UNDERSTANDING INVASIVE PLANT MYCOPARASITES AND THEIR REMEDY THROUGH ADVANCED MOLECULAR APPROACHES

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ABSTRACT

Fungi are historically notorious pests that have threatened availability of quality food. Several invasive species have appeared to be destructive for valuable crop species and even led to famine in certain severe cases. Surveillance and eradication of these disastrous microbial invaders is dependent on their sentinel behavior. Molecular Biology has helped to great extent in understanding these epidemic agents. Plant defense system as well as plant microbe interaction have well been explored and proved quite fruitful in understanding metabolic pathways involved in pathogenesis and defense response. Ultimately, researchers are able to define strategies for the control of these invasive pathogens. Genome editing has successfully been employed to develop pathogen resistant crops. Antifungal proteins have been expressed through transgenic technology to develop disease resistant plants. A few have proved to be the real success stories whereas others are at the stage of infancy. This review is an update about research work accomplished to-date, for the characterization and identification of fungal pathogens, metabolic pathways activated during plant pathogen interaction, advancements in the detection of fungal pathogens and transgenic plants developed to withstand pathogen attack.

Keywords: Invasive fungal pathogens, Plant defense response, Molecular diagnosis, Transgenics, Cisgenic approaches.

INTRODUCTION

Human population is increasing at a pace of 80 million per annum which is quite alarming. Sharply increasing population coupled with shrinking cultivable land, water scarcity and expanding urbanization demands huge quantity of quality food from decreased cultivated land and lower input supplies (Nelson & Smith, 1993). Under these circumstances, provision of food to ever increasing population is really a challenge. All this can be achieved by the introduction of better performing varieties, improving cultural practices and discovering innovative methods for the control of invasive pathogens. Researchers have contributed a lot to combat all of the aforementioned limitations (Oerke & Dehne, 2004).

Fungal pathogens are destructive for almost all of the plant species including cereals, fiber crops, sugar crops, vegetables, flowers, fruits and tree plants. More than 125 million tons of the major food crops including wheat, maize, rice, soyabean and potatoes is deteriorated by fungal infections each year (Matthew et al., 2012). This lost crop produce could be enough to feed all the people who don’t have food to eat. Certain diseases i.e. potato late blight, rice blast, wheat stem rust, corn stem rust, soybean rust and smut in maize, red rot in sugarcane are known to affect 50% of the crop produced. In tree plants, too, sheesham dieback has resulted in severe destruction of this important timber tree in certain regions of the world (Anonymous, 2012). Plant yield is limited to great extent due to devastating effects of fungal pathogens. Premier management practices, to control these pests were development of disease resistant varieties, use of chemical fungicides and biocontrol agents. These management practices are of pivotal importance but have certain limitations including...
Emergence of new pathogens has added to these limitations, as a result major portion of the crop produce is lost due to fungal infections. Understanding mode of infection of newly emerged pathogens and their mode of interaction with host plant will prove a great milestone for their control (Fisher, 2013). Further employing transgenic technology to develop pathogen resistant plants against a range of pathogens is really of great importance. This review highlights such updates regarding plant pathogen interaction, resistance development against chemical fungicides and significance of transgenic technology to develop resistant plant against disastrous fungal pathogens.

**Fungal pathogens as life threatening parasites for plants:** Fungi are classified into four major phyla including Zygomycota, Basidiomycota, Ascomycota and Oomycota. Zygomycota are sexually reproduced by forming the conjugating gametangia, involving the production of zygospores and zygosporangia. They have aseptate hyphae and also called filamentous fungi. Basidiomycota have club shaped fruiting bodies called basidia which are present at the tip of hyphae. They have sexual reproduction. Most of the edible fungi belong to this phylum. Ascomycota is also called sac fungi due to formation of sac-like structure, ascus. They are produced both sexually and asexually. This group includes commercially important fungi. Oomycota are not true fungi and are sometimes ranked as lower fungi. They have many structural similarities with fungi but major difference is in the composition of cell wall that is composed of beta glucans and cellulose instead of chitin (Samanta, 2015).

