CHAPTER 1

RESISTANCE TO FUSARIUM WILT IN BANANA: A REVIEW

INTRODUCTION

In nature, plants are constantly challenged by aspiring pathogens, but disease rarely occurs because most plants are able to defend themselves against microbial attack. Plants have an extensive repertoire of responses, by which they are able to localize, inhibit and isolate infections (Beckman *et al.*, 1982; Schenk *et al.*, 2000). These defence responses are dependent upon early recognition of the pathogen and rapid mobilization of the response system (Schenk *et al.*, 2000). Although defence mechanisms have been studied extensively in several plants, little is known about either defence responses of monocotyledonous plants or, more generally, plant responses against root invading pathogens.

Fusarium wilts are economically important soil borne diseases that affect a wide variety of crops throughout the world (Beckman, 1987; Fuchs *et al.*, 1999). One of the most important Fusarium wilt diseases is Fusarium wilt of banana (*Musa* spp.). Bananas are regarded as the fourth most important staple fruit crop, and provide a food source and income to millions of people in the world. Fusarium wilt devastated banana plantations in Central America during the 1960's, and the international export industry was saved from destruction by replacing Gros Michel bananas with resistant Cavendish bananas (Ploetz, 2005a). Cavendish bananas, however, now succumb to a new race (race 4) of the Fusarium wilt pathogen that is, once again, threatening sustainable cultivation of bananas globally (Ploetz, 2005b).

A clear understanding of disease resistance is essential to facilitate the breeding or development of Fusarium wilt resistance in banana. Since the introduction of Cavendish as a replacement dessert banana to the world, very few studies addressed the fundamentals of resistance to Fusarium wilt. In fact, the topic was arguably best summarised by Wardlaw (1961) and Stover (1962) more than 40 years ago. Much of what is known about Fusarium wilt resistance today is still based on the work of Beckman (1987). The objective of this review, therefore, is to summarise the literature related to resistance in banana to Fusarium. The review first recapitulates information relevant to the Fusarium wilt pathogen, the host plant, and resistance responses in plants in general. It then discusses defence mechanisms in banana and other crops to Fusarium wilt pathogens, and considers how such resistance can be utilised to obtain disease resistance in susceptible banana varieties. Finally it proposes ways to study resistance mechanisms and resistance genes in banana to Fusarium wilt.

FUSARIUM WILT OF BANANA (PANAMA DISEASE)

Fusarium wilt or Panama disease of banana is regarded as one of the most destructive diseases of agricultural crops in the world (Wardlaw, 1961; Stover, 1962; Simmonds, 1966, Ploetz, 2005 a,b). The disease was first reported from Australia in 1874, but became renowned for the destruction it caused to export banana plantations in Central America during the first half of the 20th century (Jones, 2000). The Fusarium wilt pathogen was disseminated throughout the world in infected planting material, and today the disease occurs in all countries where banana is grown except the South Pacific Islands and some of the countries bordering the Mediterranean (Jeger *et al*., 1996).

Fusarium wilt of banana is caused by the soil-borne fungus *Fusarium oxysporum* Schlect. f.sp. *cubense* (E.F. Smith) Snyder & Hansen (*Foc*) (Stover and Waite, 1954). The fungus survives as immobile and dormant chlamydospores in decayed banana tissue and soil until it is stimulated to germinate by the host roots, root excretions from non-host roots, or contact with pieces of fresh non-colonized plant remains (Stover, 1962). The pathogen enters the plant through the root tips, moves through the xylem vessels and colonises the rhizome (Beckman, 1990). It then produces microconidia that invade the water-conducting tissue (xylem) of the pseudostem, plugging the vascular vessels and reducing the movement of water. Further spread of the conidia is hindered by sieve cells and, consequently, the spores germinate and grow through the sieve cells to continue their spread until the entire xylem system is blocked (Stover *et al*., 1961; Jeger *et al*., 1995). After the plant dies, the fungus grows into the surrounding tissue where they form chlamydospores that are released back into the soil when the plant decays (Jones, 2000).

Classical external symptoms of Fusarium wilt of banana appear as a yellowing of the leaf margins of older leaves (Moore *et al.*, 1995). As the disease progresses, the yellowing advances from the oldest to the youngest leaves. Leaves gradually collapse at the petiole or towards the base of the midrib and hang down to form a "skirt" of dead leaves around the pseudostem. A longitudinal splitting of the outer leaf-bases of the pseudostem is often observed just above soil level (Brandes, 1919; Wardlaw, 1961; Stover, 1962). Internal symptoms are characterised by the discoloration of vascular tissue. Discoloration begins with yellowing of the vascular tissue in the roots and corm, which then progresses to form continuous yellow, red or brown discoloured vascular strands in the pseudostem (Wardlaw, 1961). No disease symptoms have been observed in or on fruit. Susceptible banana plants infected with *Foc* rarely recover.

Three races of *Foc* cause Fusarium wilt to a set of differential banana cultivars (Stover and Buddenhagen 1986; Ploetz, 2005). The race that devastated almost 100 000 acres of Gros Michel bananas in Central America during the first half of the 1900's, is race 1. Race 1 also attacks cultivars of the AAB genotype, 'Silk' and 'Pome'. The outbreaks of Fusarium wilt in Gros Michel bananas were stopped by replacing them with Cavendish varieties, which are resistant to *Foc* race 1 (Ploetz, 2005). However, Cavendish bananas are attacked by race 4 of the pathogen in countries such as Taiwan, Canary Islands, Australia, South Africa, Philippines and Malaysia (Gowen, 1995; Bentley *et al*., 1998). Strains of *Foc* that attack Cavendish bananas in the subtropics are referred to as 'subtropical' race 4, and those attacking Cavendish bananas in the tropics are referred to as 'tropical' race 4. Race 4 also attacks bananas susceptible to all other races of *Foc*. Another race of *Foc*, race 2, attacks Bluggoe and other closely related cooking bananas, while a fourth race, *Foc* race 3, was recently discarded as a race of *Foc* (Ploetz, 2005b). This race attacks *Heliconia* spp., a plant distantly related to *Musa*, and was found to be genetically distinct from *Foc* (Waite, 1963).

Numerous disease control strategies have been investigated for the control of Fusarium wilt. Of these, quarantine and exclusion practices have been successful in preventing pathogen dissemination by restricting the movement of infected corms, suckers and soil (Moore *et al.,* 1995). Flood fallowing, crop rotation and the use of organic amendments were unsuccessful in controlling the disease effectively (Moore *et al.*, 1995). Chemical control measures do not exist, and partial control of the pathogen has largely relied on the use of methyl bromide. However, fumigated areas were thoroughly reinvaded by the pathogen (Herbert and Marx, 1990), and the use of methyl bromide has now also been restricted in many countries. Biological control and the use of chemical or biological products to induce systemic resistance in plants have not been properly investigated. It is now generally accepted that an effective way of combating the disease is by the use of resistant genotypes (Jones, 2000).

THE HOST: BANANA

The banana plant is a large perennial monocotyledonous herb consisting of an underground stem (rhizome), a pseudostem and a terminal crown of leaves (Jones, 2000). Both the root system and the aerial parts arise from a sympodial rhizome (Gowen, 1995). The terminal growing point or meristem of the rhizome extends within the pseudostem and is eventually transformed into an inflorescence that forms the bunch (Robinson, 1996). The bunch consists of female, male and hermaphrodite flowers, of which only the female flowers develop into fruit. Most edible bananas are seedless due to female sterility and a lack of pollen due to triploidy (Robinson, 1996). Reproduction in banana, therefore, occurs in the form of vegetative propagation by the production of suckers, which are outgrowths of vegetative buds formed on the rhizome (Simmonds, 1959).

More than 1000 varieties of bananas have been identified in the world. The greatest diversity of bananas is found in Southeast Asia, while secondary centres of evolution include India and the east African Highlands (Simmonds, 1959). All bananas and plantains (cooking bananas) belong to the genus *Musa* (Family *Musaceae*, Order *Zingiberales*), which includes five sections, divided into 40 species. *Eumusa* is the largest and best known section and includes *M. acuminata* Colla and *M. balbisiana* Colla, which are the principal progenitors of most edible banana cultivars (Simmonds, 1959; Stover, 1962; Waite, 1963). Their origin is considered to be Southeast Asia for *M. accuminata* (genome AA) and the Indian subcontinent for *M. balbisiana* (genome BB) (Simmonds, 1966). *Musa acuminata* is generally considered to be sweet, and *M. balbisiana* starchy with resistance to biotic and abiotic stresses.

Inedible, seed-bearing diploids are still found in Southeast Asia and the western Pacific regions and are regarded as the ancestors of modern-day bananas and plantains (Stover, 1986; Ploetz and Pegg, 1997). Natural evolution over time has resulted in many inedible diploids crossing naturally and resulting in intraspecific hybrids (Buddenhagen, 1990; Robinson, 1996). To obtain seedless, edible bananas and plantains, early breeders crossed *M. acuminata* with *M. balbisiana* to produce diploid, triploid and tetraploid hybrids (Simmonds, 1966). A triploid banana consisting of one set of chromosomes from *M. acuminata* and two sets from *M. balbisiana* is referred to as ABB, and a tetraploid banana consisting of three sets of chromosomes from *M. acuminata* and one set from *M. balbisiana* is referred to as AAAB. Bananas in the Gros Michel and Cavendish groups are classified as AAA, signifying that they are triploid with all chromosomes coming from *M. acuminata*.

