Chapter 2

Literature review

1. Description of barley

Barley is a monocotyledonous plant that belongs to the family *Poaceae.* The stems of these plants are usually between 120cm and 150cm in length. The leaves are single and usually with a waxy or glossy appearance. The leaves comprise a sheath, ligule, auricle and a blade (Figure 2.1). The auricle is an ear-shaped appendage and the sheath is glaborous. Barley is a self-pollinating cereal and the chances of cross-pollination are limited. The inflorescence consists of a spike that has three spikelets attached to the central stem (rachis). The inflorescence may be soft, which is an adaptation to humid conditions during maturation (Arnon, 1972).

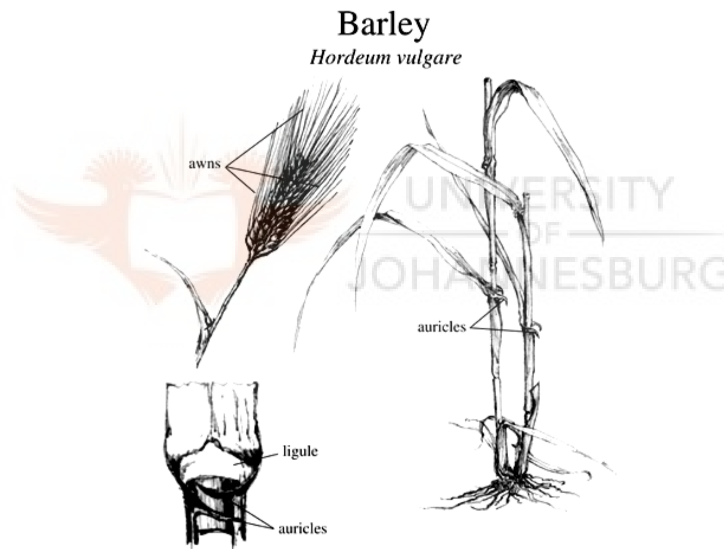

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Figure 2.1: An illustration of barley. Taken from www.cdl.umn.edu/interaction/hosts.html Retrieved in February 2007.

The average stems number between three and five under normal conditions, such as when the nitrate and moisture concentrations are sufficient. Barley has higher tolerance to drought and diseases than wheat. Arnon (1972) published that barley could grow in poor soil as it has a high salt tolerance, and only need 200 – 250mm of rainfall per year.

It needs well-drained clay soil with a pH of 6.0 or more for optimum growth. Although barley is a stronger plant than wheat, very dry and hot temperatures will have an effect on the seeds when they mature. The optimum temperatures for normal seed germination are between 15℃ and 20℃. The optimum growth of young plants to ripening of seeds is between 16℃ and 17℃. Studies were done in the field with barley, indicating that soil temperatures as high as 40℃, did not affect the growth of the young plants. However, the rate of maturity was increased and the final yield percentage was lessened. A lower yield production also has an effect on the quality of malting, decreasing the value (Arnon, 1972).

The genome structure of barley differs from other cereals in the *Poaceae* family. Barley is a diploid, with two copies of each chromosome. It has a basic chromosome number of 2n=2x=14 and a genome size of C = 6.18pg. Rice (*Oryza sativa*) is also a diploid and contains a basic chromosome number 2n=2x=24 and a genome size C = 0.45pg (Arumuganathan and Earle, 1991). The chromosome number of wheat is 2n=6x=42, which is a hexaploid and have a genome size of C = 17.33pg (Fay *et al*., 2005; Huang *et al*., 2003).

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2. Plant pathogens

A variety of micro-organisms (e.g. bacteria, viruses, fungi and nematodes) are constantly exploiting plants (Hammond-Kosack and Jones, 1996). Barley is no exception and various pathogens attack it (Table 2.1). For a pathogen to be successful during infection, it must have mechanisms to penetrate the host. These mechanisms include mechanical means to penetrate, such as penetration pegs and haustoria, and the production of toxins and enzymes in order to destroy the cells. This provides an area of infection and prevents the activation of the defence mechanism. (Lamb *et al.*,

Table 2.1: The characteristics and descriptions of diseases that occur in barley plants (Arnon, 1972)

Pathogen	Disease	Symptoms
Fusarium graminearum	Head blight (Scab)	Water-soaked brownish spots, decolourises the spike.
Helminthosporium gramineum	Barley stripe	Brownish stripes occur on the leaves, damaging the tissue causing the leaf to tear at stripes.
H. teres	Net blotch	Brown net patterns occur on the leaves.
H. sozokinianum	Spot blotch	Dark brown spots first occur and form a blotch later on the leaves.
Rhynchosporium secalis	Scald	Big round spots occur on the blade and sheath of the leaves with a blue-green colour turning to light grey.
Blumeria graminis var*. hordei*	Powdery mildew	Characteristic white growth occurs on the abaxial side of the leaf first.
Septoria passerinii	Septoria leaf blotch	Speckles appear on the leaf causing death.
Puccinia graminis	Stem rust	First seen as elongated elliptical blisters on the leaves and stems of the plant, breaking open some days later to reveal a mass of rust-coloured spores.
P. hordei	Leaf rust	Orange-red pustules erupting from the leaf surface containing spores
Barley yellow dwarf virus	Yellow Dwarf (BYDV)	Leaf yellowing and stunted plants.
Barley stripe mosaic virus	Stripe mosaic (BSMV)	Leaf yellowing and stunted plants.
Ustilago hordei	Covered smut	Top part of plant covered with black spots and a grey membrane around the spots.
U. nigra	Loose smut	Top part of plant covered with black spots, without any membrane.
U. nuda	Nude loose smut	Top part of plant covered in black spots.

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1989; Keen, 1992). Senescent leaves are the most common organs for most necrotrophic pathogens to attack (Agrios, 1997; Steiner-Lange *et al*., 2003). Fungal pathogens evolved from infecting numerous plants to infecting specific plants. These pathogens can be divided into three levels. The first level is the opportunistic pathogen that needs mechanical wounds to penetrate a host. The second level is true pathogens that can survive with or without a living host and the third level is the obligate pathogen. It needs the host to complete its life cycle. *F. graminearum* is an example of a true pathogen (Knogge, 1996).

2.1 *Fusarium graminearum*

Fusarium head blight (FHB) is caused by *F. graminearum* Schwabe [teleomorph = *Gibberella zeae* (Schwein.) Pertch; synonym = *G. saubinatti*]. This ascomycete fungus is a necrotrophic pathogen occurring on wheat, barley and rice. The disease caused by *F. graminearum* results in yield and quality losses across the world. In barley it reduces the grain feed value and use for malting. Once it has infected cereals it usually causes discolouration of the kernels (KD) (Capettini *et al*., 2003). *Fusarium* can be divided into two groups according to the pathological differences between the perithecia formation on carnation leaf agar (CLA) or carrot agar. The first group is *F. pseudograminearum.* It is heterothallic and cannot form perithecia from single-spore cultures on either of the medium. The second group is *F. graminearum,* which is homothallic and can form perithecia from single-spore cultures on both of these media (Leslie and Summerell, 2006).

Environmental conditions such as temperature, relative humidity and rainfall determine the development of the pathogen each year and subsequent deoxynivalenol (DON) accumulation. DON is a toxin produced by the pathogen that causes hazardous effects in animals and humans such as diarrhoea and nausea (McMullen *et al*., 1997). Other factors such as genetics, plant height, canopy density and spike morphology influences the fungal colonisation (Prom *et al*., 1999; Hill *et al*., 2006). Spores spread through

arthropod vectors, such as mites, and wind and rain dispersal to other plants (Parry *et al*., 1995). Favourable growth conditions for *F. graminearum* are rainy, humid summers (Pekkarinen *et al*., 2003). Optimum growth occurs between 24℃ - 26℃ and at 100% humidity (Booth, 1971).

In barley, flowering occurs when the spike is closed inside the sheath. Infection usually occurs on the exposed spike after emergence from the sheath (Prom *et al*., 1999). Mycelium and chlamydospores over-winter on the cereal, releasing conidia or ascospores when conditions are favourable (Figure 2.2) (Parry *et al*., 1995). The first signs of infection occur in the middle of the glume and rachis as water-soaked brown spots. From the initial infection, it spreads to other surrounding areas. Later during the infection, pink-red fungal growth can be seen on the edge of the glumes or at the base of the spikelets (Booth, 1971).