We discuss here, few of the plant pathogens which affect crop plants seriously and may even lead to complete crop failure(Dean et al., 2012). Irish potato famine is one of the familiar examples to understand level of disastrousness of fungal pathogens. Potato has been the main staple food in European countries. One third of Irish population was dependent on potato as a staple food at times (Reader, 2009). Unluckily, *Phytophthora infestans* (which was first appeared in North America) moved to this region during 1845 and spread rapidly in Great Britain and Ireland etc. The disease extent was so severe that it led to complete failure of the crop resulting in historic Irish famine. Almost one million people died whereas more than one that were forced to migrate to other countries (Turner, 2005). Still, Irish population is one third of the actual population as was at the time of famine (1840s) (Yoshida et al., 2013). The casual agent of Irish famine (*Phytophthora infestans*) is an extremely virulent pathogen and exists alongwith other noxious species i.e. *ipomoeae* and *mirabilis* (Haas et al., 2009). It normally multiplies through sexual-reproduction and has adapted a high level of genetic and phenotypic diversity. *Magnaportha oryzae* is a filamentous ascomycetic fungi which causes the most disastrous disease of rice blast (Ou, 1980). Blast may result in 10-30% losses to the crop yield and may even be epidemic in conditions favorable for pathogen. In addition to rice, this pathogen also infects grasses millet, wheat and barley (Couch et al., 2005). *Botrytis cinerea* (grey mold) is a broad spectrum fungal pathogen known to infect more than 200 plant species. These fungi have been most drastic for wine and table grapes and caused heavy economical losses in South Africa (SA Rand 25 million/year), Australia (AUS $52 million/year) and Chile (US$ 22.4 million/year) (Scholfield & Morison, 2010). The fungus is a typical necrotroph which causes infection by inducing programmed cell death (PCD) in the host (van Baarlen et al., 2007). Advancements in genomics approaches have helped a lot to explore this pathogen by characterizing its genome and by employing molecular tools to understand its mode of infection. *Puccinia* species including *Puccinia tritici*, *Puccinia striiformis* and *Puccinia graminis* cause rust diseases in wheat including yellow rust (stripe rust), brown rust (leaf rust) and black rust (stem rust). These fungi have heteroecious, macrocyclic lifecycles and are obligate, biotrophic basidiomycete. (Rutter et al., 2017). Obligate biotrophs obtain their nutrients through haustoria (specialized feeding structures which are embedded inside the plant cells). They overwhelm the host defence response by developing particular infection structures (Voegele & Mendgen, 2011). Historically, stem rust has proved to be the most notorious resulting in complete destruction of wheat crop. In ancient Rome, people were so afraid of this disease that rituals were performed to save their crop. Wheat rust, also known as polio of agriculture, is spreading all over the globe and is causing drastic losses to this cereal food crop (Singh et al., 2004). North America has already faced similar type of threat in 1950’s where more than 40% of the crop was wiped out due to rust. Since then, rust-resistant lines have
been commercialized by investing millions of dollars, as a result wheat crop is comparatively secure and farmers are growing with confidence (Kolmer, 1996). A mild out-break was reported in Uganda during 1999 where fungus was reported to attain certain lethal mutations, as a result, most of the cultivated varieties became susceptible (Pretorius et al., 2000). This insisted researchers that consistent efforts are direly needed to conduct research to understand plant pathogen interaction and to develop new varieties with elevated level of resistance against infectious fungal pathogens. *Fusarium graminearum* is a disastrous pathogen for all cereal species which may infect a wide range of crops including cotton, tomato, banana and melon (Michielse & Rep, 2009) and is now emerging as human pathogen (Nucci & Anaissie, 2007). It normally co-infects and co-exists with other *Fusarium* species and infects floral tissues. Long time storage of infected cereals results in increased mycotoxin level in the grains (Magan et al., 2010). *Blumeria graminis* is an ascomycete that causes powdery mildew of grasses including barley and wheat. It may affect crop growth and cause severe reduction in yield. *Mycosphaerella graminicola* is another disastrous ascomycete which causes blotch disease (*Septoria tritici*) in wheat. This pathogen is more infectious in warm humid regions (Orton et al., 2011). Fungus makes intracellular colonies and remains inside the plant for more than seven days without any symptom. Then, it results in the formation of leaf lesions ultimately resulting in complete destruction of the crop (Keon et al., 2007). One of the most common and important genera of plant pathogenic fungi is *Colletotrichum*. Almost each of the crop plant is susceptible to this pathogen and it may cause blights, post-harvest rots and anthracnose (Prusky, 1996). Most of the *Colletotrichum* species initiate infection through biotrophic phase accompanying with large intracellular primary hyphae. Later on fungus switches to critical necrotrophic phase accompanying with narrower secondary hyphae. *Aspergillus flavus* is notorious to produce aflatoxins which may affect quality of almost each and every stored food product. Crop plants growing in the warm, humid climate (maize, cotton, groundnut) are more prone to be infected by this pathogen, as a result seed quality is seriously affected and may even completely be lost. Anyhow, all of the *Aspergillus* strains do not produce aflatoxins, rather few are used in the preparation of quality food products.