Edible bananas are divided into two major groups: cooking bananas and dessert bananas (Jones, 2000). Cooking bananas form 57% of the world banana production, and comprise a staple food that needs to be baked, boiled, fried or roasted before consumption (Jones, 2000). Plantains are one of the best-known cooking bananas that are produced by many small-scale farmers throughout the tropics as an important food, fibre and income source (Swennen, 1990). Dessert bananas are eaten raw when ripe, and account for the remaining 43% of the world's production. Bananas from the Cavendish subgroup are the most popular dessert bananas. The banana export market, consisting mainly of Cavendish bananas, constitutes only 13% of all bananas grown in the world. The rest are grown for local consumption in the tropics and subtropics (Jones, 2000).

Humans have been responsible for moving vegetative banana planting material (suckers) outside Asia and around the world. The mode and time of introduction of bananas to Africa is believed to be from India around the 1400s. They were then spread across the African continent from east to west (Simmonds, 1959; Robinson, 1996). The Portuguese carried the plant to the Canary Islands some time after 1402 and from there to the New World (Simmonds, 1959). Dessert and cooking varieties were introduced into the Americas from Southeast Asia before 1750 (Wardlaw, 1961). Gros Michel was first introduced into Panama before 1866, and with the expanding export industry at the time, was distributed throughout the entire Central America (Stover, 1962). The Silk (ABB) variety was introduced into Australia before 1876 and the Gros Michel cultivar was introduced only around 1910 (Stover, 1962). This set the stage for the cultivation of bananas as a dessert and as a staple crop, around the world. Edible bananas are now cultivated in many tropical and subtropical regions of the world, including, Asia, Africa, South and Central America, Oceania and the Caribbean.

RESISTANCE IN PLANTS TO PATHOGENS

Terminology

A diverse range of organisms constantly challenge plants, but not all of them are able to cause disease. When an organism is able to invade and multiply within plants, they are referred to as **pathogens**. Sometimes pathogens can live on a susceptible host without causing any disease. In such case they are called **saprophytes**. If, however, conditions become favourable for infection and disease, they are called **parasites**.

The ability of a pathogen to cause disease to a host plant is often dependent on how a plant responds. If infection takes place with subsequent disease development, a plant is considered **susceptible** to the pathogen. Susceptibility may be caused by an inability of the plant to recognise the pathogen and/or produce an effective and rapid defence response. Such an interaction between plant and pathogen is termed a **compatible** interaction. If, however, plants are able to restrict pathogen multiplication or movement from the initial site of infection they are **resistant** (Dempsey and Klessig, 1995), and the interaction is **incompatible**. The speed and extent of the defence response often establishes whether a plant is resistant or susceptible (Lamb *et al*., 1989). **Tolerance/partial resistance** is the ability of a plant to sustain the effects of a disease without suffering serious yield losses and dying as a result of infection. Partial resistance is also known as field resistance. Some crops that are resistant comprise varieties that do not develop any disease, despite challenge by a known pathogen under favourable environmental conditions. Such varieties are then considered as **immune**. When an entire plant species is resistant to a pathogen, it is called **non-host resistance** (Heath, 2000a; Nürnberger and Lipka, 2005). The prerequisite for successful invasion of a plant by a pathogen, therefore, is basic compatibility, where a potential pathogen has attained pathogenicity factors in co-evolution with the plant in order to overcome non-host resistance (Heath, 1981). Plants are able to produce or accumulate **stress metabolites** in response to stresses such as pathogen invasion, drought or heat shock. **Minor resistance gene** are present in all plants and play a role in non-specific plant defence through the production of phytoalexins, glucanases, chitinases, lignin, callose and enzymes for oxidative stress protection.

The Constitutive Defence Response

Constitutive (passive) resistance is due to the presence of preformed physical and chemical factors (Dangl and Jones, 2001). The cell wall in plants is the physical barrier that keeps most organisms from developing close contact with the plant (Johal *et al*., 1995; Ride, 1992; Nürnberger and Lipka, 2005). Preformed physical factors also include the thickness or hardness of the cuticle, the amount and quality of wax that cover the epidermal cells (Dangl and Jones, 2001; Nürnberger and Lipka, 2005), the size and shape of stoma and the root pericycle (Keen, 1992). Early barriers of defence in plants include preformed secondary metabolites, peptides, protein inhibitors and proteins that play a role in the normal programme of growth and development, but may bring about resistance to various pathogens (Heath, 2000b; Dixon, 2001). Preformed chemical defence factors in plants are often also due to the presence of high concentrations of alkaloids and phenolics in cells, as well as the presence of prohibitins or phytoanticipins (Grayer & Kokubun, 2001). Phytoanticipins are low molecular weight compounds that are present in plants before challenge by micro-organisms or are produced after infection solely from pre-existing constituents (VanEtten *et al*., 1994). Plants often also release fungitoxic exudates that inhibit the germination of spores (Agrios, 2004). If all these preformed plant weapons are not sufficient to stop pathogen invasion the plant activates inducible defences.

The Induced Defence Response

Induced (active) defence responses involve both physical and chemical factors (Agrios, 2004) that are activated upon pathogen attack. Inducible defence responses in non-host plants comprise the synthesis and accumulation of antimicrobial reactive oxygen species, phytoalexins, and translation products from pathogenesis-related genes as well as the localized reinforcement of cell walls and hypersensitive, programmed cell death (Nürnberger and Lipka, 2005). Disease resistance in plants relies on complex mechanisms of molecular recognition and cellular signal transduction (Fig. 1).

Recognition

The ability of plants to invoke defence reactions is mediated by the initial recognition of pathogens by plants (Dixon *et al.*, 1994; Schenk *et al.*, 2000). The activation of inducible plant defence responses is brought about by the recognitions of pathogen associated molecular patterns (PAMP) (Nürnberger and Lipka, 2005). Once the plant has recognized an attacking pathogen, the race is on. The plant attempts to prevent infection and to minimize potential damage, while the pathogen attempts to gain access to nutrients for reproduction and growth (Schmelzer, 2002). Resistance in plants is innate owing to the presence of genes coding receptor-related or receptor-like proteins, which can either directly bind pathogen-derived molecules or represent constituents of larger signal perception protein complexes (Schmelzer, 2002). This results in a cascade of biochemical events in the plant that lead to the induction of defence responses (Keen, 1992; Dixon *et al.*, 1994; Baron and Zambryski, 1995). Plant cells are capable of defending themselves through a wide variety of mechanisms that can either be a local or systemic, constitutive or inducible response (Dixon, 1986; Keen, 1990; Ryals *et al.*, 1994).

Hypersensitive response

The hypersensitive response (HR) is regarded as one of the most frequently occurring defence responses in crop plants against pathogens (De Wit, 1992) and is an active process of the host and may be a form of programmed cell death (Greenberg and Yao, 2004). The HR is associated with a phenomenon termed the resistance response (RR) and is considered to be the cell death component of the RR (Greenberg and Yao, 2004). The RR involves the coordinate activation of several defence responses that limit pathogen growth in the host (Greenberg, 1997)

The HR occurs only in specific host-pathogen systems in which the plant and the pathogen are incompatible and the pathogen fails to infect the host (Agrios, 2004). This might be due to the presence of a resistance (*R*) gene in the plant, which recognizes and is triggered by the elicitor molecule produced by the pathogen avirulence (*Avr*) gene (Greenberg and Yao, 2004). The HR involves only single cells or very few cells, and results in rapid cell death around the penetration site that could be responsible for restricting pathogen growth (Tomiyama, 1982; Keen, 1992; Schenk *et al*., 2000). According to Agrios (2004), an effective hypersensitive response is not always visible with the eye and may remain unnoticed when a plant remains resistant to pathogen attack. Ultrastructural analysis has revealed morphological events that occur during the HR including changes in mitochondrial morphology, membrane dysfunction and progressive vacuolization of the cytoplasm (Greenberg and Yao, 2004).

The HR is sufficient to restrict the growth of biotrophic and hemibiotrophic pathogens, which require nutrition from living plant cells for at least part of their infection cycle. However, to contain necrotrophs, the HR has to be supported by other defence mechanisms. In this case the early response is followed by a cascade of other defence responses (De Wit, 1992).

Mechanisms which accompany the HR include rapid burst of oxidative reactions, ion fluxes, especially K^+ and H^+, cellular decompartmentation and strengthening of cell walls through increased synthesis and deposition of callose, lignin, hydroxyproline-rich glycoproteins and phenolic compounds (Bowles, 1990; Agrios, 2004). Enzymes of the phenolic pathway such as peroxidases as well as glucanases and chitanases are also expressed (Dixon and Lamb, 1990). Resistance modulated by the HR is a cellular and tissue response and, therefore, the spatial and temporal expression of defence response genes are strategically important (Keen, 1992).

In addition to playing a role in limiting pathogen growth directly, the HR may have additional contributions, such as activating specific defences in neighbouring tissue. Such systemic signalling is important for protecting plants from future infections (Greenberg and Yao, 2004).

Oxidative burst

The HR has several rapid processes that involve the activation of pre-existing components rather than changes in gene expression. One of these processes is the release of reactive oxygen species (ROS) (Apostel *et al.*, 1989; Sutherland, 1991; Legendre *et al.*, 1993; Mehdy, 1994; Ebel and Scheel, 1997). The predominant species detected in plant-pathogen interactions are super oxide (O_2^-), hydroxyl radical (OH) and hydrogen peroxide (H_2O_2). These highly reactive oxygen radicals are thought to be released by the multi-subunit NADPH oxidase enzyme complex of the plant cell plasma membrane (Parker, 2000). They appear to be released in affected cells within seconds or minutes from contact of the plant cell with the pathogen or its elicitors (Bradley *et al.*, 1992; Agrios, 2004), and reach maximum activity within minutes to a few hours (Agrios 2004).

