

Fighting Wheat Stem Rust: Pathogenesis-Related Genes, From Conventional To Modern Approaches

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Abstract

Stem rust is a major threat to wheat worldwide. It causes damage to almost 85% wheat varieties and may reduce the annual yield up to 20%. It is caused by a pathogenic fungus *Puccinia graminis*. Many efforts are being carried out to eliminate this deadly disease but not successful due to less understood of disease mechanism at molecular level. Number of genes have been studied which are responsible for the disease but their interactions are not well known. In this review article, conventional and modern technologies to combat the stem rust pathogen are compared, and innovative approaches that make wheat resistant against evolving deadly strains of the pathogen are discussed.

Keywords: Stem rust, *Triticum aestivum*, Ug99, *Puccinia graminis*, PR genes, molecular approaches

Introduction

Wheat (*Triticum aestivum* L.) is a staple food that meets almost 50% of the total needs of cereal consumers worldwide (Abdul *et al.*, 2015). It contains proteins and carbohydrates and provides approximately 72% of the calories in diets (Guzman *et al.*, 2016). It is cultivated throughout the world; however, the yield varies due to many factors, including biotic and abiotic factors and the unavailability of modern technologies and breeding resources that are required to fight epidemic diseases (Nuttall *et al.*, 2017). One of the major causes of poor yield is stem rust. Stem rust is responsible for loss in grain yields up to 100% in susceptible wheat varieties and reduces the quality of the grain (Mwando *et al.*, 2012). Stem rust may occur randomly, but it destroys the entire crop once attacked (Afzal *et al.*, 2016).

Puccinia graminis is a fungus that causes stem rust. It belongs to the order Pucciniales (Patpour *et al.*, 2016). It is a complex and diverse family of pathogens incorporating severe disease symptoms in crop plants. In 1998, a new race, *Puccinia graminis* f. sp. *tritici*, commonly known as Ug99, was identified as rapidly expanding and destroying cereal crops in Uganda (Ellis *et al.*, 2014). Ug99 starts its life cycle on wheat just after the exposure of spores on wheat plants. These spores are called primary inoculum. The primary or first spore infecting young plants in the early stages is the urediniospore that comes from infected plants grown in previous seasons. Thus, the infected wheat plants serve as a link connecting the generations, which carries *Puccinia graminis* f. sp. from the harvesting season to the next sown crop (Rodriguez-Algaba *et al.*, 2014).

DEFENSE MECHANISMS AGAINST PUCGINIA GRAMINIS

Wheat cells contain different organelles that are the main targets of *Puccinia graminis*. Fungi cannot synthesize their own food so they develop the ability to live on host organisms and use their manufactured food for survival. *Puccinia graminis* develops a long-term relationship by feeding on the host and thus is called a biotroph. This species absorbs food and nursery molecules from host plants (Olori-Great and Opara, 2017). Wheat is a major host of *Puccinia graminis*, but before infecting a wheat plant, there is a complex mechanism of interaction or fight between the fungus and host plants. Wheat plants fight to protect their cells and tissues, while the fungus fights for its survival. Wheat has its defense mechanism to prevent complex disease, which consists of three steps. The three steps of defense in plants are usually called the three branches of the innate immune system. As soon as pathogens attack plants, they recognize them as non-self-particles due to the presence of specific cell wall components that are specific only to the microbes that may or may not be pathogens. If the pathogens obtain entry into the inner side of the plant cells and escape the first line of defense, plants respond to the pathogens by secreting pathogen virulence effector proteins that either suppress pathogen growth or kill them directly. If this second step fails to target pathogens, defensive genes are activated as the third line of defense (Garcion *et al.*, 2014).

PHYSICAL BARRIERS

The cuticle layer or waxy layer, the cell wall and the stomata act as physical barriers for fungal attack. Closed stomata also inhibit the entry of fungal spores inside the plants (Micali *et al.*, 2011). These physical barriers protect wheat against fungal diseases. This is the first line of defense against fungi. When the uredospore falls on the surface of the wheat plant, it starts germinating toward the stomata. This germination movement is in the shape of a tube called the germ tube on the surface of the wheat leaf. On reaching a stoma, its tip swells and forms a vesicle-like structure called the appressorium. The protoplasm of the germ tube migrates into the appressorium that is separated by the septum from the germ tube, producing a narrow hypha. It enters inside the sub-stomatal chamber through the stoma. The tip of the hypha again swells and forms a sub-stomatal vesicle. The contents of the appressorium migrate to the vesicle through the hypha. It develops branches and produces hyphae, which spread between the cells (Leonard and Szabo, 2005).

Puccinia graminis must break the first step of defense to come closer to its host. If we could control the stomatal opening and closing, then resistance could be achieved. *SNAC1* and *OsSRO1c* can be engineered to control the closing of the stomata. Resistance can also be achieved by excreting chitinase, which helps to destroy hypha (You *et al.*, 2013). Another path for fungal entry in the plant is via an injury or pre-existing openings on the plant surface. In this situation, the plant must resort to a different set of defense mechanisms, such as toxins and enzymes (Micali *et al.*, 2011).

PLANT IMMUNE SYSTEM

The second step of defense is the plant's immune system. The plant immune system is different from that of the mammalian, but both share a basic function to recognize and differentiate the cells and molecules of the invading pathogens and their own. This ability to differentiate their own molecules from pathogens is the first step of immune defense. Interaction with conserved molecules or structures accessible to pathogen cells prompts the first level of inducible defenses. Such eliciting molecules are referred as PAMPs or MAMPs (pathogen- or microbe-associated molecular patterns) (Doehlemann and Hemetsberger, 2013; De Wit, 2015). PAMPs work when a plant recognizes a molecule or any activity belonging to a fungus-triggered immune system. This triggered immune system is known as PAMP-triggered immunity (PTI). Different patterns are activated in response to different molecules.