**Fungi: plant interaction and defense response:** Fungal pathogens are one of the serious disastrous entities that cause serious yield losses to crop plants. Scientists have worked out that how these phytopathogens interact with plant cell and ultimately cause death of the invaded cell. Plant pathogenic fungi are categorized into biotrophic, necrotrophic and hemibiotrophic phases of interaction (Selin et al., 2016). During the biotrophic phase of interaction, plasma membrane of plant invaginates around intercellular hyphae (IH). Similar type of interaction has also been observed during haustoria formation. A few of the plant species don’t follow this interaction i.e. *Colletotrichum lindemuthianum* – bean interaction (Green et al., 1995). This interface results in plant-fungal interaction which ultimately plays crucial role in the establishment and maintenance of biotrophy and ultimately helps to find out ways to control fungal pathogens. Necrotrophic fungi produce cell wall degrading enzymes such as pectin lyases, endo-PG (endo-polygalacturonase), α and β-galactopranosidase, α-arabinofuranosidase. During hemibiotrophic phase of interaction, the pathogen first establishes biotrophic phase followed by necrotrophic phase which shifts to necrotrophic phase, ultimately resulting in death of the host cell (Perfect et al., 1999).

Fungal pathogens trigger death of the host cells, either by releasing toxins or inducing PCD (programmed cell death) in response of ROS (reactive oxygen species) production (Barna et al., 2012). Some necrotrophic fungi also promote susceptibility of host cell by releasing toxic effectors. These effectors down-regulate the resistance proteins and defence enzymes by interrupting regulatory/signaling pathways (Wang et al., 2014). *Aspergillus* mycotoxin induces necrotic lesions through down-regulation of antioxidant defence enzymes and oxidative burst induction at high ROS level (Peng et al., 2010). *Fusarium* releases nivalenol, T-2, diacetoxyscirpenol, HT-2, SMs fusarenon, DON and deoxynivalenol which inhibit seed germination and induce PCD (programmed cell death) in tomato protoplast (Paciolla et al., 2004). In *Arabidopsis thaliana*, *Fusarium sporotrichioides* induce production of hydrogen peroxide, callose deposition, cell death and accretion of salicylic acid and DON (deoxynivalenol) toxin repressed translation in *Arabidopsis* cells without
inducing the elicitor-like signaling pathway (Nishiuchi et al., 2006).

**Defensive strategies opted by plants to combat fungal pathogens:** No doubt, fungal pathogens are the most notorious ones and have even resulted in famine during certain eras. Evolution of certain defense mechanisms have helped plants to cope with these disastrous pathogens. These defense strategies may be categorized into chemical and physical barriers that obstruct pathogen entry and infection. Morphological, biochemical and molecular changes improve defense ability of plants including upregulation of defense related genes, oxidative bursts, programmed cell death and production of antimicrobial compounds (Bari & Jones, 2009).

Plants have evolved a two-layered innate immunity. Pattern recognition receptors (PRRs: a set of defined receptors) are used to attain the primary innate immunity that is first line of defense. These receptors are involved in the recognition of conserved MAMPs (microbe-associated molecular patterns). MAMPs recognition persuade the primary defense responses that include deposition of callose, amendments in cell wall and accretion of defense related proteins such as chitinases, glucanases and proteases by which pathogens are negatively affected. Elongation Factor Tu (Zipfel & Felix, 2005), lipopolysaccharides, flagellin, chitin and β-glucans are other microbe-associated molecular patterns (MAMPs) effective against plant pathogens (de Wit, 2007).