The oxidative burst leads to the cross-linking of cell wall proteins, rendering plant cell walls more resistant to attack by fungal enzymes (Bradley *et al.*, 1992; Keen, 1999). Researchers have reported that soybean and bean suspension cultures treated with fungal elicitors have shown an increase in lignifications and cross-linking of cell wall hydroxyproline-rich glycoproteins, responses linked to increased H_2O_2 levels (Bradley *et al.*, 1992; Brisson *et al.*, 1994). ROS have also been considered as signalling agents for the induction of hypersensitive cell death and additional active defence responses (Levine *et al.*, 1994; Alvarez *et al.*, 1998). The ROS may serve as second messengers for the activation of genes expressing protective proteins, such as glutathione peroxidase, glutathione S-transferase and polyubiquitin, as well as for enzymes involved in scavenging ROS (Lamb and Dixon, 1997). The highly reactive oxidants, O_2^- and OH may exert potent antimicrobial activity and contribute to lipid peroxidation of both plant and pathogen membranes (Vera-Estrella *et al.*, 1994; Parker, 2000). ROS are also said to be toxic to pathogens (Peng and Kúc, 1992; Mehdy, 1994). Peng and Kúc (1992) showed that spore germination for a number of different fungal pathogens was inhibited by micromolar concentrations of H_2O_2. ROS has further been connected with the production of phytoalexins (Mehdy, 1994; Lamb and Dixon, 1997), but there may be a ROS-independent phytoalexin synthesis pathway (Mithöfer *et al*., 1997).

Ion fluxes

Changes in ion fluxes and membrane depolarisation in plant cells occur within minutes in response to pathogen attack (Zimmermann *et al.*, 1997). Ion fluxes are caused by changes in the permeability of the plasma membrane and *R* gene- and receptor-mediated regulation of the ion channels within the plasma membrane, resulting in calcium (Ca^{2+}) and proton (H^+) influx and potassium (K^+) and chloride (Cl^-) efflux (Scheel, 1998; Nürnberger and Scheel, 2001). It appears that ion fluxes are necessary in most plant-pathogen interactions for the induction of the oxidative burst and defence gene activation (Scheel, 1998).

Cell wall strengthening and vascular occlusion

In several plant diseases the cell walls of the host are able to produce, modify or accumulate defence-related substances that reinforce the walls, resulting in enhanced resistance. Among the substances produced or deposited by plant cell walls in reaction to infection are callose, glycoproteins, amino acid hydroxyproline, phenolic compounds such as lignins and suberin, and mineral elements such as silicon and calcium (Agrios, 2004). Many of these substances react and cross-link with one another to form more insoluble cell wall structures that confine the pathogen. Plant cells also respond to invading pathogens by producing vascular occlusions such as tyloses (Agrios, 2004) and gels (Mace, 1963). They serve to cut off the transpiration stream in the xylem, thereby immobilising the pathogen. Tyloses and gels are infused with stress metabolites and become lignified and highly resistant to physical and chemical degradation (Mace, 1963).

Lignification

Lignification is one of many biochemical events resulting in an ultra-structurally modified reinforced cell wall (Walter, 1992). Lignin provides rigidity and mechanical support to plant tissue, waterproofs xylem elements and allows for defence strategies against pathogen attack. It is commonly formed in response to microbial penetration and mechanical damage (Wardrop, 1971), and is resistant to degradation by most micro-organisms (Kirk, 1971; Kirk *et al.*, 1979). Lignin is a matrix polymer enclosing cellulosic fibrilic material that restricts the diffusion of enzymes and toxins from the fungus to the plant, and of nutrients and water from the plant to the fungus, thereby starving the fungus (Ride, 1978).

Lignification follows the elicitor-receptor theory. Vance *et al.* (1980) proposed that more than one elicitor might interact with a single receptor and that two or more receptors might independently activate a resistance mechanism. Low molecular weight lignin precursors are produced by the plant and could act as elicitors. These lignin precursors are possibly able to react with fungal walls and membrane polymers to activate a recognition system, or to block the activation by other compounds (Vance *et al.*, 1980). Lignin and lignin-like polymers of host plants have indefinite structure and configuration and are unlikely candidates for receptors. There is no evidence that minor changes in lignin molecules lead to major property changes. Unlike other cell wall proteins and saccharides, lignin is not a preformed constituent of epidermal cells prior to initial pathogen penetration (Vance *et al.*, 1980).

Callose deposition

Callose deposition is known to be a very rapid and localized event in response to mechanical injury or pathogen invasion (Bowles, 1990). In many host-pathogen interactions callose appears to be locally deposited directly from the plasma membrane onto the adjacent cell wall (Aist, 1976). Localised deposition of callose may be important in the first line of defence against pathogens. Callose is also a major component of papillae or cell wall appositions which are formed at the sites of attempted penetration by invading fungal hyphae (Aist, 1976; Bell, 1981).

Callose is a polysaccharide containing a high proportion of 1, 3 - β-linked glucose. Callose synthetase or 1, 3 - β-glucan synthase (GSII) catalyses the formation of callose, and this protein is a functional component of the plasma membrane (Bowles, 1990). GSII is abundant in plant homogenates but is latent in healthy living cells. Callose synthesis can be initiated in a Ca^{2+}-dependent or in a Ca^{2+}- independent manner. Localised deposition of callose may start when Ca^{2+} influx increases and activates the GSII (Bowles, 1990). Callose deposition can also be caused by polycations such as chitosan and poly-L-lysine (Köhle *et al*., 1985). These elicitors were shown to interfere with the negatively charged phospholipids of the plasma membrane, generating a local Ca^{2+} influx into cells, K^+ efflux and an external alkalisation of the medium, followed by callose formation (Köhle *et al*., 1985; Waldmann *et al*., 1988). In the absence of Ca^{2+}, callose synthase can be activated by polyamines, ruthenium red, trypsinization (Kauss *et al*., 1990) and by phospholipids.

Phenolic compounds

Antibiotic phenols have been found in all plants that have been investigated (Nicholson and Hammerschmidt, 1992). Specialized plant cells synthesize phenolics and store them in their vacuoles during normal processes of differentiation. These phenolic-storing cells are distributed within most plant tissues, either uniformly in all cells or randomly scattered or strategically located at potential points of entry (Beckman, 2000). During injury or infection, phenolic-storing cells burst with a chemical reaction that oxidizes the plant phenolics. This then serves to lignify and/or suberize the site of infection.

One of the main roles of phenolics in plants is to protect them against diseases caused by bacteria, fungi and viruses (Swain *et al*., 1979). Some phenols occur constitutively and function as preformed inhibitors associated with non-host resistance (Mansfield, 1983; Nicholson and Hammerschmidt, 1992). Others are formed in response to pathogen invasion and are considered to form part of the active induced defence response (Nicholson and Hammerschmidt, 1992). The rapid accumulation of phenols may result in the effective isolation of the pathogen at the original penetration site (Friend, 1981; Ride 1983). Phenolics also serve as signalling molecules during vascular defence (Wink, 1997).

Many phenolic compounds have anti-microbial activity *in vitro* (Friend, 1981). Certain common phenolic compounds, such as chlorogenic acid, caffeic acid and ferulic acid, are toxic to pathogens, and are produced and accumulate at a faster tempo after infection, especially in resistant plants compared to susceptible plants (Agrios, 2004). Walker and Link (1935) showed that catechol and protocatechuic acid had a toxic effect on onion parasites. Although individual phenolics may reach toxic concentrations, several of them appear concurrently in the same infected tissue, and it is possible that their combined fungitoxic effect is responsible for the inhibition of pathogen infection in resistant plants (Agrios, 2004).

Indole acetic acid

Indole acetic acid (IAA) builds up rapidly in plants when decompartmentation occurs (Beckman, 2000). Gordon and Paleg (1961) made the discovery that phenolics mediated the conversion of tryptophan to IAA (3-indoleacetic acid). Two processes cause the level of IAA to raise sharply in the affected xylem tissues. Firstly, the release of flavenols inhibits the ATPase pumps that are responsible for downward transport of IAA, thus causing accumulation of IAA above the infection point (Lomax *et al*., 1995; Normanly *et al*., 1995). Secondly, oxidized phenolics have been shown to inhibit the destructive oxidation of IAA, therefore permitting a severe build-up of IAA (Sequeira, 1964). This type of build-up in the host plant has been demonstrated to occur in infected vascular tissues of tomatoes infected with *Fusarium* (Matta, 1970).

The action of IAA in affected tissue is indicated by an oxidative burst (Lamb and Dixon, 1997), the activation of H^+ pumps, and a drop in apoplastic pH (Cleland, 1987; Grignon and Sentenac, 1991). Beckman (1969) demonstrated such a drop in pH in the walls of banana parenchyma cells that were in contact with vessels infected with *Fusarium*. Studies on cotton infected with *Verticillium* indicated that there is a dramatic shift in IAA/cytokinin balance in infected vascular tissues at and around the site of perturbation (Misaghi *et al*., 1972). According to Fosket (1994) and Kaufman and Song (1987) this kind of shift in hormone balance in plants was shown to promote lateral growth of cells in the affected area. The treatment of banana roots with IAA promotes lateral growth in adjacent paravascular parenchyma cells to form tyloses in the lumina of vessels (Mace and Solit, 1966), and Beckman (1987) showed that an oxidative burst occurs in plant cells during this period.