Reactive oxygen species

The first weapon used by plants is the reactive oxygen species (ROS). Several roles of ROS in plant defense have been proposed. ROS destroy any

molecule that comes in contact with them. ROS are used as signaling molecules by wheat (Mittler *et al.*, 2011). A fungus may avoid this situation by neutralizing the ROS with the help of the *Yap1* gene (Torres, 2010; Lehmann *et al.*, 2015).

Modifications in the cell wall

One of the most important responses of PAMPs is the reinforcement of cell wall where the fungus has attacked. The cross-linking of components of the cell wall is ensured by H₂O₂ connecting glycoproteins, lignin and suberin (Almagro *et al.*, 2009).

Pathways in signal transduction

Different plant hormone signaling pathways are regulated by ROS. However, induction of salicylic acid (SA) is one of the best defense signaling pathways, with a well characterized role of regulation through oxidation events and their responses for two main key regulators, TGA transcription factors and NPR1 as a receptor of SA (Fu and Dong, 2013; Shi *et al.*, 2012). SA accumulation occurs on pathogen attack, thus altering the redox state of the cell (Tada *et al.*, 2008). Interestingly, ROS accumulation is not always mediated by induction of SA-dependent defense feedback.

Programmed cell death

During fungal attack, programmed cell death (PCD) is a resistance mechanism. PCD in plants is well characterized, and many molecular cascades have been described to date, including ROS, Ca²⁺, nitric oxide (NO) and endoplasmic reticulum (Williams *et al.*, 2014). In plants, PCD occurs at the time of leaf senescence or photosynthetic reactions and as part of the built-in immune responses initiated by plant-pathogen interactions, termed as the hypersensitive response (HR). In specific plant-pathogen interactions, ROS and NO both participate in a coordinated way in planned HR (Bellin *et al.*, 2013).

Much literature has been reviewed that discusses the roles of RNS (reactive nitrogen species) and ROS, as they not only are crucial players during HR but also participate in a multifaceted crosstalk where they can regulate and interact with each other (Wang *et al.*, 2013). However, depending on the biotic interaction, ROS can be directly involved in PCD regulation after induction or might not be involved at all (Torres, 2010). This highlights the fact that PCD is under enigmatic regulation, where ROS have a significant role. Fungi may avoid this situation by neutralizing the ROS with the help of the *Yap1* gene, which helps to fight the ROS and protects the fungus from severe damage (Lehmann *et al.*, 2015).

Chitinase Activity

Most importantly, PTI is activated by chitin, which is a key molecule in the fungal cell wall. Different PAMPs, such as chitin, the main component of the *Puccinia graminis* cell wall, are well recognized by wheat receptor proteins. These receptors trigger the different defense mechanisms to kill the invading pathogen (Gao *et al.*, 2016). *CERK1* or *CEBiP* genes in wheat are reported as a chitin-induced defense and target the cell wall of the fungus (Lee *et al.*, 2014). The fungus avoids damage by targeting chitinase and producing its inhibitors. The *Avr4* and *Ecp6* genes

help the fungus to defend against attack (Van Esse *et al.*, 2007).

Glucanase Activity

In addition to chitinase, glucanase is also excreted by wheat cells in response to fungal attack and is reported to inhibit fungi with the help of the *GIP1* gene (Ekchaweng *et al.*, 2017). The next level of the defense system is highly specific to specific pathogens (Doehlemann and Hemetsberger, 2013) (Table 1).

Table-1: Genes of wheat and fungus involved in defense mechanism

S. No.	Plant genes activated by PTI	Fungus response (genes)	Stage of Action
1	ROS	<i>Yap1</i>	Apoplast
2	Chitinase	<i>Ecp1, Avr4</i>	Apoplast
3	Glucanase	<i>GIP1, FBI</i>	Apoplast
4	Stomata Closing	<i>SNAC1, OsSRO1c</i>	Apoplast

Jasmonic acid (JA) and Salicylic acid

Some phytohormones are produced in the immune response, such as jasmonic acid (JA) and salicylic acid, which play a key role in signal transduction pathways. SA and JA are activated for different purposes, but their goal is the same: to protect the plant against pathogens (Glazebrook, 2005). SA in response to any contact of the biotrophic pathogen

with the host produces a massive amount of ROS, which induce programmed cell death. Herbivores and necrotic pathogens, by distinction, are proficiently regulated by signaling dependent on JA, resulting in phytoalexins secretion, such as terpenes and flavonoids, which are known to act directly as intruders or toxins (Memelink *et al.*, 2001).