True pathogens may suppress innate immune system of plants. Several mechanisms inhibit activation of primary defense responses and recognition of MAMPs in fungal, bacterial and viral pathogens (Vivier & Malissen, 2005). Plants have developed secondary defense responses to defend themselves against true pathogens that are triggered by identification of effectors or effector-mediated trepidations of host targets (Jones & Dangl, 2006). These effectors and their trepidations are surveilled by resistant proteins (RPs) and secondary defense responses are initiated which frequently inhibit hypersensitive response (HR) with further defense responses that blocks growth of pathogens. RP detects the specific host targets whereas induction of hypersensitive response (HR) is non-specific and is generally activated against drastic plant pathogens (de Wit, 2007).

Plants produce a wide variety of pathogenesis-related proteins including glucanases, chitinases, chitosanases and metallothionine. Thirty-three PR proteins have been explored in tobacco, more than thirty in Norway spruce and twenty in sugar beet (Van Loon, 1997). Chitinases are enzymes involved in the breakdown C1-C4 linkage of two successive chitin N-acetyl-D-glucosamine monomers. They exist in plants, animals, fungi and bacteria. Chitin is commonly present in the exoskeleton of arthropods and fungal cell wall (Bartnicki-Garcia, 1968). Plant chitinases inhibit fungal growth and have ability to degrade chitin that is major component of fungal cell wall (Broekaert et al., 1988). β-1,3-glucanase play a major role in plant defense response and belongs to PR-2 family of pathogenesis-related proteins. It is also present in fungi, bacteria, yeast, insects, fish and actinomycetes (Pan et al., 1989) and is involved in the cleavage of β-1,3-glucan by breaking down β-1,3-glucosidic bonds (Simmons, 1994). β-1,3-glucan is an important structural component of cell wall in many pathogenic fungi but major constituent is chitin (Adams, 2004). PG (polygalacturonase) is a cell wall degrading enzyme that catalyzes release of oligosaccharides from the plant cell wall, thus elicits to activate defense mechanism in plants (Walker-Simmons et al. 1983). PGIP is a protein present in the plant cell wall that precisely hinders PG activity (Albersheim & Anderson 1971). It has also been proved to slow down hyphal extension rate which results in activation of other defense responses (Figure: 1) (Cervone et al., 1986).

Systemic acquired resistance helps to protect plants from pathogen attack at sites away from primary and secondary immune responses. Whenever pathogen infects any plant part either leaves or stem, systemic resistance is induced simultaneously alongwith local primary and secondary immune responses. Systemic acquired resistance (SAR) depends upon various plant hormones i.e. salicylic acid, ethylene, jasmonic acid and abscisic acid or combination of these and is effective against a wide range of infectious pathogens (Grant & Lamb, 2006). These hormone dependent defense systems are active against insect pests and microbial pathogens (De Vos et al., 2005). Anyhow, the level of effectiveness of these resistance responses and their dependence on hormones varies from plant to plant or species to species.
Figure 1: Pictorial representation showing interaction of fungal pathogens with plant and various defense strategies opted by plants to combat mycoparasites.

Pathogen infection produces methyl salicylate which acts as an inducer for the activation of systemic acquired resistance. Exogenous application of salicylic acid enhances resistance by the induction of pathogenesis related genes (Park et al., 2007). Besides, ethylene and jasmonic acid are linked with defense against necrotrophic pathogens and pests. Jasmonic acid/ethylene and salicylic acid defense mechanisms interact synergistically against pathogen infection although they are antagonistic (Beckers & Spoel 2006). Nature of pathogens decides about the activation of defense signaling pathways. These pathways may be activated synergistically depending upon the extent of infection (Adie et al., 2007). However, under field conditions, plants are attacked by multiple pathogens simultaneously which require diverse defense mechanisms to be triggered.