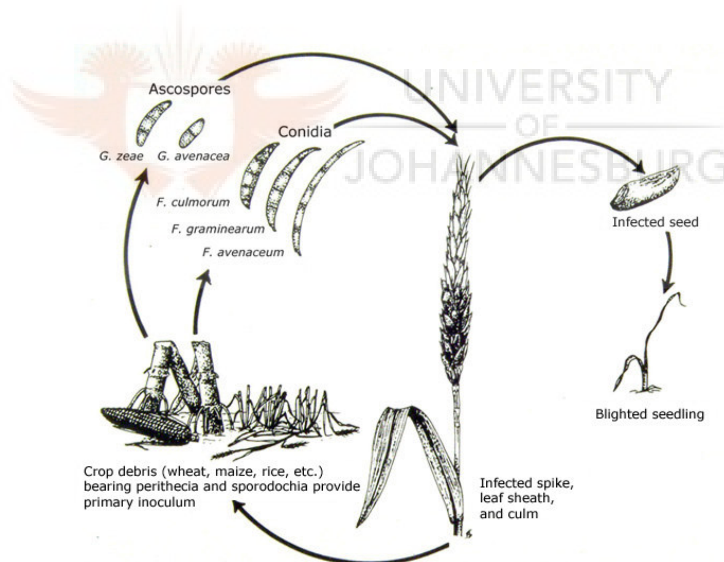

Figure 2.2: The disease cycle of *Fusarium graminearum*. Taken from www.apsnet.org/.../Fusarium/discycle.htm, Retrieved in November 2007.

Cell death will not be induced if fungal growth stays between the cuticle and walls of the epicarp. When the fungus grows into the cytosol, cell death occurs (Jansen *et al*., 2005). Infected seeds will decrease in size and become grey-brown to blue-black in colour when harvested (Mathre, 1997). Because of the decrease in kernel size it was

hypothesised that the pathogen targets the starch reserves in the endosperm (Skadsen and Hohn, 2004). Multiple enzymes are needed for the pathogen to invade its host. These enzymes include the proteinases that are needed to break down the host's proteins. The role of the different proteinases differs during different interactions (Knogge, 1996). Thus the pathogen uses alkaline proteinase to degrade the proteins in the plants cells in order to obtain nutrients for growth (Pekkarinen *et al*., 2003a).

It is thought that initial infection occurs on the brush hairs on the seed tip. The hyphae move towards the pericarp epidermis to the cross-cell (testa). Other resistance mechanisms of the host involve the formation of a barrier between the cross-cell and aleurone that reduces the growth of hyphae into the endosperm. This could be due to antifungal compounds in the testa and aleurone. Antifungal proteins, such as PR5 thaumatin, occur in the aleurone and are highly toxic to *F. graminearum* (Skadsen and Hohn, 2004).

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3. Plant defences

There are many ways for a plant to defend itself against pathogens. The use of barriers is the most frequent strategy employed by plants. The first external barrier is a physical structure, such as the cuticle and epidermal cell walls. This prevents the pathogen from entering the plant (Keen, 1992). Some bacteria and viruses need a physical opening such as a wound or open stomata on the leaves to penetrate. Insects, nematodes and some fungi can penetrate the host through wounds created by mechanical damage, by secreting enzymes or toxins, or sometimes a combination of the two (Figure 2.3). Substances such as pectic enzymes that are secreted by *Blumeria* spp., damage the plant cells, causing the nutrients to leak out and thus providing optimum growth conditions for the bacterial pathogen. Cell defences can also be affected and reduced (Keen, 1992).

A host-response to pathogens is the formation of papillae. Fungal spread is prohibited

by the formation/deposition of callose at the site of penetration. The hypersensitive response (HR) is another barrier that plants employ. This is a biochemical reaction, which includes signal transduction. A pathogen contains elicitors, which are products of avirulence (*Avr*) genes that a plant recognises when an infection takes place. These elicitors elicit a response in the plant. Once these elicitors are recognised by the plant's resistance (*R*) gene products, it sends a signal to the host cell proteins and nuclear genes, activating them, to produce substances that inhibit the growth and mobility of the pathogen (Agrios, 1997). Therefore, it was thought that the *R* gene product could be a receptor of a specific elicitor activity, leading to the defence mechanism (Keen, 1990).

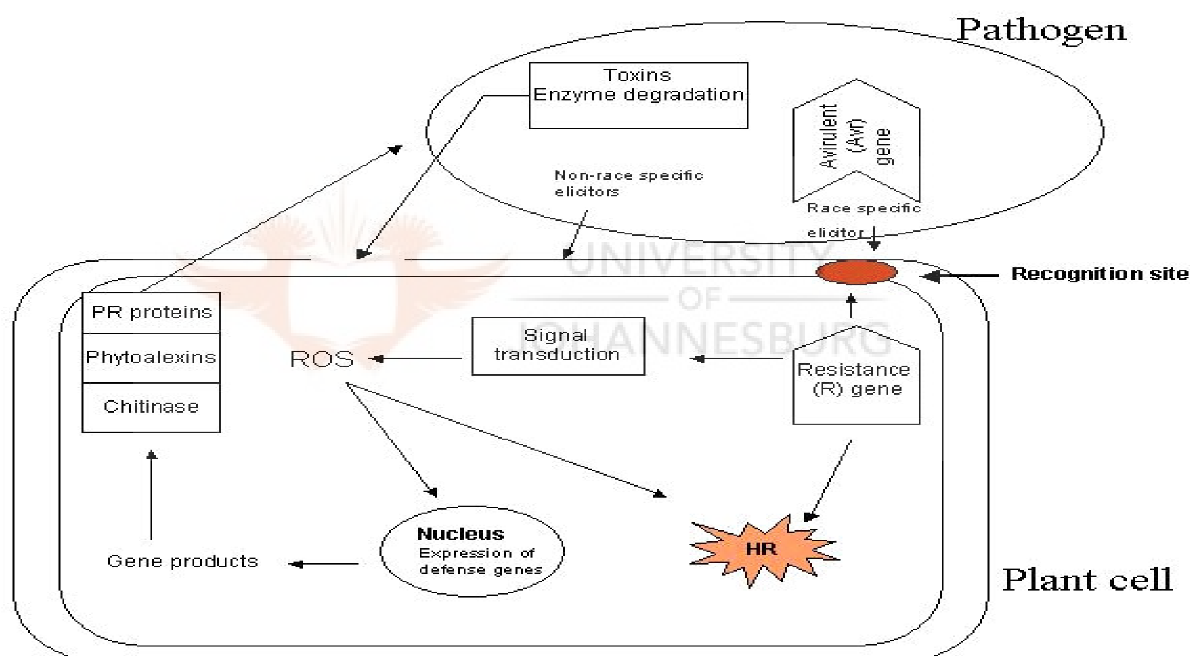

Figure 2.3: The interaction between plants and pathogens. Adapted from Slater *et al*., 2004.

3.1 The "Gene-for-Gene" concept

Flor suggested the gene-for-gene interaction while working on the flax rust fungus*, Melampsoma lini.* This hypothesis suggests that a plant contains an *R* gene and the pathogen an *Avr* gene. For each *R* gene in the plant there must a corresponding *Avr* gene in the pathogen (Flor, 1971). The resistance or susceptibility of a plant to a pathogen is mainly determined by the presence of dominant or recessive alleles of these genes in the plant and pathogen. A plant contains either *R* (dominant) or *r* (recessive) alleles. The pathogen contains either *Avr* (dominant) or *avr* (recessive) alleles (Table 2.2). If the interaction contains an *Avr* allele from the pathogen and an *R* allele from the plant, it would mean that the plant would be resistant to the pathogen as stipulated by the gene-for-gene interaction hypothesis. If the interaction contains an *Avr* allele and an *r* allele, the plant would be susceptible. If the interaction takes place between an *avr* allele product and an *R* allele product, the plant would also be susceptible because of the presence of the virulence gene from the pathogen. The *R* gene from the plant would not signal a defence response. The last interaction would be if there were *avr* alleles and *r* alleles present. The plant would also be susceptible because it lacks the resistance gene and the pathogen is virulent. There are two types of resistance, gene-for-gene interaction and field resistance. This could also be referred to as specific or non-specific resistance (Agrios, 1997).

