limited number of successes have been achieved. The use of chemicals and biological control agents for controlling Fusarium wilt in soil has become popular as environment as friendly approach (Ploetz *et al.*, 2003).

2.4 Induced systemic resistance in plants

Induced systemic resistance (ISR) in plants is a defensive capacity against a broad spectrum of pathogens induced by certain stimulus such as primary infection by a weaker strain of a pathogen. The consequential resistance is due to an inducing agent upon infection by ISR (Bakker *et al.*, 2003).

Defense mechanisms are triggered by a stimulus prior to infection by a plant pathogen to reduce the disease. The most intriguing forms of resistance and it is the basic theory of induced resistance, in which a variety of biotic and abiotic treatments prior to infection can turn a susceptible plant into a resistant one (Bakker *et al.*, 2007). In contrast, induced resistance is not the creation of resistance where there is none, but

the activation of latent resistance mechanisms that are expressed upon subsequent infection (Van Loon, 1997). Microorganisms and chemicals that ISR are commercially successful and available to control the plant diseases (Oostendorp *et al.*, 2001; Kim *et al.*, 2001; Zehnder *et al.*, 2001; Reuveni *et al.*, 2002; Bednarz *et al.*, 2002).

Infections caused by plant pathogens can be suppressed through biotic or abiotic elicitors that induce resistance are categorized as either systemic acquired resistance (SAR) or induced systemic resistance (ISR). Induced resistance (SAR and ISR) involves the synchronized action of defence signalling pathways which can be either activated by non-pathogenic microorganisms (for example, some rhizobacteria) or pathogenic microorganisms. In other plants, the type of defence can be induced by certain groups of chemicals (Van Loon *et al*., 1998). Moreover, plants can develop resistance against pathogens through active or passive means (Huang, 1998). Passive defense mechanisms are those that are present before contact with the pathogen, while active defense mechanisms are activated only after pathogen recognition however in reality this distinction is not always clear, as many pre-existing defenses are modified after infection (Huang, 1998).

The initiation of systemic resistance by rhizobacteria is referred to ISR, while it known as SAR by other parties (Van Loon *et al.*, 2006). ISR or SAR is mainly used to afford protection against pathogenic fungi, bacteria, nematodes and viruses that may affect the growth of the plant. In addition, many experiments have been conducted on a large number of defense enzymes associated with ISR including phenylalanine ammonia lyase (PAL), chitinase, glucanase, peroxidase (PO), polyphenol oxidase (PPO), superoxide dismutase (SOD), catalase (CAT), lipoxygenase (LOX), ascorate

peroxidase (APX) S-nitrosoglutaionereductase (GSNOR) and proteinase inhibitors peroxidase (APX) S-nitrosoglutaionereductase (GSNOR) and proteinase inhibitors (Schneider *et al.*, 1996;Schisler *et al.*, 1997; Van Loon *et al.*, 1998). Figure 2.2 shows the mechanisms of ISR and SAR.