Plant-pathogen interaction produces AOS (active oxygen species) such as superoxide anions (O⁻²⁻), hydroxyl radicals (OH•) and hydrogen peroxide (H2O2). Baker and Orlandi, 1995). AOS play pivotal role in strengthening plant cell wall and improves signal transduction for enhanced resistance against pathogens. In addition, chloroplasts also play significant role in plant defense mechanism as is the site for biosynthesis of signaling compounds such as salicylic acid, jasmonic acid, nitric oxide and reactive oxygen species (ROS) (Lee et al., 2015). PCD (programmed cell death) is also regulated by above mentioned signaling compounds.

Advanced approaches to overcome fungal pathogen infestation: Since the beginning of agriculture, farmers have been striving hard to protect crops from harmful pests including fungi, bacteria, viruses, mites, rodents, nematodes, insects and weeds. Various chemical, biological and integrated approaches have been employed to combat these pathogens. With the advancements in research, innovative strategies have been developed and employed to develop plants having ability to withstand these drastic yield limiting agents (Oerke, 2005).

No doubt, breeding has contributed a lot to develop resistant crop varieties, better tolerant to fungal pathogens and other devastating agents. Conventional techniques have been used to develop pathogen resistant genotypes by stacking antifungal genes in various crops. Conventional methods to control pathogens had been very effective but insecticides, fungicides and bactericides are toxic for plant invaders as well as for the ultimate consumer of these plants (Thakur & Sohal, 2013). Further, resistance
development and species barrier are the major constraints limiting implications of breeding. So, it is direly needed to explore alternative strategies which are human friendly and provide broad-spectrum control of pathogens. Transgenic technology is only state-of-the-art technology that addresses all of the above-mentioned limitations paving way to develop environment friendly plant varieties having broad spectrum resistance against plant pathogens including fungi (Jach et al., 1995).

Engineering endogenous plant genes for fungal pathogen resistance: Overexpression of endogenous plant genes, inspite of alien genes is more attractive as far as commercialization of the engineered plants is concerned. Since various defense mechanisms have been explored in plants to withstand fungal pathogens. These mechanisms can further be improved by engineering the most critical genes involved in these mechanisms. Sheath blight in rice may be controlled by the overexpression of two defense-related genes OsCHI11 and OsOxO4. OsCHI11 encodes for chitinase whereas OsOxO4 encodes for oxalate oxidase (Karmakar et al., 2016). Chitinase plays important role in degradation of chitin and hydrolyzes cell wall of pathogenic fungi (Muthukrishnan et al., 2001). Oxalate oxidase degrades oxalic acid by forming CO2 and H2O2. At the site of infection, it destroys fungal virulence factor (Lane, 2000). Expression of these genes was controlled by green tissue specific promoters including rice cis-acting 544-bp DNA element and maize phosphoenolpyruvate carboxylase gene promoter (PEPC). In comparison with wild type, transgenic plants showed increased level of hydrogen peroxide, higher activity of ROS dependent enzymes and a remarkable reduction in infection and disease symptoms.

In potato, over-expression of endochitinase gene inhibited hyphal growth of Alternaria solani by 40% to 60% when assessed through quantitative in vitro assay. In comparison with wild type, there was a decrease in number of necrotic spots on the leaves of transgenic potatoes (Khan et al., 2016). PR-3(I) rice chitinase gene constitutively expressed in rice plants increased the protection against Rhizoctonia solani (Lin et al., 1995). Overexpression of PR-5 in potato resulted in a delay in disease progression upon infection by Phytophthora infestans (Liu et al., 1994). Mixtures of purified PR-3(I) chitinase and β-1,3-glucanase (PR-2 family) from tobacco showed synergistic antifungal activity in vitro (Sela-Buurlage et al., 1993). In tobacco the resistance against oomycete pathogens Peronospora tabacina and Phytophthora parasitica was increased by constitutive expression of the PR-1a gene (Alexander et al., 1993). Germins and germin like proteins (GLPs) play significant role in development and growth of plants. Expression of Oryza sativa root GLP2 promoter in potato. This promoter is activated in response to Fusarium solani and Alternaria solani. OsRGLP2 promoter activity was observed to be increased by 15 folds and 12 folds after infection with Fusarium solani and Alternaria solani Sorauer infection (Munir et al., 2015). Cultivated potato transformed with receptor-like protein ELR (elicitor response) taken from wild potato resulted in enhanced resistance to Phytophthora infestans. BAK1/SERK3 (immune receptors) associated ELR mediates broad-spectrum recognition of elicitor proteins from several Phytophthora species, including four diverse elicitors from P. infestans (Du et al., 2015). In orange fruits the monoterpene level was altered by down-regulation of a D-limonene synthase gene which leads to resistance against Penicillium digitatum infection. A resistant variety of ecotype Columbia against Fusarium oxysporum by overexpressing the thionin under constitutive promoters was developed. It was evaluated that phytopathogenic fungi induces the thionin Thi2.7 gene in Arabidopsis. The fungi with irregular hyphae including hyperbranching was grown on cotyledons of transgenic lines in comparison with wild type (Epple et al., 1997).