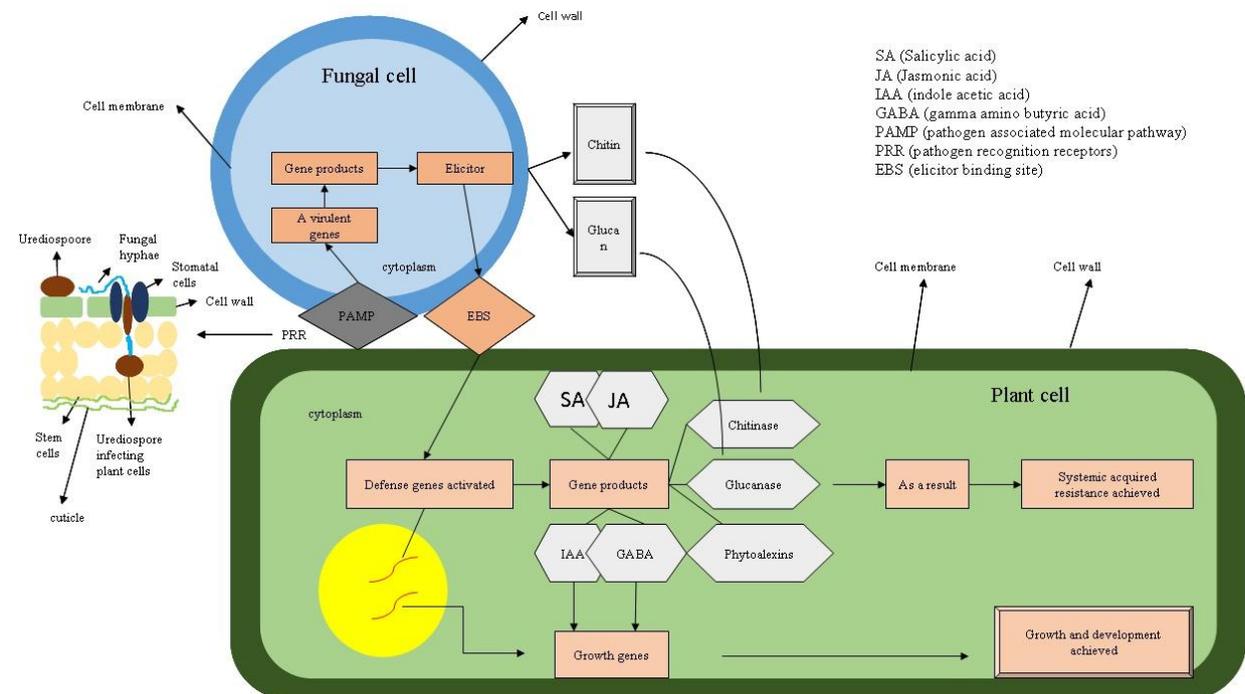


Figure 1: Mechanism of Interaction of Wheat and Fungi

EFFECTOR-TRIGGERED IMMUNITY

When PTI fails to stop the pathogen from invading, the fungus releases proteins that enter the host cell and prepare it for fungal attack (Hewezi and Baum, 2013). These proteins change the physiology and development of host cells. Effectors are an

example of extracellular proteins that perform their function outside the pathogen and in the host. These effectors are highly specific to the pathogen and are important factors to cause disease (Dodds and Rathjen, 2010). Effectors are recognized by the host and activate specific resistance patterns. Wheat has a

specific hypersensitive response (HR) against *Puccinia graminis* with the help of R genes. These R genes cause host cell death when the pathogen comes in contact with the cell. This response is against the biotrophs, so they could not feed on the host for a longer time. HR is the characteristic defense reaction in effector-triggered immunity (ETI), initiated by the direct or indirect recognition of pathogen proteins called effectors with the help of specific R proteins of plants. The specificity of R-gene races is a consequence of the specific interaction between R proteins themselves and highly diverse effectors. This is contrary to the wide-ranging resistance provided by PTI (pattern-triggered immunity) that is induced through the recognition of PAMPs. Unlike effectors, MAMPs are highly conserved between species and races. Although plant immunity has been an interesting field of study, many questions remain unanswered about the molecular mechanisms of resistance, mainly in wheat (Pujol *et al.*, 2016).

R genes and proteins

R genes recognize the pathogens and their molecules and interact with them to detoxify or denature. These genes regulate a wide range of defense responses (DR) (Dmochowska-Boguta *et al.*, 2015). Seven wheat defense-associated genes, including *TaPIE1*, *defensin*, *PR1.2*, *PR2*, *PR10*, *chitinase1* and *chitinase2*, have been reported in response to pathogen attack (Zhu *et al.*, 2017). Stem rust-resistant genes have been studied previously, including *Sr13*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, *Sr27*, *Sr28*, *Sr32*, *Sr33*, *Sr35*, *Sr36*, *Sr37*, *Sr39*, *Sr40*, *Sr42*, *Sr44*, and *SrTmp* (Jin *et al.*, 2007). The effective genes, including *SrTmp*, *Sr22*, *Sr24*, *Sr25*, *Sr26*, and *Sr36*, are dominant in defense responses. The other genes either require a reduction in size in large alien chromosome segments or may lack durability (Jin *et al.*, 2007).