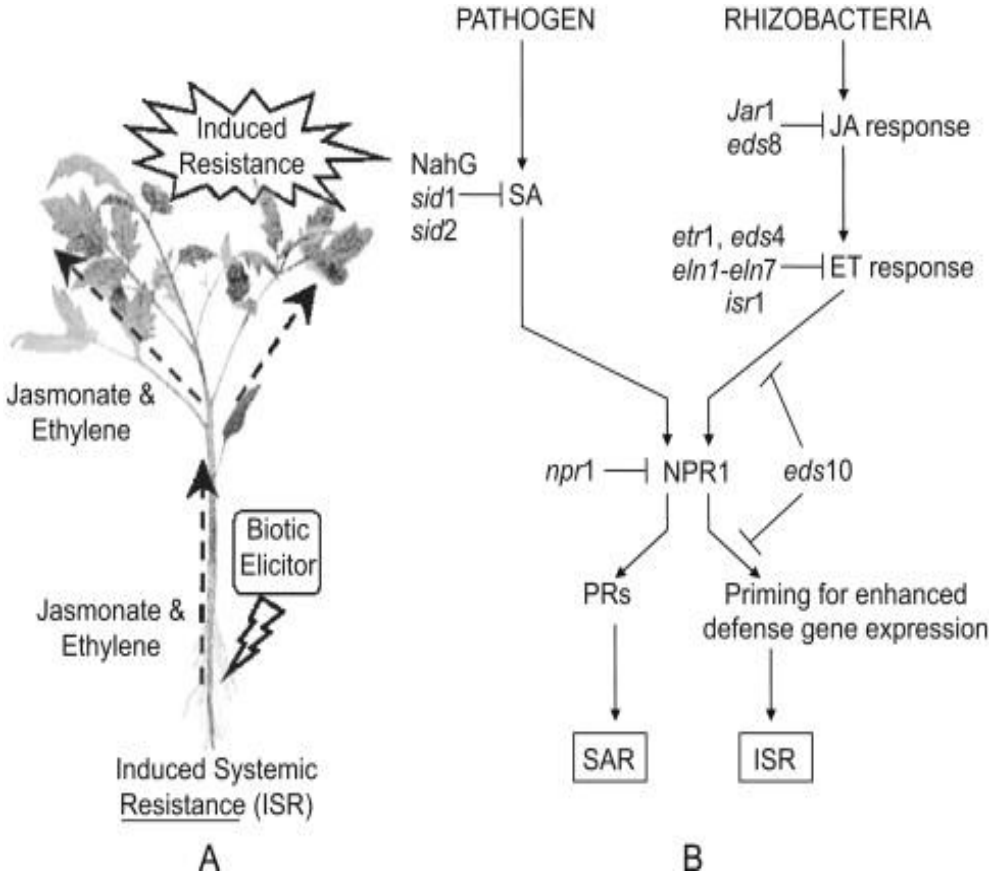

Figure 2.2 Mechanisms of induced systemic resistance (A), and systemic acquired resistance (B) (Choudhary and Johri, 2009).

Necrotizing pathogenic organisms trigger SAR and non-pathogenic rhizobacteria activate ISR under natural conditions. Both SAR and ISR were shown to be effective against a broad range of pathogens (Pieterse and Van Loon, 2004). SAR

and ISR are phenotypically similar, but genetically and mechanistically different. Like SAR, ISR has been systemically demonstrated against fungi, bacteria, and viruses in *Arabidopsis thaliana*, beans (*Phaseolus vulgaris* L.), carnation (*Dianthus caryophyllus* L.), cucumbers (*Cucumis sativus* L.), radishes (*Raphanus sativus* L.), tobacco and tomato (Kang *et al.*, 2007).The pathways of SAR and ISR are modulated by NPR1 protein which is master regulator of defence related portions (Figure 2.3) (Saskia *et al*., 2000).