Cisgenic approaches to control fungal pathogens: Since, transgenic technology has no species barrier and any of the transgene may be expressed across the species. Ectopic expression of transgene has its own value and proved to be very effective in engineering crop plants for valuable traits. For fungal pathogen resistance, various genes have been recognized in yeast, fungi, bacteria and higher plants to have great antipathogenic activity. These genes may be exploited through transgenic technology and some research groups have attained excellent results (Table 1) (Grover & Gowthaman, 2003).
Table 1. Antifungal protein genes used for making fungus-resistant transgenic plants.

| Fungal pathogens | Name of the Gene | Source | Host plant | Reference |
|------------------|------------------|--------|------------|-----------|
| *Rhizoctonia solani* | PR3 (Class Ichitinase) | Bean (Phaseolus vulgaris) | Canola (Brassica napus) | Broglie et al. (1991) |
| *Pythium aphanidermatum* | PR5 | Tobacco | Potato (Solanum tuberosum) | Liu et al. (1994) |
| *Phytophthora infestans* | PR3 (Class II Chitinase), type I RIP | Barley | Tobacco | Jach et al. (1995) |
| *Phytophthora megasperma* | PR2 (Class II Glucanase) | Alfalfa (M. sativa) | Alfalfa (Medicago sativa) | Masoud et al. (1996) |
| *Phytophthora infestans* | PR5 | Potato (S. commersonii) | Potato (Solanum tuberosum) | Zhu et al. (1996) |
| *Cercospora nicotianae* | PR3 (Class I Chitinase), PR2 (Class II Glucanase) | Alfalfa, Rice | Tobacco | Zhu et al. (1994) |
| *Botrytis cinerea* | PR2 (class I glucanase) | Soybean (Glycine max) | Kiwifruit (Actinidia chinensis) | Nakamura et al. (1999) |
| *Wheat* (Triticum aestivum) | PR3 (Class II Chitinase) | Barley | Wheat (Triticum aestivum) | Bliffeld et al. (1999) |
| *Elisinoe ampelina* | PR3 (class I chitinase) | Rice (Oryza sativa) | Grapevine (Vitis vinifera) | Yamamoto et al. (2000) |
| *Rhizoctonia solani* | Chitinase(RC7) | Rice | Rice | Datta et al. (2001) |
| *Magnaporthe grisea* | Stress-inducible b-glucanase (Gns1) | Rice | Rice | Nishizawa et al. (2003) |
| *Sclerotinia homoeocarpa, Rhizoctonia solani* | Chitinase(RCH10); Glucanase(ALG) | RCH10 from rice; ALG from alfalfa | Creeping bent grass | Wang et al. (2003) |
| *Rhizoctonia solani* | Chitinase(chi11) | Rice | Rice | Kumar et al. (2003) |
| *Phytophthora infestans, Phytophthora erythroseptica* | Cationic peptide (msrA3) | Synthetic preparation | Potato | Osusky et al. (2004) |
| *Rhizoctonia solani* | Chitinase(ech42); Chitinase(nag70); Glucanase(gluc78) | *Trichoderma atroviride* | Rice | Mei et al. (2004) |
| *Magnaporthe grisea* | Antifungal protein (Afp) | *Aspergillus giganteus* (chemically synthesized) | Rice | Coca et al. (2004) |
| *Rhizoctonia solani* | Chitinase(BjCHI1); Glucanase(HbGLU) | HbGLU from rubber tree; BjCHI1 from mustard | Potato | Chye et al. (2005) |
| *Verticillium dahliae* | Chitinase(Chi) | Bean | Cotton | Tohidfar et al. (2005) |
Three barley proteins i.e. class-II β-1,3-glucanase, class-II chitinase and a class-I ribosome inactivating protein were expressed in tobacco under the control of CaMV35S promoter. Chitinases and glucanases were observed to be accumulated in intracellular spaces. Fungal infection assay confirmed expression as well as antipathogenic activity against *Rhizoctonia solani*. Co-expression of these genes appeared to be effective for the control of pathogens as compared with single gene (Jach et al., 1995). Co-production of chitinase and β-1,3-glucanase resulted in a substantially higher protection against *Cercospora nicotianae* than transgenic plant expressing single gene (Zhu et al., 1994). Expression of PR-3(I) chitinase or PR-2(I) β-1,3-glucanase genes in tobacco plants showed no protection to infection against *Fusarium oxysporum*. However, in tomato plants simultaneous expression of both of these genes decreased disease severity to 40–60% (Jongedijk et al., 1995). Another study explained the combined expression of a PR-3(II) chitinase gene (encoding an extracellular isoform of chitinase) and a PR-2(II) β-1,3-glucanase gene (encoding an extracellular isoform of β-1,3-glucanase) from bean in transgenic tobacco plants increased the protection to *Rhizoctonia solani* infection as compared with the tobacco plants expressing the single transgene (Jach et al., 1995). Transgenic tobacco plants expressing the barley class II chitinase and barley RIP gene simultaneously showed improved protection to *R. solani* attack as compared with the transformants with single gene expression (Jach et al., 1995).