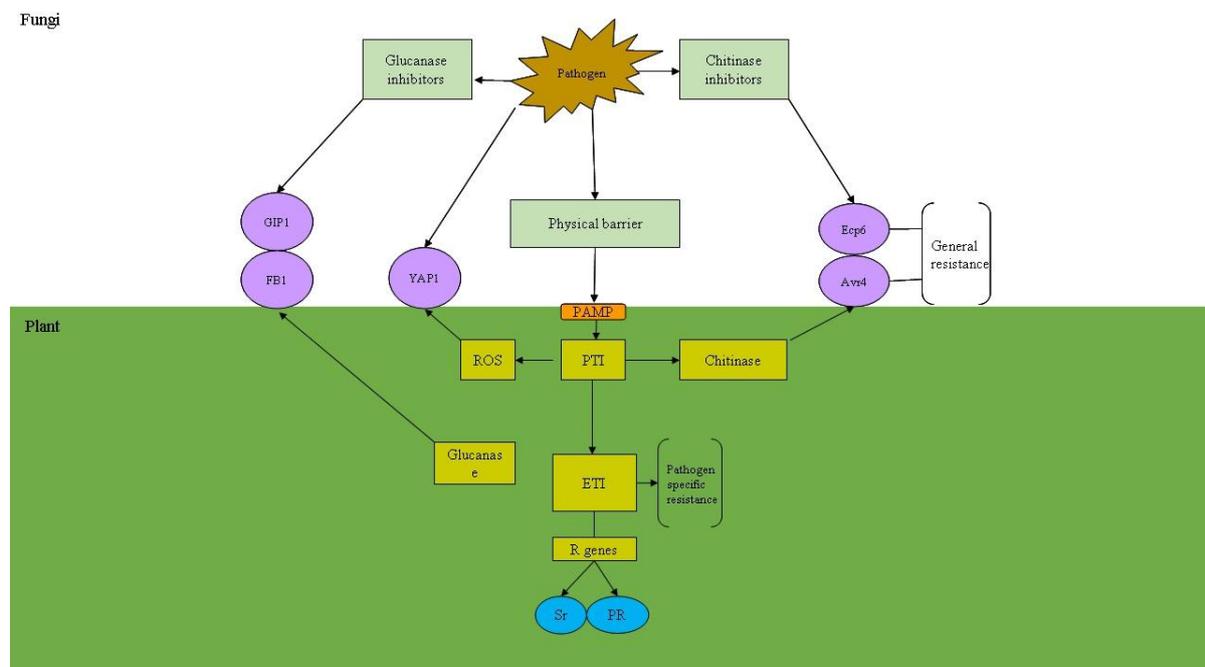


Figure 2: Defense Mechanism of Wheat against *Puccinia graminis*

PLANT DEFENSE RESPONSE

Plants are often threatened by a wide range of pathogens, including bacteria, viruses, fungi, and oomycetes. They have evolved an array of response systems to protect themselves from pathogen attack, in which external cues are deciphered and translated into effective defense feedbacks. RLKs (receptor-like kinases) play vital roles in the recognition of outside stimuli and activate defense-associated signaling pathways, thereby regulating cellular responses to pathogen infection (Antolín-Llovera *et al.*, 2012). Regrettably, new rust races are evolving rapidly, reducing the efficiency of major genes and convincing

breeders to adopt new resistant sources (Singh *et al.*, 2011). In addition, minor gene resistance contributes more in durability and precision because such genes are difficult to identify and transfer into corresponding cultivars (Kolmer, 2013). New resistance sources are usually found in the wild relatives, but transferring them to adapted cultivars is problematic and time consuming.

About sixty (60) stem rust resistant genes are identified in wheat up till now (McIntosh *et al.*, 2006). They tolerate stem rust to different extent and to different strains, i.e. *Sr25* is resistant to *Ug99* strain (Singh *et al.*, 2011). It is widely being used by the CIMMYT wheat breeding programs. *SrWeb* is also

resistance to *Ug99* (Hiebert *et al.*, 2010). *Sr50* along with other genes like *Sr2* confers resistance against the disease.

PATHOGENESIS-RELATED GENES

There are approximately 17 families of pathogenesis-related genes that are activated in plants upon infection by insects or pathogen attack. The first identified family was *PR1*, which was screened in tobacco plants infected by the tobacco mosaic virus in 1970, showing the symptoms of hypersensitive response (van Loon and van Kammen, 1970). This discovery is considered as a hallmark in the plant defense responses showing hypersensitive responses toward pathogens (Buchel and Linthorst, 1999; van Loon and van Strien, 1999; van Loon *et al.*, 2006). Today, many studies have revealed the multigene families that are responsible for encoding proteins, e.g., *PR1* (van Loon *et al.*, 2006; Mitsuhashi *et al.*, 2008; Lu *et al.*, 2011). There are other eukaryotes that are known to contain homologues of pathogenesis-related genes, including humans, animals, insects and fungi. One of the best examples in humans is glioma pathogenesis-related protein (GliPR), associated with cancer development in human cells (Szyperski *et al.*, 1998).

Functional studies of PR genes have enlightened many gene activities that are induced upon invasion, including (i) activation of enzymes involved in oxidative burst, (ii) regulation of enzymes involved in phenylpropanoid pathways, and (iii) triggering of defense-related proteins (Boddu *et al.*, 2007). Similarly, there are many rust-resistant genes found in the wheat cultivars that are concerned with the invading fungus, as the levels of resistance varied by the variation in the structural differentiation of the attacking fungus (Periyannan *et al.*, 2013). Many studies have favored the role of biocontrol agents in imparting resistance to plants by triggering many mechanisms involved in the achievement of systemic resistance, which in turn upregulates the PR genes causing the phytoalexins to accumulate in the infected area (Shoresh *et al.*, 2010).

Pathogenesis-related gene 1 (PR1)

PR1 proteins in plants are categorized as basic or acidic on the basis of their isoelectric points (van Loon and van Strien, 1999). Various plant species, including tomato, tobacco (Bol and Linthorst, 1990) and barley (Bryngelsson *et al.*, 1994), contain both groups of these proteins. However, antifungal activities are possessed by some *PR1* proteins that are basic in nature, e.g., the proteins in tomato named *P14c* and those in tobacco named *PR1g* (Niderman *et al.*, 1995). The activation of *PR1* proteins upon infection and with a hypersensitive response in various tissues of plants also highlights their role in defense-related pathways, but their exact underlying

mechanism is still unknown. There is a conserved *PR1* domain in all *PR1* proteins that is also known as the extracellular SCP-like domain pfam00188. There is a catalytic triad found in human Golgi cells called Golgi-associated pathogenesis-related proteins (*GAPR1*) in which Ser-73 contacts His-72 and Glu-77, forming a dimerized interface (Serrano *et al.*, 2004). There are five assumed active sites in the domain of the *PR1* protein, suggesting their enzyme-related functions. However, the functional roles of individual active sites are not yet validated in *PR1* proteins.