Other Defence Responses

The expression of defence-related genes such as those involved in lignin and suberin pathways, those for signal transduction proteins, pathogenesis related (PR) proteins as well as proteins that are necessary for the accumulation of phytoalexins and phenyl propanoids, is induced concurrently or downstream of the HR (Reymond and Farmer, 1998; Greenberg and Yao, 2004). Defence responses, such as the production of PR proteins (1, 3 β-glucanases and chitanases), can be induced many hours or even days after infection (Lamb *et al.*, 1989; Bowles, 1990).

Pathogenesis-related proteins (PR proteins)

PR proteins have been identified in many monocots and dicots and appear to be ubiquitous in higher plants (Cutt and Klessig, 1992). They can be induced in response to a diverse range of pathogens and biotic and abiotic elicitors (Bowles, 1990). PR proteins are induced both locally and around the infection site and systemically away from the initial infection site. Some PR proteins inhibit spore release and germination, while others are associated with strengthening of the plant cell wall and its outgrowths and papillae (Agrios, 2004). PR proteins are either extremely acidic or extremely basic and are, therefore, highly soluble and reactive (Agrios, 2004). They are grouped into families based on acid solubility and their resistance to proteinases (Bowles, 1990). Currently there are 11 recognised groups of PR proteins (Van Loon *et al.*, 1994; Koiwa *et al.*, 1997; Van Loon, 1997). The better-known PR proteins are PR-1 proteins, β-1,3-glucanases (PR-2), osmotin-like proteins, thaumatin-like proteins (PR-5), glycine and cysteine-rich proteins, proteinase inhibitors (PR-6), proteinases (PR-7), chitosanases (PR-8) and peroxidases (PR-9) (Agrios, 2004).

Peroxidases

The induction of plant peroxidases appears to be an early event in plant-microbe interactions (Cook *et al*., 1995; Harrison *et al*., 1995). Peroxidases are haem-containing proteins that catalyse the reduction of hydroperoxides, especially hydrogen peroxide, to water. Most higher plants possess a number of different isoenzymes and at least 12 distinguishable isoenzymes that fall into three sub-groups have been characterised from tobacco: the anionic (pI 3.5-4.0), moderately anionic (pI 4.5-6.5) and the cationic (pI 8.1-11) isoenzymes. Peroxidase isoenzyme expression is tissue-specific, developmentally regulated and influenced by environmental factors (Lagrimini *et al*., 1987). Each group is thought to serve a different function in the cell.

Peroxidases are involved in several plant defence responses including lignification (Walter, 1992), suberization and wound-healing (Sherf *et al*., 1993) as well as in the production of antimicrobial radicals (Peng and Kúc, 1992; Kobayashi *et al*., 1994). The function of highly anionic peroxidase isoenzymes is understood best. This group is cell wall-associated and has high activity for the polymerization of cinnamyl alcohols in *vitro* (Mäder, 1986). They function in lignification and the cross-linking of cellulose, pectin, hydroxy-proline-rich glycoproteins and lignin during secondary cell wall formation (Lagrimini *et al*., 1987). Anionic peroxidases show a high affinity towards cinnamylalcohols *in vitro*, but can probably also cross-link extensin monomers and feruloylated polysaccharides (Walter, 1992). They may also function in suberization or wound healing by forming water-tight barriers over the wound (Espelie *et al*., 1986). The moderately anionic peroxidases are localized in the cell walls, only have moderate activity towards lignin precursors, and may be involved in suberization and wound healing (Lagrimini and Rothstein, 1987; Walter, 1992). The cationic isoenzymes efficiently catalyse the synthesis of H_2O_2 from NADH and H_2O and have been localized to the central vacuole (Mäder, 1986). It is speculated that this group of isoenzymes regulates auxin levels, form ethylene from 1-aminocyclopropane-1-carboxylic acid and provides H_2O_2 for other peroxidase isoenzymes (Lagrimini *et al*., 1987). Peroxidases are also involved in other processes, such as the inactivation of host and pathogen enzymes by oxidized phenolics (Matern and Kneusel, 1988).

Phytoalexins

Phytoalexins are toxic antimicrobial substances produced in appreciable amounts in plants after stimulation by phytopathogenic micro-organisms, or by chemical or mechanical injury (Agrios, 2004). They are thought to be important components of the active plant defence response, despite the absence of genetic proof using plant mutants (Keen, 1999). Phytoalexins accumulate in healthy plant cells surrounding wounded or infected cells and are stimulated by substances diffusing from damaged cells into the adjacent healthy tissue (Agrios, 2004). Resistance occurs when one or a few phytoalexins reach a concentration sufficient to restrict pathogen development. Most phytoalexins are produced in response to fungal infection and inhibit the growth of the fungus, but some are also toxic to nematodes and bacteria.

Signal Transduction

In most eukaryotic organisms pathogen recognition and defence responses are linked by means of signal transduction cascades (Nürnberger and Scheel, 2001). Plant tissues acquire resistance by relying on transmissible signal molecules that, at low concentration, can activate resistance mechanisms in cells not directly invaded by the pathogen (Ross, 1961; Schenk *et al.* 2000). Molecules are classified as signal molecules if they are synthesised in the plant, increase systemically following pathogen attack, move throughout the plant, induce defence-related proteins and phytochemicals and if they enhance resistance in the plant against pathogens.

The events following pathogen recognition are still poorly understood. Downstream pathways seem to vary for different host plants, resistance genes, elicitors and pathogens (Bent, 1996; Ebel and Mïthofer, 1998; Glazebrook, 2001). Consequently, general models are unable to explain signal transduction and resistance for every plant-pathogen interaction. Despite the complexity and diversity of plant-pathogen interactions, signalling events and resistance mechanisms for many similar plant responses have been identified.

During early signal transduction events, cells in and around the recognition site experience large ion fluxes and undergo changes in lipid oxidation, protein phosphorylation, and concentration and accumulation of ROS (Dixon *et al.*, 1994; Hammond-Kosack and Jones, 1996; Ebel and Mïthofer, 1998; Holt *et al.*, 2000). Different kinases are involved downstream of pathogen recognition in different plant-pathogen systems. These include receptor-like kinases, protein kinases, calcium-dependant protein kinase (CDPK's) and mitogen-activated protein kinases (MAPKs) (Fig. 1) (Torii, 2000; Guillaume *et al.*, 2001; Romeis, 2001; Asai *et al.*, 2002).

Following the early defence responses and subsequent biochemical and signalling alteration within the infected plant cells, a second signalling wave induces specific defence responses. These defence responses are induced either by positive feedback loops or signal cross-talk and influence the metabolic flow or activity of housekeeping genes to ensure high levels of required precursor compounds (Hammond-Kosack and Jones, 1996). The induction of defence-related genes seems to be regulated by a small number of signalling pathways that are dependent on low molecular mass regulators. Salicylic acid (SA), jasmonic acid (JA), ethylene (ET), possibly hydrogen peroxide (H_2O_2) and nitric oxide (NO) play a role in cross-talk and convergence points between pathways (Reymond and Farmer, 1998; Kumar and Klessig, 2000).

Salicylic acid

SA, a phenolic signalling molecule, has been shown to play a central role as a signalling molecule involved in efficient generation of the local HR (Gaffney *et al.*, 1993), local acquired resistance (LAR) and systemic acquired resistance (SAR) (Chamnongpol *et al.*, 1998; Reymond and Farmer, 1998). An intricate relationship between SA and cell death has been discovered, indicating that SA is both required for and induced by cell death (Nawrath and Métraux, 2002). Whether or not SA acts as the primary systemic signal for SAR, however, is still under debate. Still, SA accumulation and activity almost invariably precede the expression of *PR-1* transcripts (Cao *et al.*, 1994; Delaney *et al.*, 1994). Little progress has been made in characterising the SA biosynthesis pathway itself, although a number of experiments have demonstrated that SA is synthesized from phenylalanine via cinnamic and benzoic acid (Lee *et al.*, 1995; Coquoz *et al.*, 1998). Additionally, oxidative stress caused by ultraviolet light or ozone also triggers SA biosynthesis (Yalpani *et al.*, 1994).

Jasmonic acid

The signalling molecule JA and its counterpart methyl jasmonate (MeJA) are involved in several plant biological aspects such as pollen and seed development, fruit ripening, tuber formation and defence reactions against wounding, insects and microbial pathogens (Creelman and Mullet, 1997; Reymond and Farmer, 1998; Li *et al.*, 2001; Lorenzo and Solano, 2005). JAs are fatty acid derivatives with a 12-carbon backbone and are involved in basal resistance against specific pathogens (Pieterse *et al.*, 2002). JA and MeJA accumulation is associated with lipid oxidation mediated by either ROS or by lipoxygenases and are, therefore, part of the lipid-based intracellular signalling pathway (Farmer and Ryan, 1992; Ebel and Mithofer, 1998). JA signalling is important in limiting the growth of certain pathogens, such as *Alternaria* spp. and *Pythium* spp. and has systemic effects resulting in gene expression throughout the plant (Glazebrook, 1999). JA and ET-mediated defence responses are associated with induced expression of an antifungal plant defensin (*PDF1.2*), *PR3* and *PR4* (Penninckx *et al.*, 1996; 1998) and a thionin (*Thi2.1*) (Epple *et al.*, 1997). JA is also involved in induced systemic resistance (ISR) against pathogen infection in plants first challenged with a non pathogenic, root colonizing bacterium, *Pseudomonas fluorescens* Migula (Pieterse *et al.*, 1998).