Table 2.2: A quadrant that determines the resistance or susceptibility between plants and pathogens. (Agrios, 1997).

Virulence / avirulence genes in the pathogen	Resistance / susceptible genes in the plant	
	R (dominant)	r (recessive)
Avr (dominant)	AR	Ar
avr (recessive)	aR	ar

The interaction between matching *Avr* gene and *R* gene products culminates in a strong resistance response (Keen, 1990). Recognition between these two products results in a

signal transduction and an active response that ends in the HR. These signal transductions include responses such as ion fluxes, changes in protein phosphorylation, activation of reactive oxygen species (ROS) and the release of salicylic acid (SA) (Figure 2.4). If the *Avr* gene or *R* gene is absent, it causes a compatible interaction. This results in the penetration of the pathogen and finally plant disease (Halterman *et al.*, 2001). The full function of most of the avirulence genes has not yet been determined (Lahaye and Bonas, 2001). The proposed function of *Avr* genes is the reduction of the defence system and accessibility to nutrients for the pathogen (Luderer and Joosten, 2001).

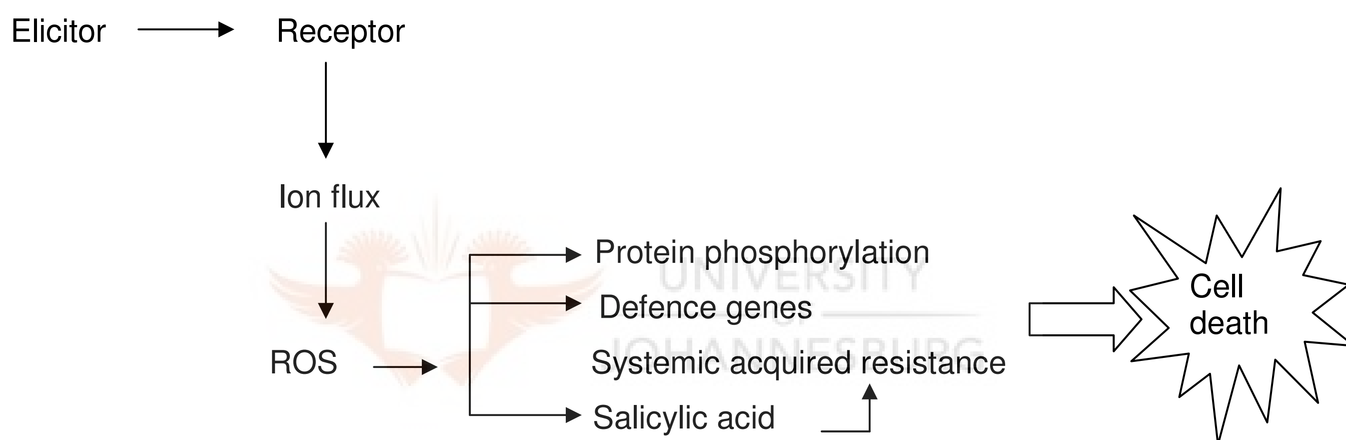

Figure 2.4: A representation of some of the responses in signal transduction in the host during infection. Adapted from Slater *et al.*, 2004

3.2 Guard hypothesis

The guard hypothesis is a model in which a plant's R proteins would stimulate its defence mechanisms upon recognition of the Avr protein. In this model the R protein guards specific receptors for each of the Avr proteins. The R protein binds and activates the resistance response once one of these receptors binds an Avr protein. If the R protein in the plant is absent, the plant is susceptible to the pathogen, thus not guarded (van der Biezen and Jones, 1998; Dangl and Jones, 2001). There are two variations of

the guard hypothesis. The first variation is that R proteins can respond to two or more different types of effectors that will target the same receptor. Secondly, the host protein complex that is targeted by pathogens can be guarded by more than one R protein (Mackey *et al.*, 2003).

A well-known example of this system is the interaction between tomato and *Pseudomonas syringae.* The tomato plant carries an R protein, Pto that is responsible for a defence response. The corresponding AVR protein in *P. syringae* is AvrPto, where the main function is to target the Pto protein and suppress the defence response. Another protein that is needed for the activation of the defence response is Prf; a nucleotide-binding site leucine rich repeat (NBS-LRR) protein that guards Pto. The function of Prf is to detect the interaction between Pto and AvrPto and activate a defence response (van der Biezen and Jones, 1998; Dangl and Jones, 2001).

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3.3 Hypersensitive response

When pathogens attack a certain host, it will result in a defence response from the plant. One of the most important defence mechanisms that a plant will employ is the HR. The HR is a mechanism or biochemical reaction to initiate death of the plant cells through programmed cell death (PCD) to prevent further pathogen infection. These reactions are stimulated inside the cell and tissue when a pathogen has entered. According to Jabs (1999) the activation of the HR is caused by the response of the plant and not the invasion of the pathogen. A cascade of events is activated inside the cell, followed by the manifestation of the HR through the development of necrotic lesions. This prevents further spread of the pathogen. These events are usually a defence response when chemicals such as phytoalexins are formed and structural barriers are established (Keen, 1992). The HR can occur in one cell or in all the surrounding cells to prevent the spread of the pathogen to neighbouring cells (Heath, 2000a). Necrotrophic fungi gain nutrients that they require for survival from dead cells. Thus the HR would not be a viable source of defence because they may take advantage of this system (Govrin and Levine, 2000). *R* genes

could activate an HR response or it could prevent infection by means of other responses. These responses could be so efficient that it is not necessary to activate the HR. An example would be in the case of *Rhynchosporium secalis,* where the *Rrs1* gene stops fungal infection by preventing subcuticle stroma from developing, thus not activating the HR (Lehnackers and Knogge, 1990).

When the hypersensitive response is activated, certain changes occur inside and around the cells. If the plant is susceptible to the fungus, the nucleus will move to the site of infection first. From there it will move away when the fungus comes in contact with the plasma membrane and return as the fungus penetrates deeper inside the cell (Heath *et al.*, 1997). In resistant plants, the nucleus does not move to the penetration site, but stays intact with only differences in size and appearance. After that, cytoplasmic streaming will occur and the protoplast will fractionate. After this step the plant cell cannot recover and particles appear in the vacuole and cleavage of the nuclear DNA occurs. The plasma membrane will lose its permeability and the protoplast shrinks due to tightening of the cell (Heath *et al.*, 1997; Heath, 1998). The HR also initiates more responses, such as the production of phytoalexins; increased concentration of SA, ROS, membrane damage, ion fluxes, pathogenesis-related (PR) protein expression and more (Heath, 2000a). The HR causes the resistant cells to lose turgor pressure and turn brown as they die off. The cells around the infected area die, and this prevents further penetration of the pathogen (Agrios, 1988).

3.4 Resistance genes

There are three types of gene classes involved in the defence mechanism. The first class of genes is not directly involved in the defence, but is there for protection, mostly through the formation of a waxy cuticle. Secondly, there is the class of non-specific responses, where the plant will produce phytoalexins and oxidative enzymes. The last class is the race-specific resistance genes, where it will activate the defence mechanisms by the *Avr-R* gene interaction (Hammond-Kosack and Jones, 1996).

For a plant to have a good defence system, it requires resistance genes. In order for the plant to show resistance to a specific or specialised pathogen, the plant must contain *R* genes and the pathogen must contain corresponding *Avr* genes. Thus, both these genes have to be present for the plant to have resistance against specific pathogens. If one or both of these genes are absent, the plant will be susceptible to the infection (Bent, 1996). Two known examples of *Avr* genes in the bacterial Avr protein class are AvrBS3 and AvrRXV/YOPJ, which are linked to transcriptional and protease activities. Clearly when the *Avr* gene product is recognised by the *R* gene product it will lead to a defence response. Thus the *Avr* gene can be a disadvantage for the pathogen as it limits the host range (Luderer and Joosten, 2001).