There are approximately 23 types of *PR1* such as genes in the hexaploid wheat genome comprising the three main groups of basic, acidic and basic with an extension of the C-terminus, all of which are upregulated upon attack by pathogens (Lu *et al.*, 2011). The role of PR proteins in wheat-performing biochemical as well as defense-related functions toward pathogenesis was also investigated by in vitro analysis (Lu *et al.*, 2011). The basic *PR1* group was also studied for aspects of characterization and heterologous expression in *PR1-1* and *PR1-5*, also revealing their functions. There are some active sites in the *PR1* domain that also contain conserved sequences for the apoptosis process by caspase-like cysteine proteases. This feature is present in both the basic PR proteins existing as monomers, as well as dimers, being resistant (mainly through dimerization) to proteolytic attack. Many functions are associated with *PR1* proteins such as their antiviral and anti-herbivory nature in tobacco and maize, respectively (Antoniw and White, 1980; Zhang *et al.*, 2015). Moreover, many studies have suggested their role in programmed cell death in affected tissues through protease mediation (Lu *et al.*, 2013). *PR1* genes are also activated when the fungus *U. maydis* infects the maize plant, repressing the signaling of SA in the early stages of intrusion and enhancing auxin production (Doehleemann *et al.*, 2013).

Genes responsible for encoding *PR1* proteins are mainly activated by SA, a defense-related hormone that is also a marker for the plant immune response, providing a wide range of protection against many pathogens. Favorably, there are some MAMPs that trigger the expression of PR genes, especially *PR1*, being ergosterols in nature (Klempner *et al.*, 2014). The stability of tobacco is increased toward pathogenic attack by its basic *PR1* proteins (transgenically expressed) that make it more resistant to fungal species such as *Phytophthora parasitica* and *Peronospora tabacina*. Although the *PR1* genes have been used as markers in many ways after they were discovered approximately 50 years ago, their exact mode of action is still enigmatic (Van Loon *et al.*, 1970). Recently, a novel feature of *PR1* has been identified, binding the sterols of invading fungi (Gamir *et al.*, 2017).

Nevertheless, extracellular or vacuolar locations of different *PR1* proteins make them effective

components of basal defense, directly countering pathogens either in the extracellular spaces or upon disruption of host cellular structures during infection. Given their ability to bind sterols, PR1 proteins can potentially affect plant membranes as well. However, plants are most likely to protect themselves from PR1 auto-toxicity by synthesizing these proteins in a pathogen-inducible manner and/or storing them in the vacuole until they are needed. The PR1 proteins of tobacco have a remarkable role in binding the sterols of invading fungi by sequestering them (especially the auxotroph oomycetes) in their membranes (Gamir *et al.*, 2014).

The immune response of PR proteins is more for oomycetes than for *Botrytis cinerea* and *Aspergillus niger*. Therefore, it can be deduced that the capability of microbes to produce sterols directly correlates with the antifungal effects of these proteins. The localization of PR1 proteins may be extracellular or intracellular depending on the attack either on the surface of the cell or in the intracellular compartment disruption stage during the infection stage. The vacuolar proteins provide a basal defense against various fungi. To protect themselves from the auto-toxicity of the PR proteins, plants have developed a system of producing PRs in vacuoles or in the pathogen-inducible storage manner that is activated only on invasion.

Pathogenesis-related gene 4 (PR-4)/Chitinase

Plant chitinases can obstruct the growth of fungal hyphae by cleaving the chitin of the fungal cell wall (Grover *et al.*, 2012) and are also known to detach chitin oligomers of the fungal cell wall for the recognition of fungal pathogens. These oligomers are actually MAMPs (microbe-associated molecular patterns) recognized by the membranes of host plants at the point of attack to activate the immune response (Kaku *et al.*, 2006). Several defense reactions are triggered by fungal MAMPs comprising oligosaccharides having chitin in them and contributing in many host species, mainly crops, providing them resistance against a broad range of pathogens. Many responses are triggered by chitin only, including the production of PR proteins named TLPs (thaumatin-like proteins), chitinases and proteases, to initiate the phenylpropanoid pathways involved in plant defense (Boller and Felix, 2009). Many other defense responses, such as the synthesis of H₂O₂ and NO (Zhao *et al.*, 2009), phytoalexin accumulation, octadecanoic pathway of abscisic acid, activation of many TGA, deposition of callose and production of PR proteins, are triggered by chitosan and its fragments (Chujo *et al.*, 2007), including hypersensitive responses upon microbial attack.

Chitinases are reported to have antifungal properties in many plant species. They were first screened in *Solanum tuberosum* (Stanford *et al.*, 1989), followed by in other plants (Bravo *et al.*,

2003). The classes were named class I and class II on the presence and absence of the N-terminal cysteine rich domain (sometimes named hevein-like proteins), respectively. Extensive research on class I has revealed that it has a greater affinity to bind chitin and show a strong antifungal response (Odintsova *et al.*, 2007). In any event, class II has been less studied and its functions are also contrary to the former, as its functions vary in accordance with the host species showing divergent biological activities (Bertini *et al.*, 2009). This class is also responsible for various biotic and abiotic stress-induced responses, eventually activating the defense mechanism as well as the regulation of metabolism (Wang *et al.*, 2011).