In tobacco and canola the constitutive expression of a vacuolar isoform of chitinase (PR-3(I) family) from bean reduces the susceptibility to *Rhizoctonia solani* (Broglie et al., 1991). A chitinase gene was isolated from the soil pathogen *Seratia marcescens* and was overexpressed in tobacco. The resultant transgenic plants depicted improved resistance to *R. solani* infection (Jach et al., 1992). Constitutive expression of PR-3(I) chitinase genes from rice in cucumber showed enhanced resistance against *Botrytis cinerea* infection (Tabei et al., 1998).
Similarly, expression of class I rice chitinase gene (RCC2) was studied in the somatic embryos of grapevine and resultant transformants showed enhanced resistance against *Uncinula necator* (causal agent of powdery mildew) (Yamamoto et al., 2000). Transgenic tobacco plants with recombinant barley RIP protein exhibited increased protection against *Rhizoctonia solani* infection (Logemann et al., 1992). Expression of pokeweed RIP gene (which encodes an antiviral protein that does not have ability to inhibit fungal growth when applied *in vitro*) in tobacco showed increased resistance against *R. solani* (Zoubenko et al., 1997).

Defensins are another important group of genes which play significant role in plant defense. In tobacco plant the expression of defensin gene from radish enhanced the tolerance against *Alternaria longipes* (Broekhuis et al., 1997). Similarly, expression of alfaAFP from *Medicago sativa* to potato showed a great degree of resistance against *Verticillium dahlia* in green house as well as field conditions (Gao, et al., 2000). Transgenic potato (*Solanum commersonii*) plants expressing chimeric gene constructs comprising of sense or antisense RNAs under the control of cauliflower mosaic virus 35S promoter and a pA13 cDNA (for an osmotin-like protein) was developed. An increased level of resistance to *Phytophthora infestans* was observed in transgenic potato plants expressing pA13 (Zhu et al., 1996). The expression of *Streptomyces griseus* ChiC gene in combination with wasabi defensin (from *Wasabia japonica*) in potato plants was reported. Higher level of resistance against *Fusarium oxysporum* (causal agent of Fusarium wilt) and *Alternaria solani* was shown in transgenic lines (causal agent of early blight) (Khan et al., 2014).