In 1992, Johal and Briggs were the first to isolate a plant *R* gene, namely the *Hm1* gene from maize plants displaying resistance to the fungus *Cochliobolus carbonum.* The fungus contains HC toxin, which it uses to infect certain hosts. The host (*Zea mays)* contains the *HM* gene that encodes HC toxin reductase (HCTR), which reduces the activity of the HC toxin. Since then, several genes have been isolated and identified. Examples include the *Pto R* gene in tomato plants that display resistance to *Pseudomonas syringae* carrying the *Avr* gene *AvrPto* (Agrios, 1997); and the pathosystem between tomato plants and *Cladosporium fulvum*, causing leaf mould, (Parniske *et al.*, 1997). The tomato plant carries the *Cf* gene, which shows resistance to the fungus *C. fulvum* containing the corresponding *Avr* gene (Jones *et al.*, 1994). Many *Cf* genes have been identified that are expressed in the presence of the corresponding *Avr* genes (Hammond-Kosack and Jones, 1996). The *Cf* genes encode transmembrane proteins with extra cytoplasmic LRR's. The *Avr* genes encode short 28 amino acid peptides that are secreted by the fungus. These peptides kill cells resulting in the formation of necrotic spots on the leaves of the plant when infected (Jones *et al.*, 1994). Mutations in these *Avr* genes ensure that the plant does not recognise the fungus, thus creating an opportunity for the fungus to penetrate. The *Cf-Avr* interaction is a classic example of the gene-for-gene interaction. The Cf proteins recognise the Avr product, leading to a defence response with certain enzymatic activities, ethylene and SA

biosynthesis and pathogenesis-related (*PR*) gene transcription. The end result of this interaction could be PCD (Hammond-Kosack and Jones, 1996).

3.5 Classes of R proteins

In order for a plant to recognise an invading pathogen, it requires receptor proteins. There are six classes of R proteins that are identified as receptor proteins. The class that is the most important is the NBS-LRR class and forms the biggest R protein class in plant defence (van der Biezen and Jones, 1998). They could also play a role in activation of genes during signal transduction but are mostly known for their recognition in defence responses (Pan *et al*., 2000). According to Dangl and Jones (2001), it is possible that the NBS-LRR proteins are located inside the cell and that it can be an avirulent receptor. It detects the interaction between the receptor protein and the Avr protein, leading to a defence response. Thus it could be said that LRR proteins are involved in a specific protein-protein interaction (Jones, 1997).

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NBS-LRR proteins can be divided into two groups according to the sequence of the N-terminus. The first group has a homology with the *Drosophila* Toll or human interleukin (IL)-1 receptor (TIR) regions. This is also called the TIR-NBS-LRR. This region plays a role in the defence response and signal transduction (Ellis *et al*., 2000). The second group has a coiled-coil at the N-terminal domain (CC-NBS-LRR) (Dangl and Jones, 2001). The coiled-coils are bundles of α-helices that are wound into super helices (Lupas, 1996). The NBS forms the central domain of the same sequence motif as ATPase and GTPase. The P-loop or phosphate-binding loop is found in those regions of NBS genes (Saraste *et al*., 1990). When an LRR unit align with the homologues, it forms a backbone of conserved repeats. This motif or repeats (xxLxLxx), where L represents the leucine or aliphatic amino acids and x represents the variation in amino acids, form a β-strand-β-turn in each repeat (Ellis *et al*., 2000). The C-terminus consists of the LRR domain and function as protein-protein interactions in the gene-for-gene recognition. The TIR-NBS-LRR is not usually found in the cereal species, but the CC-

NBS-LRR is found in both the monocotyledons and dicotyledons (Pan *et al.*, 2000).

3.6 Elicitors

Some elicitors such as biotic elicitors are molecules from the pathogen or host that induces a defence response in the plant (Dixon and Lamb, 1990). These elicitors can elicit signal transduction pathways such as protein kinase and protein phosphorylation reactions (Heath, 2000). These can be race specific where it can activate a response in a specific host containing an avirulent pathogen. Non-race specific elicitors are released from the cell wall fragments from either the host or pathogen during infection and play a role in the defence system of non-host plant-pathogen interaction (Heath, 2000).

3.7 Signal transduction in plants

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When a plant is infected a series of physiological changes occur in the infected area. An example would be when a race-specific elicitor binds to a receptor in the host, followed by a cascade of signalling events that leads to PCD, ROS accumulation, phytoalexin synthesis and hormonal changes (Scheel, 1998). Signal transduction and downstream events seems to differ between plants infected with different pathogens (Bent, 1996). Thus, it is more difficult to use a standard or basic system that can explain the events happening in the signal pathway.

3.7.1 Oxidative burst

The oxidative burst is a response from the plant upon infection by a pathogen. This response is through the production and accumulation of ROS, which occur, extra-cellularly, such as super oxide (HO_2/O_2^-) and hydrogen peroxide (H_2O_2) (Hammond-Kosack and Jones, 1996). The ROS can be detected at one or two time points during

the infection. The first time point being an immediate response occurring upon infection and the second, where the response only occurs a few hours after infection. This could be due to a longer period of time for the plant to recognise, deliver and process the signals (Baker and Orlandi, 1995; Hammond-Kosack and Jones, 1996).

The role that ROS play is very important in the defence mechanisms. ROS is considered to be toxic to certain pathogens when infection occurs (Peng and Kuć, 1992). They also help with cell wall reinforcement. An excess of ROS in neighbouring cells can be damaging (Bradley *et al*., 1992; Levine *et al*., 1994) as it can cause browning and cell death in plants (Able, 2003). ROS can also be involved in the mechanisms such as the HR and PCD. ROS is produced in susceptible responses and a number of enzymes play a role in this production. Xanthine oxidase is responsible for purine catabolism; NAD(P)H occurs during the activation of HR and peroxidase plays a role in the plant-pathogen interaction (Montalbini, 1995; Xing *et al*., 1997).

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3.7.2 Ion fluxes

Changes in plasma membrane permeability occur when the cells react to external stimuli such as pathogen elicitors. Calcium (Ca^{2+}) enters the cytoplasm of the plant cell through ion channels from either cell organelles or from the apoplasm (Bush, 1995). This causes a Ca^{2+} and proton influx and potassium (K^{+}) and chloride (Cl^{-}) efflux (Ebel and Scheel, 1997). The role of this ion flux is to induce the oxidative burst, to activate the defence response, and the production of phytoalexins (Jabs *et al*., 1997).

G-proteins are molecular signal transducers, which bind to GTP or GDP, making it active or inactive. It is thought that the G-protein sends a signal through the α and β subunits to the Ca^{2+} channels activating a flow of cytosolic Ca^{2+} into the cytoplasm (Gelli *et al*., 1997; Aharon *et al*., 1998). G-proteins can be divided into two groups namely heterotrimeric G-proteins which can be divided further into α, β and γ subunits. The second group consists of small G-protein containing only the α subunit (Gilman, 1987).

G-proteins are activated through the signalling of the cAMP pathway. When a hormone binds to the receptor in the plasma membrane, a G-protein will bind to the receptor by changing the shape of the receptor (Hopkins, 1999) (Figure 2.5).

The function of Ca^{2+} is to control cell elongation and division, secretion of enzymes, hormone activity and defence responses. For Ca^{2+} to function properly, the cytosolic Ca^{2+} concentration must be kept low through the action of membrane-bound calcium–