Pathogenesis-related gene 5 (PR-5; Thaumatin-like proteins)

Thaumatin-like proteins (TLPs) with a molecular weight of approximately 23 kDa are found in plants, nematodes, arthropods and fungi (Petre *et al.*, 2011). TLPs share sequence similarity with thaumatin, a monomeric sweet-taste protein. TLPs cause inhibition of fungal xylanases and sometimes have weak glucanase activities that are otherwise shown by PR-2 (beta-1,3 glucanase) proteins (Fierens *et al.*, 2007). TLPs have cleft-forming domains in their structure that are acidic in nature and cause deprivation of beta-1,3 glucans (glucose polymers) in fungal cell walls (Liu *et al.*, 2010). The expression of TLPs is directly related to fungal attack, so they are called pathogenesis-related type 5 proteins (PR5 proteins) and are mostly studied in plants (Van Loon *et al.*, 2006). However, information about TLPs is increasing.

TLPs are also known to cause autotoxicity in *Lentinula edodes* (a species of edible mushrooms) by degrading its own cell wall (Sakamoto *et al.*, 2006). Moreover, an interesting feature of *Puccinia graminis* (wheat stem rust fungus) is the deletion of the C-terminal peptide depriving their TLPs from their typical acidic cleft to form small TLPs of 16-17 kDa (Petre *et al.*, 2011). Extensive studies of fungal genomes have confirmed the presence of TLPs in basidiomycetes. This information supports the fact that TLPs of fungi provide protection and defense against fungal competitors and pathogens. Similarly, MpTLP1 also encodes a gene for glucan synthesis. Fascinatingly, they are also known to show opposite activity by secreting glucanases (Van Loon *et al.*, 2006).

The genomic studies of defense-coding genes describe the same pathway in all the fungal species involving their secondary metabolism (Dhingra *et al.*, 2013). Stem rust pathogen *Puccinia graminis* also has a large number of small TLPs (Petre *et al.*, 2011). TLXI is the only small TLP characterized from wheat showing xylanase inhibitor activities (Fierens *et al.*, 2007). Moreover, TLXI from wheat is also capable of binding zymosan (a fungal polysaccharide containing

glucan) as well as the β -glucans (Fierens *et al.*, 2007). Plants also produce xylans as the pathogens belonging to GH10 and contribute largely to plant physiology, from the germination of the seed to the ripening of the fruit (Simpson *et al.*, 2002). Simultaneously, plants produce xylanase-inhibiting proteins. Studies have revealed that there are two categories of proteinaceous compounds in cereals, named TAXI and XIP after *Triticum aestivum* xylanase inhibitor and xylanase inhibitor protein, respectively. They are screened and extracted on the basis of their structure, biochemical roles and genetics. TAXI is known to occur in rye (*Secale cereal*), durum wheat (*Triticum durum*), barley (*Hordeum vulgare*) and common wheat (*Triticum aestivum*) (Goesaert *et al.*, 2003 and 2004).

Pathogenesis-related gene 10 (PR10)

PR10, a well-characterized gene, encodes the first enzyme of phenyl propanoid mechanism PAL (phenylalanine ammonia-lyase), an important enzyme in the production of lignin, flavonoids and phenyl propanoids. LTPs (lipid transfer proteins) also play a major role in plant defense as they have antibacterial properties (Boutrot *et al.*, 2005) and are required in the formation of the mechanical barrier of cutin. Whenever the cell wall or associated membranes are infected by microbial attack, LTPs are required for the repair of damaged tissues. Lignin is a polymer of the cell wall. It is phenolic in chemical nature and linked covalently to hemicellulose and cellulose in the cell wall of plants. It also assists in water and nutrient transport (Tu *et al.*, 2010), strengthening plant immunity. A prominent defense mechanism is carried through lignification in common wheat (*Triticum aestivum*) to stripe rust causing organism (*Puccinia striiformis*) f. sp. Tritici (Moldenhauer *et al.*, 2008) and leaf rust (Casassola *et al.*, 2015).

Many plant species have an inducible response of PR10 genes after microbial invasion. Fungi, bacteria and viruses contribute equally to provoke host plant PR10 genes. While studying the immunocytochemical interaction of *Cronartium ribicola* (blister rust fungus) with sugar pine needles, it was seen that the cell wall of the attacking fungus was tightly bound by PR10 proteins. Pathogen attack followed by a fungal elicitor or any kind of stress also induces the promoter Ypr10 to regulate GUS activity in young leaves of apple (*Malus domestica*). PR10 expression is mostly induced while treating a wound. \hat{A} -glucuronidase fusion and northern blot analysis of the PR10 promoter confirmed the accumulation of asparagus PR10 proteins after wounding (Liu *et al.*, 2006). Remarkably, PR10 proteins play a vital role not only in plant defense but also in growth and development, as shown by their RNase and ligand binding activities that are triggered on infection. However, studies of the overexpression of PR10 proteins in transgenic plants and their accumulation on interaction with pathogens are still ongoing. The advancements in functional

genomics and development in technology can hopefully increase the ability to assess the exact contribution of all the members of the PR10 family in the development of the immune system in plants.

Pathogenesis-related gene 12 (PR12; defensins)

Defensins, with a length of approximately 45-54 amino acids, are small peptides that are cationic in nature. They have four disulfide bridges that impart stability to their structure, comprised of a dominant alpha-helix and triple stranded beta sheets that are antiparallel, making a cysteine-stabilized alpha-beta motif (CS $\alpha\beta$) (Fant *et al.*, 1998). Defensins have a subclass containing five disulfide bonds formed using 10 cysteine residues. The non-covalent (hydrophobic) bonds are replaced by covalent bonds by the fifth disulfide bond that reinforces a hidden hydrogen bond, conferring more thermodynamic stability in defensins compared to other classes (Janssen *et al.*, 2003). This exceptional cysteine pair was screened in two floral defensins named PhD1 and PhD2 in *Petunia hybrid* (Lay *et al.*, 2003). Plant defensins are divided into two classes based on their precursor proteins. A signal sequence and mature defensin domain is found in the first class. This signal is responsible for targeting the protein to the endoplasmic reticulum, where it is ready to enter the secretory pathway after folding. The second group is less common, containing an extra C-terminal prodomain, which is removed proteolytically after or during its passage in the secretory pathway (Lay *et al.*, 2005). This class is well characterized in *Petunia hybrida* and *Nicotiana glauca* (Lay *et al.*, 2003).