Ethylene

ET is involved in many plant development processes such as root hair development, root nodulation, seed germination, flower senescence and fruit ripening, but has also been implicated in the defence response to pathogen attack and abiotic stresses, including wounding, ozone, chilling and freezing (Johnson and Ecker, 1998). Five ethylene receptors (ETR 1, ETR 2, ERS 1, ERS 2 and EIN 4) have been identified and were shown to play a role in ET signalling (Solano and Ecker, 1998). Additionally, the *Ctr1* gene encoding for a serine/threonine protein kinase is required for ET signalling. ET may play a very different role in disease resistance, depending on the type of pathogen and the plant species (Thomma *et al*., 1999). It is involved in symptom inhibition and resistance after infection of plants by necrotrophic pathogens, but enhances cell death caused by other pathogens (Wang *et al*., 2002).

Interaction between the SA, JA and ET pathways

Many stress responses in plants require the coordinated interaction of signalling pathways such as JA, ET, SA and abscisic acid (Lorenzo and Solano, 2005). Little is known about how plants integrate signals that are generated by different inducers of resistance, into specific defence responses (Pieterse *et al*., 2002). A well-accepted hypothesis is that this might be accomplished by the modulation of different signalling pathways (Fig. 2). Despite the fact that the SA-dependent and JA-ET-dependent pathways induce the expression of different *PR* genes and also confer resistance to different pathogens, there is ample evidence that they share common pathways and interact with each other, either positively or negatively (Feys and Parker, 2000; Schenk *et al.*, 2000 Ton *et al.*, 2002; Wang *et al.*, 2002; Lorenzo and Solano, 2005). Pieterse *et al*. (1998) identified a convergence point between different pathways in NPR1, which is required for both SA-dependent SAR and JA-ET-dependent ISR. The gene product SSI1 (suppressor of SA insensitivity) possibly acts as a switch in the cross-talk between the SA- and JA-ET-resistance signalling pathways (Shah *et al*., 1999). JA and ET can either cooperate or act as antagonists in the regulation of defferent stress responses such as pathogen attack and wounding (Lorenzo and Solano, 2005). ET and JA also act synergistically in the wound-response and stimulate the biosynthesis of each other (O'Donnell *et al*., 1996). This so-called cross-talk between pathways provides a regulatory potential for activating multiple resistance mechanisms in varying combinations. This enables the plant to prioritise the activation of a specific defence pathway over another, thereby providing optimal defence strategies against the invading pathogen (Pieterse *et al*., 2002).

Systemic resistance

Systemic resistance (SR) is the activation of defence responses in tissues far removed from the initial site of pathogen attack or wounding (Ryals *et al*., 1994; 1996; Maleck and Dietrich, 1999). This is due to the expression of a characteristic set of defence-related proteins that are induced systemically throughout the entire plant (Schmelzer, 2002). A number of genes associated with SR have been characterised as ones coding for β-1,3 glucanases, chitinases and thaumatin-like proteins (Bowles, 1990; Ward *et al*., 1991; Ryals *et al*., 1994). Linthorst (1991) also confirmed that at least five families of PR proteins have been associated with SAR.

It is hypothesised that the HR and other pathogen-induced local necrosis trigger SR (Kessmann *et al*., 1994; Greenberg and Yao, 2004), and that SR targets the majority of fungal, bacterial and viral pathogens. Underlying the systemic response must be a long distance signal transduction network which is capable of initiating a complex set of coordinated events resulting in a broad spectrum defence barrier (Cutt and Klessig, 1992). It has been reported that the accumulation of SA in local and systemic plant tissues contributes to SR (Malamy *et al*., 1990; Métraux *et al*., 1990; Rasmussen *et al*., 1991).

Certain micro-organisms can protect plants by inducing SR to diseases (Kúc, 1982; Matta, 1989). Dependent on the organism and signalling pathway involved, either SAR or ISR is obtained. SAR to pathogenic forms of *F. oxysporum* has been induced by using non-pathogenic strains (Mandeel and Baker, 1991; Hervas *et al*., 1995) and formae speciales of *F. oxysporum* (Gessler and Kúc, 1982; Kroon *et al*., 1991). In 1997, a non-pathogenic *F. oxysporum* strain (Fo47) was shown to induce resistance to Fusarium wilt in tomato (Fuchs *et al*., 1999). Isolates of the rhizobacterium *Pseudomonas fluorescens* systemically induced resistance against Fusarium wilt of chickpea caused by *F. oxysporum* f.sp. *ciceri* and significantly reduced the wilt disease by 20-50% compared to the control (Saikia *et al*., 2003). ISR requires JA and ET and is independent of SA accumulation (Pieterse *et al*., 1996; van Wees *et al*., 1997). *PR-3*, *PR-4*, *Thi2.1* and *PDF1.2* are the local and systemic marker genes of ISR activation (Thomma *et al*., 1998). ISR also appears to provide protection against pathogens such as *B. cinerea* and *Alternaria brassicicola*, for which SAR is ineffective (Thomma *et al*., 1998). Evidence exists for overlap between the mechanisms regulating resistance in ISR and SAR (Barker, 2000).

COMPONENTS OF PLANT DEFENCE MECHANISMS AGAINST FUSARIUM WILT

Each plant cell has acquired the capability to respond to infection and must possess a preformed and/or inducible defence strategy (Hammond-Kosack and Jones, 1997; Ebel and Mithöfer, 1998). These defence strategies consist of structural or biochemical defence systems.

Structural Defence

The epidermis, of young roots, forms the first constitutively formed structural barrier to fungal invasion. The endodermis and later the phellem surrounding the vascular tissues further form a particularly strong barrier to colonization (Baayen, 1987). A study by Brammall and Higgins (1988) shed light on constitutive defence responses in tomato against *F. oxysporum lycopersici* (Sacc.) W.C. Snyder & H.N. Hans. (*Fol*). Results suggested that the hypodermis was an important constitutive barrier hindering colonization of the inner cortex of the tomato. It is generally accepted that Fusarium wilt infection of bananas takes place though the roots. Evidence form one study has shown that root injury and cutting, to exposes the xylem, is a major factor in the infection of banana roots (Sequeira *et al*., 1958).

Plants are able to respond to pathogens by forming physical barriers that enclose and exclude them from nutrient access (Schmelzer, 2002). These responses are induced when the plant recognises a structural and/or chemical feature of the pathogen, or the damage associated with pathogen invasion. The depositioning of substances like suberin, hydroxyproline-rich glycoproteins, callose and many mono- or polymeric phenolic materials, on or into the cell wall all contribute to barrier formation (Walter, 1992). A typical example is the local apposition of material to the cell wall, resulting in a massive plug, the papilla.

Banana tissue responds to *Foc* conidial uptake in the xylem by forming vascular occluding gels 24-48 hrs after inoculation (Beckman, 1987). Light microscopy studies indicated that gels might arise from perforation plates, end-walls and sidewalls of vessels (Beckman and Zaroogian, 1967). These gels are produced just above the spore-trapping sites to cut off the transpiration stream and immobilise the secondary spores of the pathogen. After 2-3 days, the vascular parenchyma cells form tyloses that block the lumena of infected vessels. The walls of tyloses also become infused with stress metabolites so that the gel and tylose occlusion is lignified and highly resistant to physical and chemical degradation. These gels and coatings may contribute to the failure of the water transport system, thus contributing to the typical wilt symptoms.

Vascular occlusion represents a general, non-specific defence response that is the norm in both susceptible and resistant hosts. They persist for several days in resistant reactions, during which time they become infused with phenolics at the infection-response interface (Mace, 1963; Beckman and Talboys, 1981). In susceptible plant-pathogen interactions, gels seem to weaken and shear, thereby failing to stop the advance of the pathogen (Vander Molen *et al.*, 1977; 1987). Only, when a virulent pathogen was introduced into a susceptible host at the optimum temperature for disease development did the defence response become disrupted and permit extensive systemic colonisation (Beckman, 1987).

In carnation infected with *F. oxysporum* f.sp. *dianthi* (Prill. & Delacr.) (*Fod*), microscopy studies of stems and roots have revealed constitutive and induced structural defence responses. The epidermis, endodermis and later the phellem surrounding the vascular tissues formed constitutive structural barriers to fungal invasion (Baayen, 1987). Higuera and Ebrahim-Nesbat (1999) showed that *Fod* is able to colonize both the susceptible and resistant carnation cultivars. Carnation stems of resistant varieties responded with vascular gelation and hyperplasia of xylem parenchyma bordering infection sites, while susceptible varieties were intensively colonized by the pathogen (Baayen, 1988). Baayen *et al.* (1989) also described occlusion of infected root xylem vessels with gums and lignification responses in the xylem parenchyma. Vascular plugs, vessel coatings, callose deposits and phenolic compounds were observed in resistant pea plants when infected with *F. oxysporum* f.sp. *pisi* (Lindf.) (Tessier *et al.*, 1990).

The hypodermis of tomato serves as an important constitutive barrier to colonization of the inner cortex by *Fol* (Brammall and Higgins, 1988). Histochemical studies of tomato parenchyma infected by *Fol* provided evidence that deposits of callose-containing substances were laid down in response to infection and that they later became lignified (Mueller and Beckman 1988; Beckman *et al*., 1989). The formation of papillae is also induced as soon as the pathogen is able to penetrate the preformed defence barrier, the hypodermis. Papillae may protect the hypodermal protoplast of tomato from both physical and chemical contact with *Fol* (Brammal and Higgins, 1988). Other features of great significance involved deposition of phenolics, lignins and nonlamellar suberin in the cortical cells. The authors concluded that phenolic structural defence barriers (papillae and modified cortical cell walls) appear to be important in limiting *Fol* colonization in tomato cultivars possessing single dominant gene resistance to this disease.