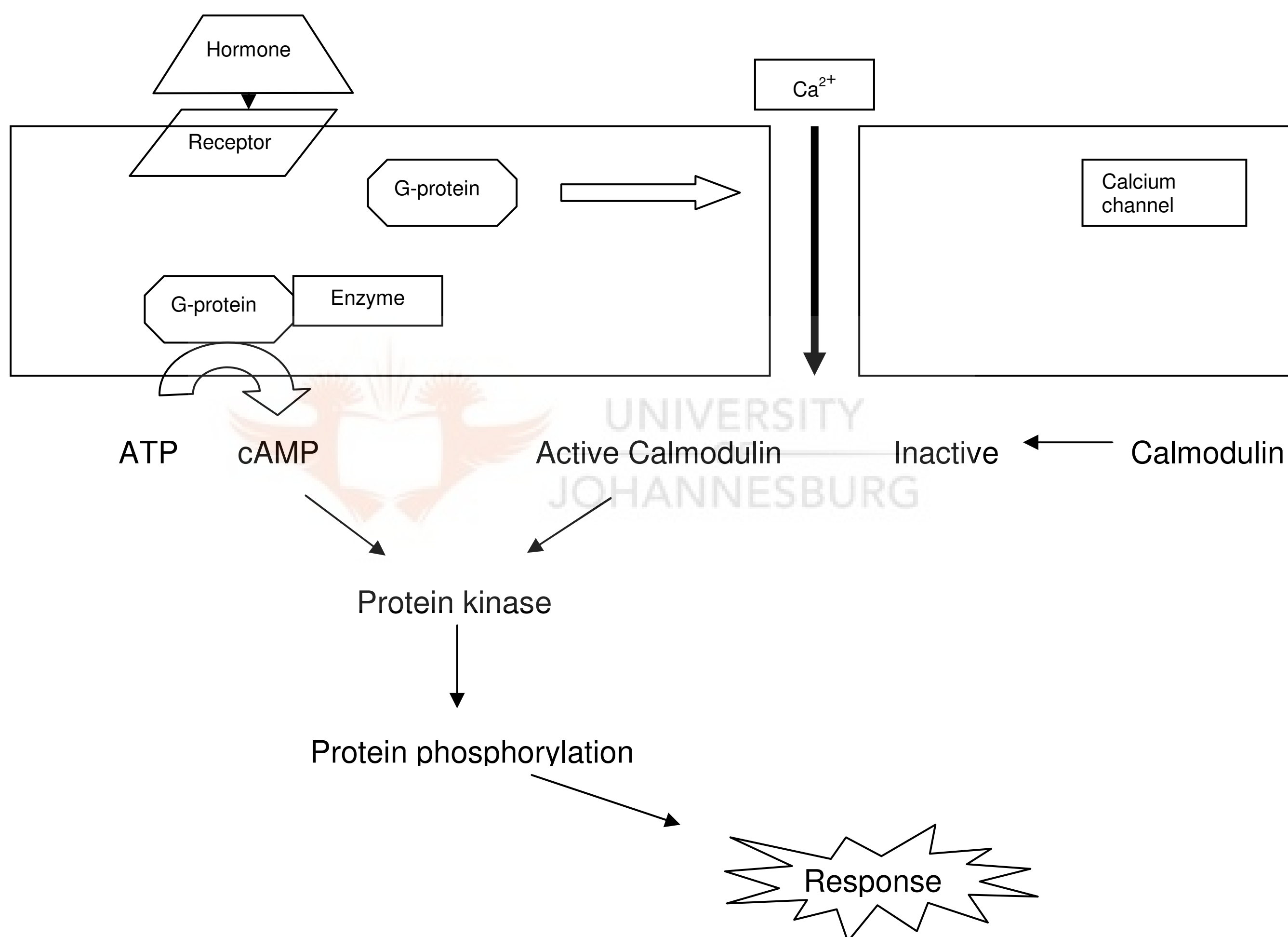

Figure 2.5: A model representing the activation of calcium channels through the binding of G-proteins to a receptor in the plasma membrane. Adapted from Hopkins, 1999.

dependent ATPase (Hopkins, 1999). Ca^{2+} plays a role in signal transduction and is transported between different cell organelles through ion channels using ATP-hydrolysing pumps and Ca^{2+} exchangers (Thuleau *et al*., 1998). For the process of Ca^{2+}

efflux it needs calmodulin, a binding agent; Ca^{2+}-dependent protein kinase/phosphatase or Ca^{2+}-activated phospholipases (Blumwald *et al.*, 1998). Proton translocation has similar roles as Ca^{2+} inhibitors that prevent the increase of cytosolic Ca^{2+} concentration thus inducing the HR (Scheel, 1998).

Abscisic acid (ABA) is a phytohormone responsible for responses in leaves during water stress and synthesis of storage proteins (Hopkins, 1999). ABA causes a hyperpolarisation in the guard cells by stimulating ROS, leading to an influx of Ca^{2+} and increased amount of cytosolic Ca^{2+} concentration. If ROS are inhibited, closing of stomata is also inhibited by ABA (Pei *et al.*, 2000).

Protein phosphorylation uses receptors with a kinase domain or Ca^{2+} to trigger protein kinases. The function of H^+-ATPase in the plasma membrane is to promote cell growth. Thus when an elicitor is introduced to the host it may or may not activate the plasma membrane H^+-ATPase (Blumwald*, et al.*, 1998). In the case of *F. graminearum* (which is a necrotrophic fungus) the elicitor stimulates the activation of the plasma membrane H^+-ATPase, thus stimulating cell death to obtain nutrients (Wevelsiep *et al.*, 1993)

3.7.3 Phytoalexins

Phytoalexins are phenolic compounds produced by plants that are toxic to fungi and bacteria. They are produced inside the plant upon infection or mechanical injury (Agrios, 1988). These compounds that are produced in the plant are toxic to bacteria and fungi thus limiting pathogen growth (Hopkins, 1999). Phytoalexins are recognised by elicitors and they form part of the chemical and biochemical defence response in plants (Agrios, 1988). Phytoalexin production is stimulated by ROS, where O_2^- signals the event (Jabs *et al.*, 1997). There is also a close relation between phytoalexins and O-methyltransferases (OMT's). Both these compounds are involved in the increase in plant responses towards pathogens (Gregersen *et al.*, 1997).

3.7.4 The role of plant hormones

The response of a plant to a pathogen relates to certain plant-hormone pathways that could regulate genes. These hormones include salicylic acid, jasmonate and ethylene (Delaney *et al*., 1994).

3.7.4.1 Salicylic Acid

Salicylic acid (SA) is an antimicrobial substance that is released in high concentration around the infected area. SA is produced through the phenylpropanoid pathway (Hammond-Kosack and Jones, 1996). The role of salicylic acid was thought to suppress the damage caused by pathogens by stimulating H_2O_2 accumulation and papillae formation; however, SA is independent from these processes. Another role would be to activate the defence response in gene-for-gene interactions (Delaney *et al*, 1994). Another important role of salicylic acid is that it activates the systemic acquired resistance (SAR) (Ryals *et al*., 1994).

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The SAR is when the defence genes are activated at the site of infection and in tissue distant from the original infection site. SAR is initiated by the HR when lesions are formed and the plant elicits a response (Ryals *et al*., 1994). Because SAR is a quantitative resistance, it protects the host against a variety of pathogens (Dangl *et al*., 1996). SAR can be divided into two phases, namely the initiating and maintenance phase (Figure 2.6). In the first phase, cells from the specific infection site release certain molecules, such as SA, into the phloem. The signal is carried to other target cells in other parts of the plant in order to raise the levels of resistance. The second phase is where the plant will undergo maintenance for a period of time to increase its resistance against pathogens (Slater *et al*., 2004).

3.7.4.2 Jasmonate and ethylene

Systemic induced resistance (SIR) is another disease resistance mechanism, which uses the jasmonate and ethylene pathways as signals to induce certain defence proteins (Slater *et al*., 2004). Jasmonic acid (JA) is a plant growth regulator and can be involved in the induction of wound healing, fruit ripening, senescence and growth inhibition (Hopkins, 1999). Jasmonate and its counterpart methyl jasmonate (MeJA) are involved in plant defence. If a pathogen enters via a wound, JA and MeJA accumulate in the infected areas and wounding response genes (e.g. proteinase inhibitors and calcone) are induced (Baron and Zambryski, 1995).