Recently, a subcellular and cytoprotective targeting function was assigned to the prodomain of these defensins (Lay *et al.*, 2014). Plant defensins perform many biological activities in the cell (De Coninck *et al.*, 2013). They are known to obstruct enzyme activities, ion channels and protein synthesis when required. Defensins can also inhibit the proliferative activities of the cancer cells or the action of HIV reverse transcriptase in plants. A large amount of data supports the antifungal activities of the defensins, whereas their antibacterial activities are poorly known (Carvalho and Gomes, 2007). Depending on their target molecules and sub-cellular location, they may perform a similar action in the cell, but their mode of action is quite diverse in each species (Van der Weerden *et al.*, 2010).

Plant defensins, when interacting with their target fungus, either stay at the cell surface from where they send signals to cause cell death, or they are internalized by the fungal cell to interact intracellularly for the death signaling cascade. Cellular uptake is well known for Psd1, MtDef4 (Sagaram *et al.*, 2013) and NaD1 (Van der Weerden *et al.*, 2008), but the latter is found for RsAFP2 (Thevissen *et al.*, 2012). To enter the cell, defensins require a RGFRRR motif, which signals

their translocation in the fungal cell, and its replacement by RGFRAA or AAAARR stops their ability to enter target fungi (Sagaram *et al.*, 2013). Several mechanisms are established in defensins for their entry in the fungal cell, as this sequence does not exist in all of them. These mechanisms include membrane permeabilization, lipid-assisted pore formation and transient permeabilization of membranes (membrane translocation) and internalization through receptors (Nicolas, 2009). Membrane permeabilization is a conditional mechanism for plant defensins, as it only occurs when a certain concentration is achieved to inhibit fungal growth or its death (Thevissen *et al.*, 2007).

Many plant defensins, such as NaD1, HsAFP1, RsAFP2 and DnAMP1, play a crucial role in fungal cell death in oxidative stress or ROS production (Van der Weerden *et al.*, 2008). However, the antifungal activity of plant defensins is reduced when the ionic strength is increased in the growth medium, preferably by divalent cations. For example, Mg^{+2} and Ca^{+2} play a significant role in reducing their efficiency. However, the condition may be contrary depending on the type of defense and the fungus (Spelbrink *et al.*, 2004). Another contrasting mechanism for decreasing the inhibitory activity of defensins through cations is also proposed (Oard and Karki, 2006). Sometimes the structure of defensin is so rigid to interact with the fungal membrane, as in wheat, that the structure of a thionin called β -purothionin is transformed by magnesium and potassium ions causing the elongation of the alpha-1 helix, unwinding the alpha-2 helix and

changing the loop conformation throughout (Oard and Karki, 2006).

RPG GENE FAMILY

The RPG gene family provides resistance against *Puccinia graminis* f. sp. tritici. The main source of this family is barley. *Rpg1*, *Rpg2*, *Rpg3*, *Rpg4*, *Rpg5*, *RpgBH* and *Rpg6* are major members of this family, which provide resistance against *Puccinia graminis*. The molecular mechanism of *Rpg1*, *Rpg4* and *Rpg5* is unknown.

RPG1

In 1942, the *Rpg1* gene was reported for stem rust resistance, and it was used for over 70 years. This gene is located on Chromosome no. 1 (7H). *Rpg1* has homology with the receptor kinase gene. The *Rpg1* gene has two protein kinase domains, protein kinase 1 (pK1) and protein kinase 2 (pK2). pK1 is a pseudokinase and helps in disease resistance. pK2 is an active domain and performs catalytic activity by phosphorylating proteins. pK1 and pK2 both are required for sustainable resistance. pK1 and pK2 are mostly present in the cell membrane, endomembrane and cytosol. When a pathogen attacks the leaves of a plant, both pK1 and pK2 disappear. This disappearance is due to the phosphorylation reaction, which is essential for sustainable resistance. This phosphorylation reaction starts a few minutes after attack and continues for 20 hours. This reciprocal response plant and stem rust is considered the ETI of the plant defense system.