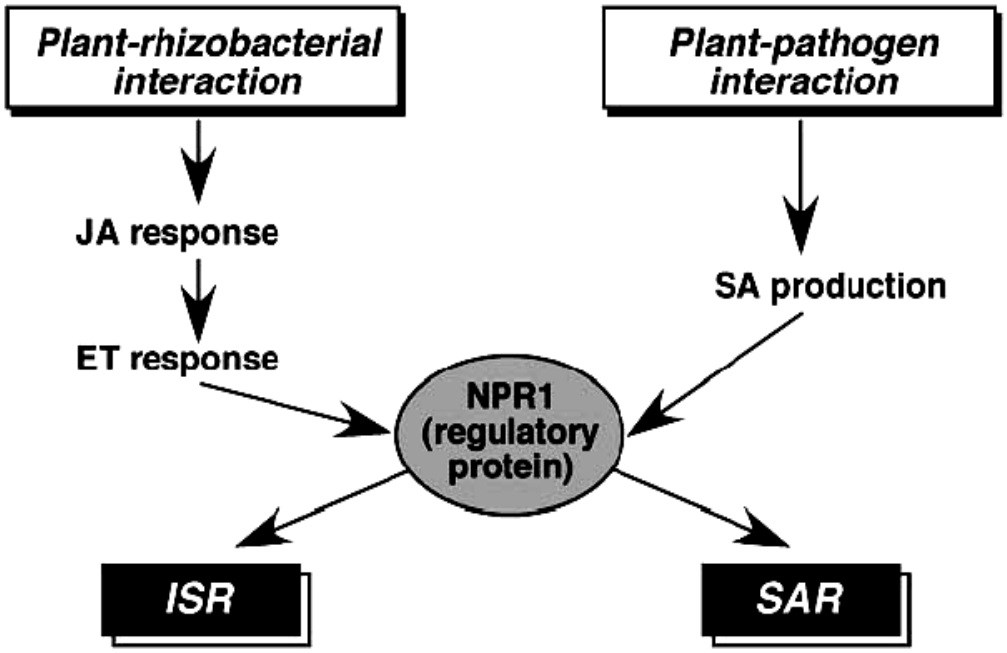

Figure 2.3 Common signalling pathways involved in induced resistance mechanism in plants (Pieterse and Van Loon, 1999).

Ali *et al*. (2002) treated the BCA strains to half of the split root system of the tomato plants, which caused a significant reduction in nematode penetration compared to the other half of the split root system. This was attributed to ISR activity of the

strain. As such, it concludes that ISR helps in enhancing the plants defense system. The split root method proved that there was no interaction between the pathogen and the non-pathogen, and that resistance is due to the non-pathogen that triggers a defence response in the plant (Larkin and Fravel, 1999; Bolwerk *et al*., 2005). The split root method involves the exposure of some roots to nonpathogens, and proving that by means of systemic translation of biochemical processes in the plant, it induces resistance to the pathogen in the other non-exposed roots.

2.5 Inducing resistance by abiotic agents (chemical control)

Chemical control of Fusarium wilt disease has yielded variable degrees of success. Chemical applications often depend on the crop and method of application. Various chemicals that can be used for the controls of different plant diseases can be divided into four different categories, namely fungicides, surface sterilises, fumigants and plant activators. Currently, Fusarium wilt disease is primarily controlled by application of synthetic fungicides. The most important, commercially and widely used chemicals for induction and enhancement plant mechanisms defense against wide variety of pathogens, are acibenzolar-S-methyl (BTH), probenazole (ORYZEMATE), beta-aminobutyric acid (BABA), 2, 6-dichloroisonicotinic acid (INA), salicylic acid (SA), and 2-deoxy-D-glucose (DDG) (Cohen *et al.*, 1994).

An application of *P. aeruginosa*, a plant growth promoting rhizobacterium alone or with crustacean chitin, fungicides (benlate/captan) or *Paecilomyces lilacinus* (a biocontrol agent) significantly suppressed *Macrophomina phaseolina*, *Rhizoctonia solani, F. oxysporum* and *F. solani.* Induced resistance against Fusarium wilt of watermelon using various abiotic inducers included different concentrations of Co as

CoSo4 or ethephon (2-chloroethyl phosphonic acid) (Sultana *et al.*, 2010). Results indicated that the most effective treatment in reducing the percentage of wilted plants were ethephon at 800 ppm, CO++ at 0.5 ppm. Treatment with ethephon at 600 ppm was highly effective with cv. Giza 1 only in field experiments (Abd-El-Kareem *et al.*, 1993). Previous studies reported by Sultana and Ghaffar (2010) studied *In vitro* and *In vivo* effects of fungicides, microbial antagonists and oilcakes in the control of *F. solani* the cause of seed rot, seedling and root infection on bottle gourd, bitter gourd and cucumber. Complete inhibition of colony growth of *F. solani* was observed where fungicides viz., Aliette, Benlate and Carbendazim at 100 ppm were used. Carbendazim completely eradicated seed borne infection of *F. solani* in bitter gourd and gave maximum reduction in cucumber and bottle gourd. On the other hands, Koppula *et al.*, (2010) tried an approach towards the development of eco-friendly antifungal compounds for controlling crop diseases using methanol solvent extracts of twenty South Indian medicinal plants against three important phytopathogenic fungi (*Colletotrichum capsici, Phythium aphanidermatum* and *F. oxysporum*).