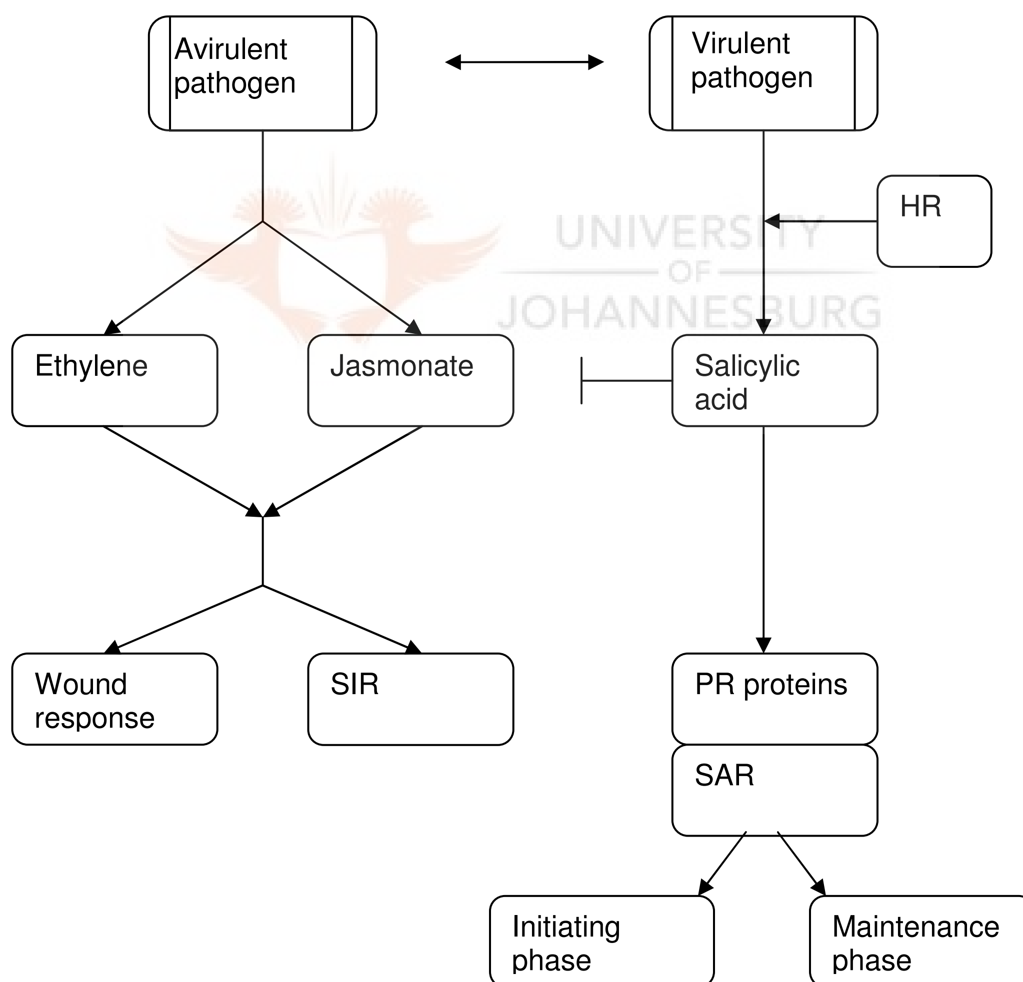

Figure 2.6: The different hormonal pathways in a disease response. Adapted from Slater *et al*., 2004.

Ethylene is an ubiquitous hormone involved in seed germination and root hair development (Hopkins, 1999). It also can be involved in resistance or inhibition of symptoms after infection or in other cases increase cell death. The role of ethylene can vary depending on the plant and pathogen (Wang *et al*., 2002).

3.7.5 Pathogenesis-related proteins

With the activation of the HR, several defence proteins are expressed. These defence proteins can be divided into three groups. The first group has a structural function such as hydroxyproline-rich glycoproteins (HRGP's), glycine-rich proteins (GRP's) and other enzymes that play a role in lignin and callose biosynthesis. The second group has an antimicrobial function. Some of these functions include enzyme inhibitors, toxic proteins and hydrolases. The third group of defence proteins are the PR proteins (Table 2.3) (Stintzi *et al*., 1993). In order for these proteins to work, two or more proteins are needed to provide resistance against the pathogen. PR proteins are located in the vacuole and their chemical structure is designed to withstand the acidic environment (Hammond-Kosack and Jones, 1996; Stintzi *et al*, 1993). The first PR protein that was identified was from tobacco plants, namely the PR-1a protein containing an αβα sandwich with high thermal stability. Most of the identified PR-proteins have destructive effects on the pathogen. PR-1 and PR-5 proteins interact with the plasma membrane; proteins from the PR-2 (β-1, 3 glucanase), PR-3, PR-4, PR-8 and PR-11 (chitinase) families interact with fungal cell walls (Fritig *et al*., 1998).

Table 2.3: Pathogenesis-Related (PR) Proteins. Adapted from http://dmd.nihs.go.jp/latex/defense-e.html and http://www.bio.uu.nl/ ~fytopath /PR-families.html. Retrieved on 16/11/2006

Family	Type member	Properties	Gene Symbol	Reference
PR-1	Tobacco PR-1a	Antifungal	*Ypr1*	Antoniw *et al*., 1980
PR-2	Tobacco PR-2	Class 1, 2, and 3 endo-beta-1, 3-glucanases	*Ypr2,[Gns2 ('Glb")*	Antoniw *et al*., 1980
PR-3	Tobacco P, Q	Class 1, 2, 4, 5, 6, and 7 endochitinases	*Ypr3, Chia*	Slusarenko *et al*., 2000
PR-4	Tobacco R	Antifungal, win-like protein, endochitinase type 1, 2 activity, similar to prohevein C-terminal domain	*Ypr4, Chid*	Slusarenko *et al*., 2000
PR-5	Tobacco S	Antifungal, thaumatin-like proteins, osmotins, zeamatins, permeatins, similar to alpha-amylase / trypsin inhibitors	*Ypr5*	Slusarenko *et al*., 2000
PR-6	Tomato inhibitor I	Protease inhibitors	*Ypr6, Pis ('Pin')*	Green and Ryan, 1972
PR-7	Tomato P_{69}	Endoproteases	*Ypr7*	Vera and Conejero, 1988
PR-8	Cucumber chitinase	Class 3 chitinases chitinase / lysozyme	*Ypr8, Chib*	Métraux *et al*., 1988
PR-9	Lignin-forming peroxidase	Peroxidases, peroxidase-like proteins	*Ypr9, Prx*	Lagrimini *et al*., 1987
PR-10	Parsley PR-1	Ribonucleases-like, Bet v 1-related proteins	*Ypr10*	Somssich *et al*., 1986
PR-11	Tobacco 'class 5' chitinase	Endochitinase type 1 activity	*Ypr11, Chic*	Melchers *et al*., 1994
PR-12	Radish Rs-AFP3	Plant defensins	*Ypr12*	Terras *et al*., 1992

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Family	Type member	Properties	Gene Symbol	Reference
PR-13	Arabidopsis TH12.1	Thionins	*Ypr13, Thi*	Epple *et al*., 1995
PR-14	Barley LTP4	Non-specific lipid transfer protein (ns-LTPs)	*Ypr 14, Ltp*	Garcia-Olmedo *et al*., 1995
PR-15	Barley OxOa (germin)	Oxalate oxidase	*Ypr15*	Zhang *et al*., 1995
PR-16	Barley OXOLP	Oxalate-oxidase-like protein	*Ypr16*	Wei *et al*., 1998
PR-17	Tobacco PRp27	Unknown	*Ypr17*	Okushima *et al*., 2000

4. Plant-pathogen interaction

This interaction between the plant and the pathogen can be divided into two groups. A compatible interaction occurs when the plant shows susceptibility to the pathogen. An incompatible interaction occurs when the plant shows resistance to the pathogen. This incompatible interaction is usually associated with necrotic lesions at the infected area (Keen, 1990). Sometimes diseases are rare although plants are exposed to pathogens. This could be due to the plant not providing enough nutrients for the pathogen to survive and it can thus be considered a non-host. Furthermore, the pathogen is unable to spread further due to the defence mechanisms or the plant contains structural barriers and toxins, preventing successful infection (Hammond-Kosack and Jones, 1996). A good model system to compare the interaction between barley and *F. graminearum* is the interaction between barley and the pathogen *Blumeria graminis*.

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4.1 The interaction between barley and *Blumeria graminis*

Blumeria graminis f.sp. *hordei* is an ascomycete fungus, causing powdery mildew disease on several cereals, including barley, wheat and oats. (Spencer, 1978). It is an obligate pathogen and multiplies mainly by the production of asexual conidia, forming colonies on the green leaves (Chaure *et al*., 2000). After conidia germination, haustoria will form, from which the mycelia develop. This stage takes approximately 6 - 10 days. Other plants can be infected with conidia through wind and water dispersal. During the sexual stage, the fungus exists as a diploid, afterwards releasing haploid ascospores, but for most of its life cycle, the fungus is haploid (Jørgensen, 1994).

