ELUCIDATE THE ROLE OF GLUCANASE GENE AGAINST FUNGAL PATHOGENS

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ABSTRACT

Fungal pathogens are one of the serious devastating agents limiting crop yield to a great extent. Premier management practices to tackle these diseases are the development of disease resistant varieties, application of appropriate fungicides, use of biocontrol agents, plant-based extracts and induction of innate host resistance. These management practices are of great value but have certain limitations i.e., side effects, high cost and decreased efficiency. Resistance development against the fungal pathogens inspired the scientists to explore modern techniques and produce plants with broad spectrum resistance against fungal pathogens. Transgenic technology holds a great potential in this regard. The advancements in molecular biotechnology have enabled the scientists to identify, isolate and characterize the plant stress responsive genes for plant transformation and also explained their role to combat stresses. Trichoderma harzianum is a potential biocontrol agent successfully employed for the control of many economically important pathogens. The biocontrol activity of Trichoderma spp. is majorly attributed to chitinolytic and glucanolytic enzymes having ability to degrade chitins and glucans. Glucanases are therefore one of the key groups of enzymes involved in mycoparasitism. They are classified on the basis of glucosidic linkage, they cleave i.e. β-1, 3-glucanases, β-1, 6-glucanases, α-1, 4-glucanases, β-1, 6-glucanases. The present review aims to explain the role of glucanase genes in the plant defense system and elaborate how glucanase genes protect plants from pathogens.

Keywords: Glucanase, β-1, 6-Glucanases, Pathogenesis-Related Proteins (PRP), Anti-Fungal Protein

1. INTRODUCTION

Fungal pathogens are one of the disastrous causative agents that limit the yield of crops. Crops are weakened by devastating diseases resulting in low yields and poor quality. A crop may be completely wiped out by fungal pathogens (Lucas et al. 1992; Anu et al. 2019). Previously they developed disease resistant varieties, applied specific fungicide, biological agent, and plant based extract were used to tackle these diseases (El-Saeid 2011) and induction of innate host resistance (Sundar et al. 2001). These management practices are of pivotal importance but have certain limitations i.e., side effects, high cost and decreased efficiency (Zafar et al. 2020). Repeated application of fungicides is causing environmental pollution, health problems, food contamination with chemicals and death of beneficial organisms. Additionally fungicides might become less effective owing to the evolution of resistant pathogens (Lucas et al. 1992; Faize et al. 2004). It is now possible to isolate, identify and characterize plant stress responsive genes for plant transformation (Azevedo et al. 2011).

Breeding for resistance reduces the need for other methods to control pathogens but is facing certain biological and financial limitations. Another difficulty coupled with the use of resistance genes is the appearance of new virulent pathotypes which are capable of conquering a previously used resistance gene (Radhakrishnan et al. 2021). Fungal pathogens are frequently becoming resistant to fungicides toward the existing genes, so other methods to control diseases are really required (Rommens and Kishore 2000).

1.1. Plants defense mechanism

Plants have evolved several mechanisms to recognize and respond against infections by activating an appropriate defense response (Zafar et al. 2022a). Primary barriers are made to serve as an initial impediment to pathogens (Osbourn 2001). Once these obstacles are overcome, elicitors from the pathogen induces a cascade of defense responses in the plant cell such as the stiffening of the cell wall with further deposition callose, lignin and suberin, acceleration of hypersensitivity reactions, production of phytoalexins, phenolic compounds pathogenesis-
include associated pathogenesis-related (PR) proteins, reactive oxygen species (ROS) and many other defense proteins (Hématy et al. 2009; Farooq et al. 2021). These defensive reactions are collectively known as “defense responses” of resistant plants. Such inducible defense developed throughout evolution. In certain situations almost all the plants produce defensive proteins, although these defensive proteins morphologically different but structurally and functionally are similar (De jonge et al. 2011; Ren et al. 2019).

1.2. Role of Trichoderma harzianum-derived hydrolytic enzymes in plants defense mechanism

Plant pathogens controlled by microorganisms have been environmentally acceptable alternatives to the existing methods of chemical treatment and considered a more natural (Baker and Paulitz 1996) as plants possess mutually basal and inducible mechanisms to defend themselves against invading pathogens. Seventeen families of pathogenesis-related proteins have been recognized, which have different properties. The PR-2 family proteins showed an endo-1,3-β-glucanase activity (Kauffmann et al. 1987), whereas the PR proteins of families 3, 4, 8 and 11 have endochitinase activity (Campo et al. 2004). *Trichoderma harzianum*-derived hydrolytic enzymes (glucanase, chitinase, chitosanase) are supposed to have more mycoparasitic potential as compared to all of the aforementioned sources of antifungal proteins. *Trichoderma* spp. are commonly secluded from soil and prevailed in the plant root ecosystem (Harman et al. 2004).