Biochemical Defence

The success of plant resistance is dependent on the rate and extent of the host response. Resistance responses in banana are based on the ability of tolerant or resistant plants to produce phenolics, deposit lignin, and increase enzymes involved in cell wall strengthening (Beckman, 1990; De Ascensao and Dubery, 2000). Phenolic compounds play an important role in the resistance process against vascular diseases and are present in the xylem tissue of banana, cotton and potato (Beckman, 2000). The role of phenolics is the inactivation of hydrolytic enzymes of the pathogen and/or their incorporation into fungal and host cell walls and vascular gels to lignify them (MacHardy and Beckman, 1981; Beckman, 1987). By inhibiting hydrolytic enzymes and lignifying these structures, phenolics lock up the infection site physically and isolate the pathogen.

Enzymes that are important in the formation of phenolics are phenol-oxidizing enzymes such as peroxidases and polyphenol oxidases, which are associated with many different vascular diseases (Pegg, 1985). Peroxidases and polyphenol oxidases are stored and preformed in various localised sites in banana and cotton (Mace and Wilson, 1964; Mueller and Beckman, 1974; Mueller and Beckman, 1978). Different isoforms of these enzymes are known to differ among different banana genotypes (Jarret and Litz, 1986). The levels and number of peroxidase isozymes produced are greatest in the roots of banana and it has been postulated that they may play a role in protecting the plant against infection by root pathogens (Bonner *et al*., 1974; Lagrimini and Rothstein, 1987). Novak (1992) cited unpublished work by Morpurgo that indicated that constitutive levels of peroxidase were present in a resistant hybrid banana. SH-3362, a *Foc* race 4 resistant, synthetic AAA hybrid produced at FHIA, had peroxidase levels 10-fold higher than in Pisang Mas, a susceptible AA cultivar.

The biochemical basis of tolerance in banana to Fusarium wilt was investigated by De Ascensao and Dubery (2000) using fungal elicitors from the mycelial cell walls of *Foc*. Root tissue of the tolerant hybrid FHIA-01 (AAAB) responded to the fungal elicitor through strong deposition of lignin, preceded by the induction or activation of the enzyme activities involved in the synthesis and polymerisation thereof, whereas only slight increases were observed in the susceptible cv. Williams. Phenylalanine ammonia lyase (PAL), peroxidase (POD) and polyphenol oxidase (PPO) activity were all induced at higher levels in the resistant cultivar. The production of stress metabolites by host plants also helps to inhibit *Foc*, and the release of PR proteins (β-1-3 glucanase and chitinase) may serve to destroy the pathogen (Pegg and Young, 1982).

Responses in cotton to *F. oxysporum* f. sp. *vasinfectum* (Atk.) include the possible biosynthesis of secondary metabolites such as lignan, syringyl lignin polymers, and terpenoid indole alkaloids in the hypocotyls (Dowd *et al.*, 2004). High syringyl content has been associated with pathogen defence response in wheat (Ride, 1975) and lignans are known to possess antifungal activity (Davin and Lewis, 1992). In roots, however, genes associated with tannin, anthocyanin and lignin biosynthesis were repressed by the pathogen (Dowd *et al.*, 2004). The importance of the preformed steroidal glycoalkaloid, tomatine, as an important resistance factor in tomato against *Fusarium* infection has been demonstrated by De Fago *et al.* (1983).

IMPROVEMENT OF BANANA FOR FUSARIUM WILT RESISTANCE

Natural sources of resistance are present in wild banana species, some cultivars and in synthetic diploids developed by breeding programs (Jeger *et al.*, 1995). *Foc* infects both resistant and tolerant cultivars, and host resistance is only expressed after infection (Beckman, 1987; Beckman, 1990).

Genes Associated with Resistance to Fusarium Wilt

At least three different classes of genes play a role in the defence strategy of a plant to disease. The first class comprises genes for constitutive (passive) defence and is not directly involved in defence responses, but may play a role in plant resistance by inhibiting pathogen entry by, for example, forming a thick waxy cuticular layer that protects against penetration. The second class contains genes that serve in the non-specific plant defence, for example the production of phytoalexins, glucanases, chitinases, lignin, callose and enzymes for oxidative stress protection. In addition to antimicrobial secondary metabolites, genes also coding for thionins, glutathionine S-transferases, lipoxygenases and phenylalanine ammonia-lyase (PAL) are induced (Glazebrook *et al.*, 1997). The third class is required for race-specific resistance and comprises genes such as *R* genes and results in the arrest of pathogen growth (Jørgensen, 1994).

Genetic analysis of host-pathogen interactions has shown that there are gene-for-gene interactions between the products of resistance (*R*) alleles and corresponding avirulence (*Avr*) alleles in the pathogen that lead to recognition and resistance (Flor, 1971). Plant *R* genes confer the capacity to recognize invasion by specific races of pathogens and to induce defence responses such as the HR (De Wit, 1992). However, plants are also able to activate several general defence responses that are under the control of a set of genes known as minor resistance genes (Dong, 1998). Major *R* genes confer immunity to the plant against specific pathogens, while the minor genes are linked to tolerance, which is a more general response that remains even though the pathogen might mutate.

R genes are grouped into six major classes, based on DNA sequence information (Jones, 2001). These classes include the following: Leucine-rich-repeat (LRR) kinase, extracellular (e) LRRs, *Pseudomonas* tomato resistance (*Pto*), Toll and Interleukin-1 receptor (TIR): nucleotide-binding (NB): LRR, coiled coil (CC): NB: LLR and signal anchor (SA):CC. The majority of *R* genes encode proteins containing a NB site and LRRs. R proteins are structurally similar and determine the recognition of a diversity of AVR proteins (Nimchuk *et al*., 2001). There are *Avr* genes in races of a pathogen for every *R* gene, defining resistance in the host plant (Baron and Zambryski, 1995; Staskawicz *et al*., 1995; Hammond-Kosack and Jones, 1997; Heath, 2000a; Agrios, 2004). When *avr* genes are lost or modified, the pathogen becomes virulent and causes disease again (Baron and Zambryski, 1995).

A gene-for-gene relationship has been proposed for the interaction between *F. oxysporum* races and plant host cultivars (Ori *et al*., 1997). This gene-for-gene relationship was confirmed for the *Fol* / tomato interaction, where the tomato resistance gene *I-2* confers resistance to race 2 of the pathogen (Ori *et al*., 1997; Mes *et al*., 2000). However, gene-for-gene interactions could not be demonstrated for the *Fol* race 1 / tomato interaction. It is apparent from both the studies of Beckman *et al.* (1982) and Beckman *et al.* (1989) that cells of the cultivar having either the homozygous recessive (ii) or the homozygous dominant (II) gene condition are capable of responding more strongly to non-pathogenic root flora than to *Fol* race 1. It therefore seems likely that the “I” gene does not directly provide for the synthesis and deposition of callose, but is rather involved in the regulation of the process through recognition, transduction or modulation.

A gene expression study on the interaction between susceptible cotton and *F. oxysporum* f. sp. *vasinfectum* demonstrated that defence-related genes were induced in the hypocotyls in contrast to being constitutively expressed in the root tissue (Dowd *et al*., 2004). Known defence genes identified in this study include two classes of *PR10* (cotton *PR10* and ribonuclease-like *PR10*), *PR5* (thaumatin), *PR2* (glucanase), *PR3* (chitinase, class I and IV) and cotton phytoalexin (gossypol) biosynthesis genes, such as those encoding for ATP citrase lyase b-subunit, Acetyl CoA C-acyltransferase, HMG CoA reductase mevalonate disphosphate decarboxylate and (+)-Δ-cadinene synthase.

In the banana/*Foc* interaction races have not been well defined (Ploetz, 1994) and should, therefore, not be confused with races in other pathosystems for which host genes for resistance and susceptibility are known (Stover and Buddenhagen, 1986). The current race structure is based on field evaluations of a limited number of banana cultivars and disease development is significantly influenced by climatic conditions. Different climatic conditions determine the disease development in Cavendish bananas caused by *Foc* in the tropics and sub-tropics (Ploetz *et al.*, 1990). 'Tropical' strains of *Foc* belong to VCG 01213/16, while 'subtropical' strains belong to VCG 0120. It is, therefore, rather difficult to propose a gene-for gene hypothesis for the banana/*Foc* interaction. The presence of tolerant banana cultivars, such as GCTCV (Hwang and Ko, 2004) further suggests that defence mechanisms are under the control of minor resistance genes, rather than one single dominant *R* gene.

Conventional Breeding

There are five major conventional banana breeding programmes in the world. These are Empresa Brasileira de Pesquisa Agropecuária – Mandioca e Fruticultura Tropical (EMBRAPA-CNPMF) in Brazil, the Centre de Coopération Internationale en Recherche Agronomique pour le Développement - Département des Productions f Fruitières et Horticoles (CIRAD-FLHOR) in Guadeloupe, the Fundación Hondurereña de Investigacón Agrícola (FHIA) in Honduras, CARBAP in Cameroon, and the International Institute for Tropical Agriculture (IITA) in Nigeria (Jones, 2000).