The conidia of *B. graminis* can form two germ tubes, namely the primary germ tubes (PGT) and the appressorial germ tubes (AGT) (Clark *et al*., 1995). Both usually form close to the leaf surface and swell up along the contact areas of the leaf. The function of the PGT is to attach itself to the host, retrieving water to produce the secondary germ tubes (Carver *et al*., 1995). The PGT penetrates the wax and cuticle layers of the

epidermal cells. If the PGT does not come into contact with the leaf surface, the PGT is suppressed and the AGT takes over the function (Clark *et al*., 1995; Wright *et al*., 2000). After the appressorial tube has differentiated, a penetration peg forms to penetrate through the cell wall by using mechanical pressure (Carver *et al*., 1995). When the fungus is successful, it will form a haustorium, developing into a haustorium body with finger-like structures within the infected epidermal cells. Secondary hyphae develop from these structures, spreading across the surface to produce more hyphae (Figure 2.7) (Ellingboe, 1972). Once the pathogen is in the epidermis or mesophyll, signal transductions are activated in the fungus by the plant to inhibit further growth. There are different types of resistance in barley towards *B. graminis*. The first resistance constitutes an external barrier, namely papilla formation. Papillae consist of complex molecules such as callose, proteins and carbohydrates (Jørgensen, 1994). An example of papilla formation for defence can be seen in the interaction between *B. graminis* with barley (Hückelhoven *et al.,* 1999).

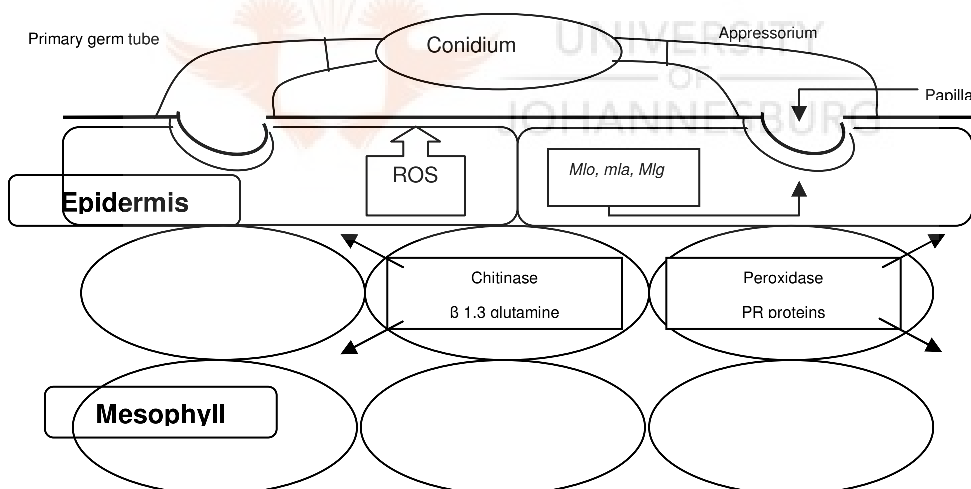

Figure 2.7: The interaction between *Blumeria graminis* and barley. Adapted from Gregersen *et al*., 1997

Papillae will form underneath the cuticle epidermal layers to prevent further penetration. Further responses such as a high production of PR proteins, chitinase and H_2O_2 production occur in the epidermal and mesophyll cells (Gregersen *et al*., 1997). The second type of resistance is race specific and non-race specific. Race specific

resistance contains the largest group of *R* genes, namely the Ml-type. A large number of *R* genes are located at or near the *Mla* locus, found on chromosome five. This group consists of 32 different *Mla* variants (Table 2.4). One of the main functions of *Mla* is to stop fungal growth after penetration and haustorium formation. *Mla* is thus dependent on the HR to fulfil this function (Jørgensen, 1994; Hückelhoven and Kogel, 1998). According to Halterman *et al*. (2001), *Mla* genes are expressed after the pathogen has made direct contact with the host membrane. *Rar* 1 and 2 are genes that are needed for *Mla* specific resistance in pathogens (Hückelhoven and Kogel, 2003).

Table 2.4: Barley resistance genes from the Ml group that shows resistance towards *B. graminis*. Adapted from Jørgensen, 1994.

Locus	Gene	Chromosome	Resistance	Function
Mla	Mla_{1-32}	5	Race-specific	Arrests fungal growth after haustorium penetration. Some requires *Rar 1* and *Rar 2*.
Mlg	Mlg	4	Race-specific	Arrests fungal growth after haustorium penetration. Independent of *Rar* 1 and *Ra*r 2.
Mlo	Mlo_{1-25}	4	Non-race-specific	Suppress HR and attacked cells survive. Independent of *Rar 1* and *Rar* 2, but require *Ror* 1 and *Ro*r 2.

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Non-race specific resistance is conferred by the *Mlo* gene (*mlo*). This resistance is unique because it does not support the gene-for-gene interaction (Jørgensen, 1994). The *mlo* resistance genes are recessive and reside on the long arm of chromosome 4. There are approximately 160 mutagen-induced *mlo* resistant mutants, where at least 25 are independent (Table 2.4) (Jørgensen, 1994).

Suggestions that *mlo* suppresses the activation of the defence response have been made, since wild type Mlo suppresses H_2O_2 production in epidermal and mesophyll cells (Piffanelli *et al*., 2002). The *mlo* resistance requires at least two additional genes, namely *Ror 1* and *Ror 2*. These genes assist the *mlo* in the response; however, these

genes are not required for race-specific resistance (Freialdenhoven *et al*., 1996). MLO forms part of a family of 7 transmembrane (7-TM) proteins (Devoto *et al*., 1999). MLO functions independently of heterotrimeric G-proteins and Ca^{2+} interactions with calmodulin to suppress the defence response against *B. graminis* (Kim *et al*., 2002).

4.2 The interaction between barley and *F. graminearum*

F. graminearum produces a type B trichothecene mycotoxin, deoxynivalenol (DON), nivalenol (NIV) and zearalenone in infected grains (Parry *et al*., 1995). Mycotoxins are low molecular weight fungal metabolites, toxic to animals and humans (Desjardins and Hohn, 1997). Trichothecenes are a large group of toxins that inhibit protein biosynthesis and could play a role in the aggressiveness of infection (Desjardins *et al*., 1993; Mesterházy, 2002). Trichothecene biosynthesis genes are clustered. These clusters include genes such as P450 oxygenase (*Tri11*) (Alexander *et al*., 1998), *Tri5*, responsible for the first step in the biosynthesis pathway (Lee *et al*., 2002), transcription factors (*Tri6* and *Tri10*) (Proctor *et al*., 1995; Tag *et al*., 2001), a toxin efflux pump (*Tri12*) (Alexander *et al*., 1999) and an acetyltransferase gene (*Tri101*) that encodes trichothecene 3-O-acetyltransferase to protect the pathogen from its own toxins (Kimura *et al*., 1998; 2003). *Tri13* was determined to be responsible for the DON-NIV switching in *Gibberella zeae* where DON instead of NIV was present in the host after deletion of *Tri13* in the pathogen. *Tri7* is responsible for further modifications in NIV (Lee *et al*., 2002). These toxins appear to vary in different geological areas, where only DON was found in North America (Abbas *et al*., 1989). DON and NIV were detected on continents such as Africa, Asia and Europe (Desjardins *et al*., 2000).

DON causes starch and protein degradation in the infected grains. A loss in barley production during brewing occurs when kernels containing detectable levels of DON are rejected through measuring the harvested seeds (Bai and Shaner, 2004). Although most of the toxins are washed away, *F. graminearum* can still re-grow saprophytically by means of mycelium or thick-walled resting spores in residues and cause

unacceptable amounts of DON in the malt and beer (Schwarz *et al*., 1995; Bai and Shaner, 2004). This is also manifested as an uncontrolled foaming known as gushing (Parry *et al*., 1995).

In barley, the *Vrs1* locus determines the spike type, namely two-rowed or six-rowed. This locus is associated with the QTL responsible for FHB resistance. It is also thought that two-row barley is more resistant than six-row barley (Mesfin *et al*., 2003). A possible reason for this could be because the two-row spikes dry faster, making it difficult for the pathogen to spread (Chelkowski *et al*., 2000). Six-row barley is used for malting and a popular breeding parent but is more susceptible to FHB. To date the most resistant two-row barley identified is CIho4196 (Bai and Shaner, 2004).

4.3 Host resistance

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Host resistance can be divided into non-host resistance and race-specific resistance. Non-host resistance is where a plant is resistant to a majority of pathogens. Race-specific (host) resistance is where the plant contains an *R* gene to a specific pathogen carrying a corresponding avirulence (*Avr*) gene (Heath, 2000). This resistance to a pathogen usually induces the HR, which manifests as PCD (Dangl *et al*., 1996).