One of the documented studies of these fungicides against fusarium wilt is Azoxystrobin. The study showed the fungicide exhibited a high efficacy on fusarium wilt of three ornamental crops namely carnation, cyclamen and Paris daisy. Azoxystrobin was shown to be similar or better than benomyl applied at higher dosages in all trials (Gullino *et al.*, 2001).The most thoroughly investigated chemical inducer is BABA (DL-ß-aminobutyric acid) (Cohen *et al.*, 1994).

2.6 DL-3β-amino butyric acid (BABA)

BABA has been identified as a non-protein amino acid that occurs occasionally in nature (Cohen *et al.*, 1999; Zimmerli *et al.*, 2001; Ton and Mauch-Mani, 2004). Since BABA is a non-protein amino acid, it has also been noted to be active as an abiotic inducer of resistance in several plants against a broad range of fungal and bacterial plant pathogens (Jakab *et al.*, 2001; Cohen, 2002). Little is known about the mod of action of BABA. Thus, the mode of action of BABA remains a matter of controversy (Figure 2.4) (Zimmerli *et al.*, 2000).The first time that BABA was addressed in the root exudates of tomato plants grown in solarized soil (Gaffney *et al.*, 1993).

It reported to protect tomato plants against *Phytophthora infestans*, tobacco against *Peronospora tabacina* and peas against *Aphanomyces euteiches* root pathogen. Furthermore, many studies have found that BABA has no fungicidal activity *in vitro* and has caused negligible or no growth inhibition of pathogens as a result, it is, considered to be a chemical capable of inducing resistance against plant pathogens (Lopez and Lucas, 2002; Nair *et al.*, 2007).

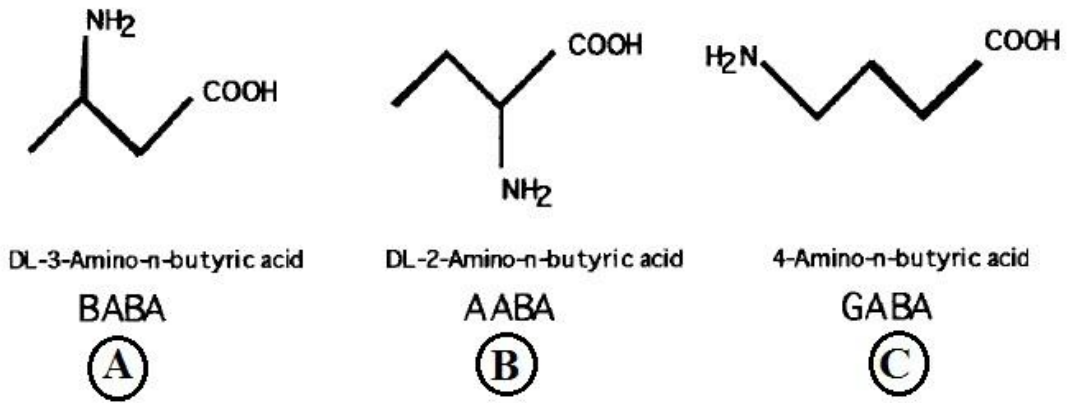

Figure 2.4 Chemical structure of amino butyric acid (Jakab *et al.*, 2001), A, BABA; B, AABA; C, GABA