The first banana breeding programme at FHIA was established in 1959. The aim of this programme was to develop banana cultivars with resistance to *Foc* race 1 (Jones, 2000). To achieve this, fertile diploid pollen was taken from resistant male plants and applied to female flowers of Gros Michel and other triploid varieties with female fertility, in order to obtain resistant tetraploid hybrids (Cheeseman, 1932; Jones, 2000). The banana breeding process, however, is extremely slow due to the low number of fertile seeds that are obtained. Still, the FHIA breeding programme has produced several hybrids with resistance to both *Foc* race 1 and 4 over the past few years, such as FHIA-01 (AAAB) (Moore *et al*., 1995; Jones, 2000) and SH-3640/10 (‘High Noon’) (Eckstein *et al*., 1996; De Beer, 1997). At EMBRAPA, conventional breeding was used in combination with unconventional banana improvement programmes. In the process, Prata, Maçã and Prata Anã tetraploid hybrids were developed that also showed resistance to *Foc* (De Matos *et al*., 1999). Although these tetraploid bananas have disease resistance, their taste is unacceptable to consumers of Cavendish bananas. Attempts are, therefore, being made to breed for triploid hybrid bananas in Honduras (Rowe and Richardson, 1975) and Guadeloupe (Vakili, 1967; Stover and Buddenhagen, 1986).

Unconventional Improvement

Cavendish cultivars do not produce seed and are, therefore, not suitable for use in conventional breeding programmes. Consequently, researchers have been investigating unconventional methods to improve disease resistance in these bananas. Programmes for the unconventional improvement of banana have been established at several institutions, such as the Taiwan Banana Research Institute (TBRI) and the International Atomic Energy Agency (IAEA) in Austria. These programmes improve banana by means of somaclonal variation, induced mutations, protoplast fusion and genetic modification.

Somaclonal variation

When meristem cultures of banana are micro-propagated for more than six cycles, natural somatic mutations occur that are genetically stable (Hwang *et al*., 1984). Such somaclonal variants can be grouped according to changes in plant stature, pseudostem colour, leaf morphology and bunch characteristics (Hwang and Tang, 2000). Researchers at the TBRI were able to select Cavendish banana clones with resistance to *Foc* race 4 from somaclonal variants since 1983. These include the moderately resistant clone (GCTCV-215-1) and the highly resistant clone (GCTCV-218), both derived from Giant Cavendish (Hwang, 1999).

Induced mutations

Mutation breeding is the use of mutagens to develop variants that increase agricultural traits. Mutations are alterations in the nucleotide sequence of a DNA molecule and can be induced by irradiation (Novak *et al.,* 1990) or chemicals (Omar *et al.,* 1989). Mutations by gamma irradiation in banana plants can be induced either by *in vitro* irradiation of the sucker or irradiation of the shoot tip culture (Novak *et al.,* 1990). Chemicals such as ethyl methanesulphonate, sodium azide and diethyl sulphate induce mutations in banana (Omar *et al.,* 1989).

Protoplast fusion

Protoplast fusion was used to form triploid banana plants by fusing a diploid banana cell with a monoploid cell (Novak *et al*., 1989; Assani *et al*., 2001). Protoplasts (single cell systems) are cells from which the cell walls have been removed enzymatically and/or mechanically. Under suitable conditions the protoplasts are able to reform cell walls and multiply. These protoplast cells form clusters that develop into callus and plants are then regenerated from the callus.

Genetic modification

At least four classes of resistance genes have been identified for cloning into susceptible plants (Walsh, 2000). The first set of resistance genes encodes components of receptor systems that directly or indirectly detect the presence of potential pathogens. Genes involved in the gene-for-gene interactions are included in this class. The activation of such receptors probably initiates a signal transduction pathway that in turn results in the induction of generic response genes (Godiard *et al*., 1994). A second class of resistance genes encode products that detoxify and inactivate compounds produced by the pathogen in order to cause disease (Walsh, 2000). The third class of resistance genes codes for altered targets for pathogen-derived molecules that are essential for pathogenesis. In this case, a resistance allele could possibly code for a product that did not interact with the pathogen (Walsh, 2000). The fourth class of resistance genes include those that encode for constitutive biochemical or structural barriers to pathogens (Walsh, 2000).

Researchers have explored the possibility of introducing foreign genes into banana by means of particle bombardment (Sági *et al*., 1995; Becker *et al*., 2000) and *Agrobacterium tumefaciens*-mediated transformation (Ganapathi *et al*., 2001; Chakrabarti *et al*., 2003). Biolistic-mediated transformation has been used to transform cv. 'Mas' banana plantlets with the pSOC1 construct which contains sequences encoding for a MADS-box transcription factor associated with early flowering (Wong *et al.,* 2004). *Agrobacterium tumefaciens*-mediated transformation has successfully been used to transform 'Rastali' bananas with a β-1, 3-endoglucanase gene. Transgenic plants exhibited three times higher enzyme activity that untransformed plants (Sreeramanan *et al.,* 2004). Khanna *et al.* (2004) also reported successful transformation of Cavendish and 'Lady Finger' bananas using *A. tumefaciens*-mediated transformation. Marker-gene expression and molecular analysis showed that the four transgenes, hygromycin phosphotransferase, β-glucuronidase, neomycin phosphotransferase and the green fluorescent protein had integrated stably in the banana genome.

CONCLUSION

Fusarium wilt of banana became known as one of the most devastating agricultural diseases of the past century, destroying thousands of virgin forests in Central America. The disease was eventually brought under control following the introduction of resistant varieties to replace the susceptible Gros Michel variety. No other means of control seemed to be effective. Today, the international banana industry faces, once again, destruction by one of the most important soil-pathogens, *Foc*. Only this time there is no replacement variety for the sweet Cavendish banana, a seedless variety that cannot be improved by conventional breeding.

The banana-*Foc* interaction, although very important for understanding the principles underlying disease resistance, has received little attention in the years that followed the solving of the epidemics in Central America. In studies by Stover (1962) and Beckman (1987; 1990) on the resistance response in banana roots, it was shown that vascular occluding gels and tyloses were produced in response to *Foc* infection. These gels and tyloses then become infused with phenolics and stress metabolites to prevent progress of the pathogen. In resistant varieties, enzymes such as PAL, POD and PPO, and the PR-proteins are produced. The genetics underlying these responses, however, are still unknown, despite the fact that Fusarium wilt resistant banana hybrids have been developed in conventional banana breeding programmes. Our current understanding of the genetics of resistance against Fusarium wilt diseases, therefore, is limited to the interaction between *F. oxysporum* and crops such as tomato (Beckman *et al.*, 1982; Brammal and Higgins, 1988; Beckman *et al.*, 1989) and carnation (Baayen 1987; 1989). In this respect, the role of transcription factors such ERF1 and antifungal compounds such as *PRs* and peroxidases have been described.

The pathosystem and plant response to variants and special forms of *F. oxysporum* are not always consistent. In tomato, for instance, a true gene-for-gene interaction between race 2 in *Fol* has been described. However, a race-for-race interaction seems to be unlikely in the case of the *Foc*-banana interaction. One of the most important questions related to resistance in banana, therefore, is whether resistance is determined by *R* genes or minor genes, such as those involved in the production of glucanases, chitinases, phytoalexins, lignin, callose, enzymes for oxidative stress protection and antimicrobial secondary metabolites. The latter scenario seems to be more likely, as various levels of tolerance and resistance in banana to certain races of *Foc* has previously been described. Race definition in *Foc* is further complicated by the role of the environment that appears to influence disease development in certain *Foc*-banana interactions.

The search for genes involved in plant resistance to pathogens and pests has become a fast growing area of interest. Techniques that are currently employed to identify resistance genes include studies on segregating plant populations and differential gene expression. To study genes that are differentially expressed, methods such as differential display, differential hybridization (Liang and Pardee, 1992), representational difference analysis (RDA) (Lisitsyn and Wigler, 1993), subtractive library construction (Tedder *et al.*, 1988), Suppression Subtractive Hybridization (SSH) (Diatchenko *et al.*, 1996; 1999), serial analysis of gene expression (SAGE) (Velculescu *et al.*, 1995), and cDNA microarray analysis (Schummer *et al.*, 1997) have been employed. For the seedless Cavendish banana, a technique such as SSH seems to be most appropriate, since the common genes that are present in the isogenic lines would be removed and only the differentially expressed genes would be isolated.

The isolation and characterization of resistance and defence genes in banana to *Foc* can be of great significance. Banana resistance genes could be used as markers for the rapid identification of tolerant or resistant traits in banana selections. The identification of banana genes associated with defence would contribute to the understanding of the molecular basis of the banana/*Foc* interaction. These banana genes can further be used for genetic improvement of susceptible bananas via transformation. These genetically modified bananas would be more acceptable to the consumer market than those improved with genes from other crops.