Molecular markers can be used to determine the amount of genes involved in the host-pathogen interaction, the morphology and development of quantitative trait loci (QTL), where quantitative resistance are involved (Zhu *et al*., 1999). Quantitative resistance means that many genes are involved to stimulate certain reactions in the plant and pathogen. These markers have been used to locate chromosomal locations of genes in crop cereals (Table 2.5) (Paterson *et al*., 1995).

Plant architecture and development such as plant height and late heading are associated with FHB and KD resistance (Mesterházy, 2002; Buerstmayr *et al*., 2002). According to Urrea *et al*. (2002), certain factors such as plant height, spike density and

the angle of the spikes are negatively correlated to FHB severity. FHB severity and DON concentrations in barley are influenced during the heading stage and maturity at any time (Prom *et al.*, 1999). Plant height influences the FHB resistance and DON concentrations. Tall plants are more resistant to FHB and contain less DON than smaller plants. The grains at heading stage can either be high or low in DON concentration, depending on the conditions and inoculation (de la Pena *et al.*, 1999).

Certain methods have been suggested to reduce FHB severity in cereals. Methods such as crop rotation, chemical control and most important, genetic resistance have been used. Partial resistance have been identified in between 40 - 100 cultivars (Steffenson, 2003; Urrea *et al.*, 2002).

There are different types of resistance in wheat and barley. They are determined according to their morphological or physiological trait (Rudd *et al.*, 2001). Type I resistance indicates resistance to the initial infection and Type II resistance to the spread of blight symptoms in the spike. Type I resistance is more important in barley since FHB does not spread initially from the infection site. Thus, Type II resistance is less important during the occurrence of FHB on barley (Schroeder and Christensen, 1963; Rudd *et al.*, 2001). Other types of identified resistance are kernel infection (Type III resistance), tolerance (Type IV resistance) and decomposition or non-accumulation of mycotoxins (Type V resistance) (Mesterházy, 2002). Different inoculation methods were used to distinguish between the different types of resistance. Type I resistance was determined by spraying with a spore suspension over flowering spikelets. Type II resistance was determined by delivering conidia into a single floret of a spikelet (Bai and Shaner, 2004). Type III resistance was measured by monitoring the damaged kernels. Type IV resistance was measured by comparing the grain yield of inoculated and non-inoculated spikes. Type V resistance was determined by measuring the DON concentration at a certain level of infection (Rudd *et al.*, 2001). The timing of inoculation and experimental location could also influence the resistance type (Bai and Shaner, 2004).

Table 2.5: QTL functions on different chromosomes of wheat and barley.

Chromosome	*Source*	*Function*	*Reference*
1	Barley	Type I resistance	Zhu *et al*., 1999
		Inflorescence density	Zhu *et al*., 1999
		FHB resistance	de la Pena *et al*., 1999; Dahleen *et al*., 2003
		Lower DON concentration	de la Pena *et al*., 1999; Ma *et al*., 2000
		KD resistance	de la Pena *et al*., 1999
		FHB severity (Type III)	de la Pena *et al*., 1999
		Nodes per cm of rachis	Ma *et al*., 2000
2	Barley	Type II resistance	Zhu *et al*., 1999
		Seeds per inflorescence	Zhu *et al*., 1999
		Lateral floret size	Zhu *et al*., 1999; de la Pena *et al*., 1999; Ma *et al*., 2000
		FHB resistance	Dahleen *et al*., 2003; Mesfin *et al*., 2003; Canci *et al*., 2004
		DON accumulation	de la Pena *et al*., 1999; Zhu *et al*., 1999
		KD resistance	Ma *et al*., 2000; Canci *et al*., 2004; de la Pena *et al*., 1999
		Plant height	Ma *et al*., 2000; Dahleen *et al*., 2003
		Late heading	Ma *et al*., 2000; Dahleen *et al*., 2003
		Lower FHB severity, spike angle and nodes per cm of rachis	Ma *et al*., 2000
3	Barley	Type I resistance, poor peaks for inflorescence density, lateral	Canci *et al*., 2004

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		florets	
		Late heading date	Ma *et al*., 2000; Dahleen *et al*., 2003
		Plant height	de la Pena *et al*., 1999; Zhu *et al*., 1999; Ma *et al*., 2000; Dahleen *et al*., 2003
		FHB resistance	de la Pena *et al*., 1999; Ma *et al*., 2000; Zhou *et al*., 2002; Canci *et al*., 2004
		DON concentration	de la Pena *et al*., 1999; Ma *et al*., 2000; Canci *et al*., 2004
		Lower FHB severity, nodes per cm of inflorescence	Ma *et al*., 2000
3	Wheat	FHB resistance	Yang *et al*., 2005
4	Barley	FHB resistance	de la Pena *et al*., 1999; Zhu *et al*., 1999;
		Plant height	Zhu *et al*., 1999; Ma *et al*., 2000
		KD resistance	de la Pena *et al*., 1999; Canci *et al*., 2004
		Seed per inflorescence lateral floret size	Zhu *et al*., 1999
		Nodes per cm of inflorescence	Ma *et al*., 2000
5	Barley	FHB resistance	de la Pena *et al*., 1999; Ma *et al*., 2000; Dahleen *et al*., 2003
		DON concentration, KD resistance	Ma *et al*., 2000
		Lower FHB severity, plant height, late heading	Ma *et al*., 2000
6	Barley	FHB resistance, DON	Ma *et al*., 2000; Dahleen *et al*., 2003; Canci *et al*.,

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		concentration	2004
		KD resistance	de la Pena *et al*., 1999; Canci *et al*., 2004
		Late heading date	Dahleen *et al*., 2003
		Lower FHB severity, kernel plumpness, plant height	Ma *et al*., 2000
7	Barley	FHB resistance	de la Pena *et al*., 1999; Ma *et al*., 2000
		DON accumulation	de la Pena *et al*., 1999; Ma *et al*., 2000; Canci *et al*., 2004
		KD resistance	de la Pena *et al*., 1999; Canci *et al*., 2004
		Plant height	de la Pena *et al*., 1999; Ma *et al*., 2000
		Lower FHB severity, nodes per cm of inflorescence, late heading date	Ma *et al*., 2000

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It has been shown that the cereal genome has a high level of synteny. This means that the order and position of certain genes are conserved from one species to another. FHB resistance was identified in barley and wheat on chromosomes 3 – 5. Chromosome 3 exhibited major effects in FHB resistance in wheat (Zhou *et al.*, 2002; Yang *et al.*, 2005). This resistance exhibited conservation of synteny between wheat and barley (Kolb *et al.*, 2001). Synteny is important or useful to compare and align genomes from other species such as cereals (Slater *et al.*, 2004). There are a number of comparative loci shared between rice and barley chromosomes that show potential synteny. Some of these include rice chromosome 1 to barley chromosome 3, rice chromosome 5 and barley chromosome 5 (Maroof *et al.*, 1996). According to Table 2.5 these chromosomes carry different genes that could be related to a defence response. Because wheat and barley also share homology in their genomes, this can also be compared to the rice genome. Chromosome 3 of wheat and barley showed homology with chromosome 1 of rice (Maroof *et al.*, 1996) Because of the homology between rice, wheat and barley, rice can be used as a model plant to study the interaction between pathogens and cereals. To study specific interactions (e.g. *F. graminearum*) it will be better to use barley as a model system as its relation to wheat is closer than rice.

5. Conclusion

The study of pathogens and their interaction with host plants are important. A variety of micro-organisms (e.g. bacteria, viruses, fungi and nematodes) are constantly exploiting plants (Hammond-Kosack and Jones, 1996). Barley is no exception and various pathogens attack it. A plant-pathogen interaction can be divided into two groups. A compatible interaction occurs when the plant shows susceptibility to the pathogen. An incompatible interaction occurs when the plant shows resistance to the pathogen. This incompatible interaction is usually associated with necrotic lesions at the infected area (Keen, 1990). There are many ways for a plant to defend itself against pathogens such as external barriers (formation of papillae) and the activation of internal signals (signal transduction). The host contains *R* genes encoding products, which recognise the

corresponding products, encoded by the *Avr* genes from the pathogen. This interaction leads to a defence response. The pathogen in return also contains defence mechanisms such as the production of toxins and secretion of enzymes (Lamb *et al.*, 1989)