Two stilbene synthase genes from grapevine were expressed in tobacco which don’t have ability to produce resveratrol. Transgenic tobacco plants were able to produce resveratrol when infected with *B. cinerea*. Disease incidence was up to 50% in engineered plants (Honee, 1999). Stilbene synthase gene from grapevine was expressed in *Carica papaya* and resultant transformants depicted high level of tolerance against *Phytophthora palmivora* (Zhu et al., 2004). The production of the human lysozyme (an enzyme which is involved in the cleavage of β-1,4- glycosidic linkage of chitin in the fungal cell wall) in tobacco plants was reported. The resultant transformants showed enhanced level of resistance to *Erysiphe cichoracearum* (Nakajima et al., 1997). In another study, transgenic potato plants were developed for the constitutive expression of H2O2-generating glucose oxidase from *Aspergillus niger*. It resulted in increased resistance *Phytophthora infestans*, *Verticillium dahliae* and *Alternaria solani* (Wu et al., 1995).

Transgenic technology in combination with conventional breeding is even more valuable to develop pathogen resistant plants. pGreenII binary vector having chitinase gene from *Streptomyces olivaceoviridis* was used to engineer pea plants with *bar* gene as a selectable marker. Similarly, β-1,3-glucanase isolated from barley was transformed into another pea line. Both of the engineered pea lines were intercrossed to stack two antifungal proteins. At the same time, transgenic pea plants were also developed expressing both the chitinase and glucanase genes under constitutive 35S promoter. *In vitro* assays of the cross bred pea plants and the plants with engineered glucanase and chitinase genes showed retardation of spore germination hence, were able to withstand fungal pathogen attack (Amian et al., 2011). Constitutive expression of *Aglu1* (acidic glucanase) and *RCH10* (rice basic chitinase) genes under CaMV35S (cauliflower mosaic virus) promoter resulted in decreased symptoms of fungal pathogen *Phytophthora megasperma* (Masoud et al., 1996).

The plant defensin NaD1, from *Nicotiana alata*, showed strong antifungal activity against a range of cotton pathogens including *Fusarium oxysporum* and *Verticillium dahliae*. NaD1 expressed in transgenic cotton plants appeared to be resistant against the infectious fungal pathogens. Crop productivity appeared to be increased by 200% as compared with non-transgenic cotton (Gaspar et al., 2014). Transgenic tomato plants expressing rolB gene evaluated its consequence on plant’s nutritional content, morphology, yield and resistance against fungal pathogens. Transgenic tomato fruits have significant improvement in nutritional quality showed by biochemical analysis. Lycopene content was observed to be increased by 62% whereas total phenolics, ascorbic acid and free radical scavenging activity were observed to be increased by 225%, 58% and 26% respectively. Furthermore, defence response of leaves of transgenic plants was observed to be increased when infected with *Fusarium oxysporum* and *Alternaria solani* (Arshad et al., 2014). Over-expression of Osoxo4 (rice oxalate oxidase 4) gene in potato resulted in enhanced activity of oxalate oxidase enzyme thus with
increased ability to degrade externally applied oxalic acid. After infection, the infectious fungal pathogens increased the levels of reactive oxygen species (H2O2) and defense related genes (anionic peroxidase and phenylalanine ammonia lyase) (Ghosh et al., 2016). Transgenic approach was used to develop sheath blight resistant varieties of rice. pZ100 gene expression cassette was developed by cloning AGLU1 (alfalfa β-1,3-glucanase gene) and RCH10 (rice basic chitinase gene) under 35-S promoter with their enhancer sequences. Agrobacterium-mediated transformation method was used to transform pZ100 cassette into rice. Transgenic plants showed resistance to both blast and sheath blight (Mao et al., 2013). Likewise, genome editing has successfully been employed to develop crop plants having better ability to withstand fungal infection.

CONCLUSIONS AND WAY FORWARD

Plant diseases are an important constraint to crop productivity worldwide and account for more than 30% losses of the global harvest each year. Conventional research has contributed a lot and played critical role in the provision of disease free healthy foods to ever increasing population. Nevertheless, advancements in Molecular Biology has not only helped to explore plant pathogen interaction but also explored defense response of the plants. Developments in disease diagnosis through PCR and ELISA is a real milestone for the eradication of diseases including seed borne pathogens. Discovery of pathogenesis related proteins has helped breeders to stack multiple genes for broad spectrum resistance. In addition, developments in transgenic technology have proved much fruitful and a wide range of plants have been developed for enhanced pathogen resistance. Another advancement in science is genome editing which has addressed all of the concerns linked with transgenic technology and a large number of fungal pathogen resistant crop plants have been commercialized within last few years.

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