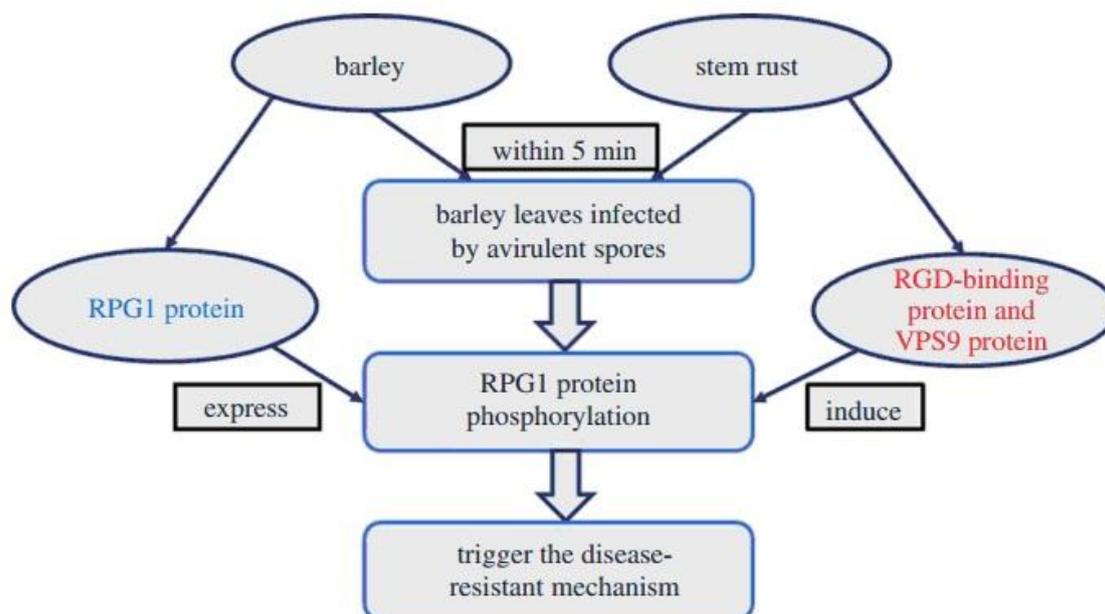


Figure 3: Interaction between *RPG1* gene and *Puccinia graminis*, *RPG1* protein activate by phosphorylation, RGD binding protein and VPS9 protein are inactive by the same phosphorylation reaction. The activated *RPG1* protein further activates race specific defence mechanism such as *Sr* and *PR* genes.

RPG4 and 5

The mapping through RAPD and RFLP markers suggested that *Rpg4* is located at the long arm of chromosome 5H (7) (Borokova *et al.*, 1995). Physical mapping suggested that *Rpg4/Rpg5* genes are present at the small region of chromosome 5H (7) (Druka *et al.*, 2000; Han *et al.*, 1997; Kilian *et al.*, 1997). *Rpg4* does not provide resistance independently, but it depends on the product of *Rpg5* for its function (Brueggeman *et al.*, 2008). Thus, *Rpg5* is the primary source for sustainable resistance against TTKSK and MCCF strains. *Rpg4* is a recessive gene, and it depends on temperature, so it has harmful effects on fungi that attack plants. Fungi use the ADF2 protein to absorb the feed from the cytoskeleton. *Rpg4* encodes the ADF2 protein that does not respond to the fungus for feeding. However, ADF2 produced by the *Rpg4* does not recognize the fungus for this, requiring the product of the *Rpg5* gene (Brueggeman *et al.*, 2008). *Rpg4* is also temperature-dependent because its protein is highly sensitive to most proteins. It is suggested that *Rpg4* works best at low temperature.

MECHANISM OF SR GENES

Sr genes are highly resistant genes and are host-race-specific. These are not housekeeping genes, so they are only activated when their specific race attacks plants. Race-specific resistant genes are very effective against their specific races, but they are not effective against all races. The race-specific resistance mechanism involves gene to gene interaction. In this mechanism, interaction between specific resistant genes (*R*) and the pathogen avirulence gene (*Avr*) is a phenomenon. The *R* gene encodes for nucleotide binding and leucine rich repeat (NLR) proteins. These NLRs act as immune receptors to the molecules that are inserted by pathogens in the host cell. These are called effector proteins. This model is also reported and studied in flax rust, but few data are available on stem rust. Wheat stem rust-resistant genes such as Sr22, Sr33, Sr35, Sr45 and Sr50 all encode NLR receptor proteins. Sr33 and Sr50 proteins recognize the defense signaling component as the N terminal coiled-coil domain. Functional studies of Sr33 and Sr50 proteins identified the minimal defense signaling component as the N-terminal coiled-coil domain and showed that dimerization of this domain is required for signaling.

Sr50 and Sr33 actively participate in triggering cell death as well as self-association in plants. Similarly, Sr33 and Sr50 full length proteins mediate a microbe-independent cell death signal in *N. benthamiana* and show self-association responses in plants when they lack a well-recognized avirulence effector. However, as these proteins are auto-active in *N. benthamiana*, it is not clear whether the self-association is a preactivated or postactivated event, thus making it possible that self-association of these NLP proteins is initiated through effector recognition. The above statements also support the fact that plant NLR protein oligomerization plays a crucial role in

their repression or activation processes. On the other hand, many immune receptors such as RGA4/RGA5 or RPS4/RRS1, Prf RB, RPS5, L6 and N reveal that a self-association platform is provided by their N-terminal domain. A prominent link between CC/TIR self-interaction and the signaling of cell death is observed in RPS4, L6 and MLA10, whereas in the NLR hetero-pairs RGA4/RGA and RPS1/RPS4, hetero-interaction is a contributing factor for the regulation activity of the signaling-proficient NLR partner (Cesari, *et al.* 2016).

Conclusions

Stem rust is an important disease of wheat which has potential to damage crop yield significantly if unattended. The present ways to combat this disease are proven to be un-satisfactory and there is a great need to work for the innovative and advanced methods to control the disease.

There are several methods to control stem rust including, chemical control, use of physical barriers, genetic manipulation, etc. However, none is effective against Ug99 strain the causing agent of the disease which is becoming more and more dangerous to the crop worldwide.

There is greater need to understand the mechanism of the disease in host plant and boosting up of the plant defense system by using molecular approaches. This indigenous defense mechanism may enable us to control stem rust.

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Figure Legends

Figure 1: Mechanism of interaction of wheat and fungi

Figure 2: Defense mechanism of wheat against *Puccinia graminis*

Figure 3: Interaction between RPG1 gene and *Puccinia graminis*, RPG1 protein activate by phosphorylation, RGD binding protein and VPS9 protein are inactive by the same phosphorylation reaction. The activated RPG1 protein further activates race specific defence mechanism such as Sr and PR genes.