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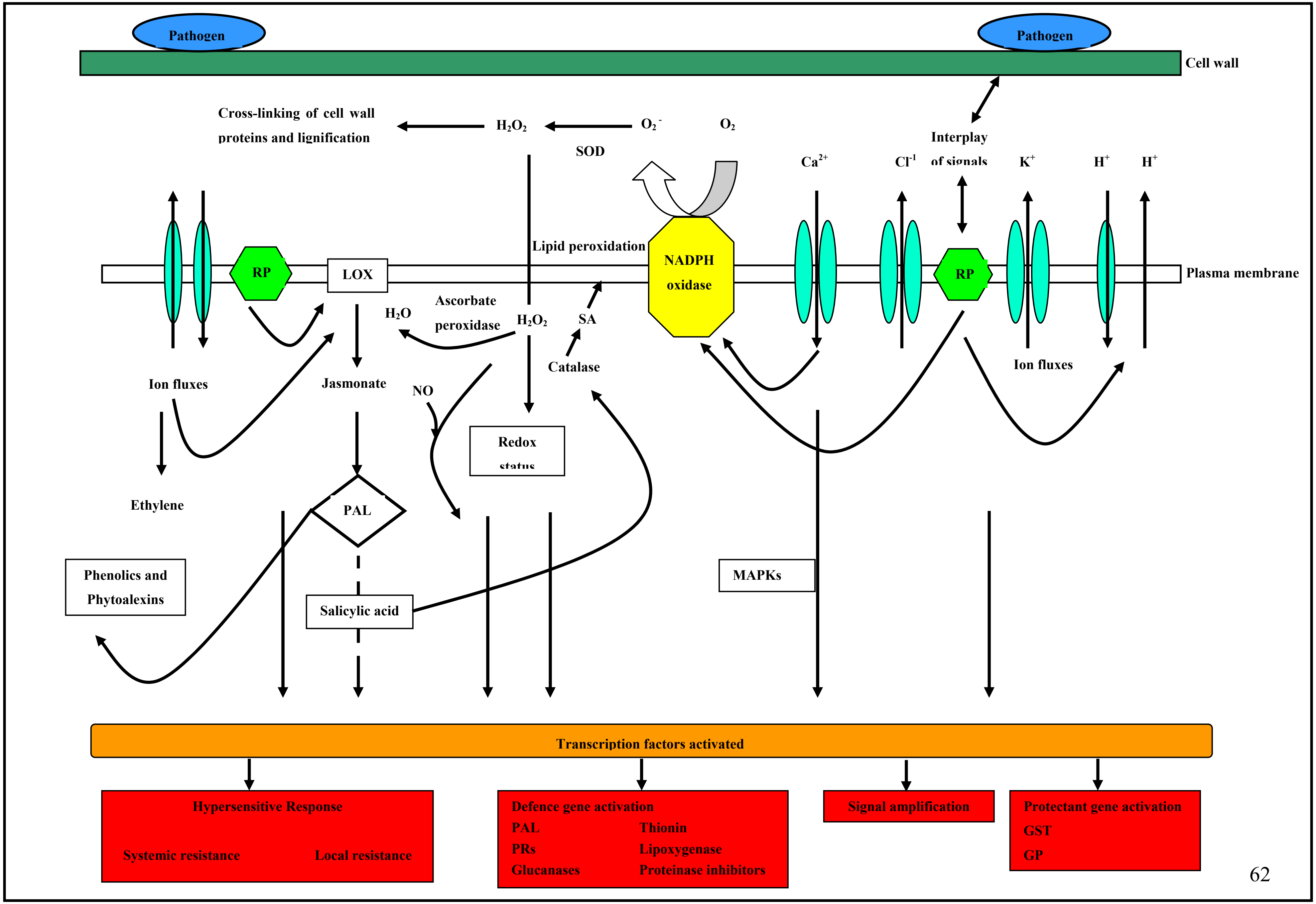
Pathogen
Pathogen
Cell wall
Cross-linking of cell wall proteins and lignification
H_2O_2
O_2^-
O_2
SOD
Interplay of signals
Ca^{2+}
Cl^{-1}
K^+
H^+
H^+
Lipid peroxidation
RP
LOX
NADPH oxidase
RP
Plasma membrane
H_2O
Ascorbate peroxidase
H_2O_2
SA
Catalase
Ion fluxes
Jasmonate
NO
Redox status
Ion fluxes
Ethylene
PAL
Phenolics and Phytoalexins
Salicylic acid
MAPKs
Transcription factors activated
Hypersensitive Response
Systemic resistance
Local resistance
Defence gene activation
PAL
Thionin
PRs
Lipoxygenase
Glucanases
Proteinase inhibitors
Signal amplification
Protectant gene activation
GST
GP

Figure 1. Overview of signalling events controlling the activation of plant defence responses.

The plasma membrane is considered to be the primary site for elicitation of different signalling cascades involved in plant-pathogen recognition and defence response. These defence responses include the production of reactive oxygen intermediates by a plasma membrane-associated complex, the induction of various ion fluxes (calcium (Ca^{2+}), proton (H^{+}) influx, and potassium (K^{+}), chloride (Cl^{-1}) efflux) across the plasma membrane. Activated signalling cascades at the plasma membrane in turn induce other plant defence mechanisms, including the accumulation of secondary signalling molecules salicylic acid (SA), ethylene (ET), jasmonate (JA), synthesis of phytoalexins and phenolics and pathogenesis-related (PR) proteins. Mitogen-activated protein kinase (MAPK) activation is placed downstream of ion fluxes and leads to defence gene induction by a process that is independent of the oxidative burst. Resistance responses are also often accompanied by the induction of the hypersensitive response (HR). (Abbreviations: superoxide dismutase (SOD), receptor (RP), lipoxygenase (LOX), phenylalanine ammonia-lyase (PAL), mitogen-activated protein kinase (MAPK), glutathione S-transferase (GST), glutathione peroxidase (GP), pathogenesis-related proteins (PRs).

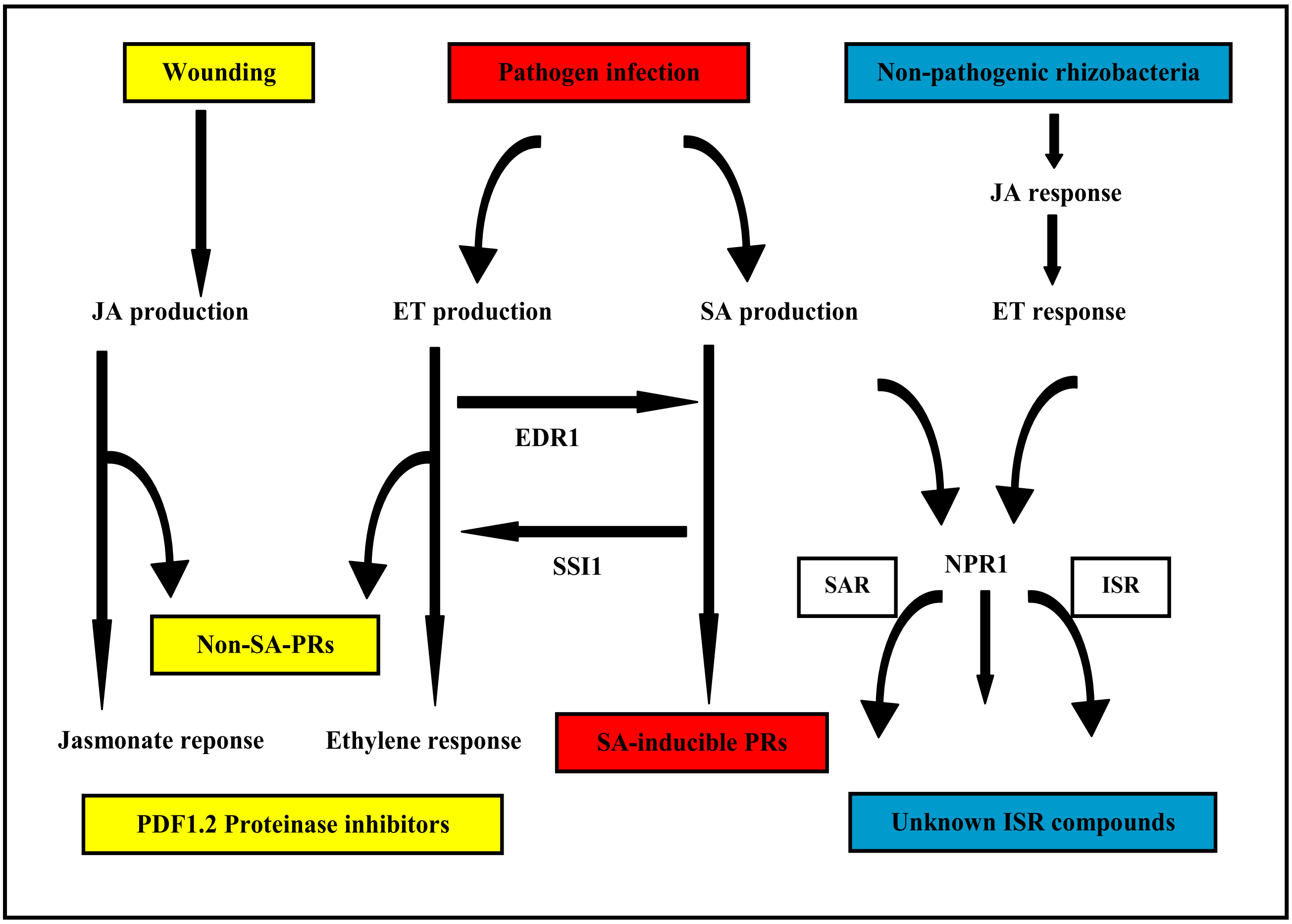

Figure 2. Simplified model of SA-,ET-, and JA-dependant signalling, convergence points and possible cross-talk between different stimuli.

The figure has been adapted from Pieterse and Van Loon (1999) and Wang *et al.* (2002). (Abbreviations: SA: salicylic acid, ET: ethylene, JA: jasmonate, SAR: systemic acquired resistance, ISR: induced systemic resistance, PRs: pathogenesis related proteins).