They produce extracellular enzymes of many types that degrade cell walls of fungi (Papavizas 1985; Sanz et al. 2004). Plant roots must be colonized by *Trichoderma* strains for stimulation of growth and to protect against infections. Colonization implies the capacity to stick and identify plant roots, pierce the plant and endure metabolites that were toxic synthesized via plants in reaction to attack by a foreign organism, whether pathogens or not. In response to fungal invasion, plants synthesize and accumulate flavonoids, phytoalexins, terpenoids, aglycones, phenolic derivatives and other antimicrobial compounds. *Trichoderma* strains are usually more resistant to these compounds than the majority of fungi, their capacity to colonize plant roots robustly depend on the ability of each strain to bear those (Harman et al. 2004).

1.3. Role of Glucanase Gene in Plant Defense System

The β-glucanases have commercial application in the textile industry as fading agent and hydrolysis of cellulose during drying of beans and processing of coffee. It is used for the oxidative degradation of biomass into biofuel. The β-glucanases are valuable medication for the allergies caused by foodstuff and environmental pollution. The β-glucanases is commonly used for detoxification of cells from array toxic compounds, codon hydrotherapy and chronic pain syndromes. Infections caused by yeast *Candida albicans*, abnormal abdominal swelling characterized as bloating, facial anxiety or paralysis are also treated by β-glucanases. The β-glucanases aids in the removal of excess cholesterol from the intestine and the breaking down of the cellulosic cell wall of plants (Alvarez et al. 1993).

There are two type of glucanases exo β-1,3-glucanases and exo- β-glucanases. Glucose monomers are produced by exo β-1,3-glucanases and oligosaccharides produced by exo-β-glucanases when hydrolyzed (Cohen-Kupiec et al., 1999). Glucanases play an important part in defense mechanism, cell differentiation (Donzelli et al. 2001)and cell development (De La Cruz et al. 1995). Glucanases are also important in plant and fungal pathogen interaction. 1-3-glucan is a substrate of glucanases that is present in laminarin and callose in the cell wall of fungi which is induced by environmental stress or by the attack of pathogens on plants. Glucanases protect plants by preventing the growth of fungal pathogens (Jach et al. 1995). Glucanase secrets elicitors from pathogen cell wall to stimulate defense mechanism (Keen and Yoshikawa 1983).β-1,3-glucanases play an important roles i.e., in higher plants as well as cell lines play role in defense mechanism when microorganisms invade. *Trichoderma* spp. secrete numerous hydrolytic enzymes having antifungal properties (Ait-Lahsen et al. 2001).

Glucanases are present in many higher plants, fungi, yeast, actinomyces, insects and fish (Pan et al. 1989) and are considerable to be one of the important component of plants defense mechanism either alone or in combination with chitinases or other antifungal proteins. Peumans et al. (2000) reported that the occurrence of the β-1, 3-glucanases in tobacco, soybean, rubber tree. Yamaguchi et al. (2002) reported parallel results in rice. A single plant might have β-1, 3-glucanases that are different in size i.e. tobacco have more than 14 types. Many (PR) pathogenesis-related proteins that are induced by the plant defense system have direct antimicrobial effects. For example, defensins, chitinases, glucanases, protease inhibitors and hydrogen per-oxide-generating enzymes are all induced as part of the hypersensitive response. Numerous attempts have been made to introduce genes encoding these into transgenic plants under the control of constitutive promoters to increase the level of the proteins and enhance resistance.

The β-1,3-glucanases and β-1,6-glucanases are semi constitutive enzymes. The ability of *Trichoderma* spp. to inhibit the growth of fungal parasites can be used as a tool for *in-vitro* screening of biocontrol candidates (Sivan and Chet 1989). Kubicek et al. (2001) reported the use of different species of *Trichoderma* as biological control agent of
plant diseases and purified enzymes in the form of expressed genes in transgenic plants. The mechanisms involved in the antagonistic effect of Trichoderma species against the plant pathogen are important in the selection of suitable biological control agents for safe utilization and more effective as an arsenal for chemical control of plant pathogens (Vinale et al. 2008).

Genetic engineering holds the potential to improve disease resistance by inserting genes from any species that produce resistance proteins to any crop (van der Biezen 2001; Razzaq et al. 2021a; Zalar et al. 2022).

<p>| Table 1: Described the plasmid, genes and their gene delivery method for developing transgenic plants resistant to fungus |</p>
<table>
<thead>
<tr>
<th>Name of the Gene</th>
<th>Source</th>
<th>*Method of Transformation</th>
<th>Vector</th>
<th>Host plant</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitinase (chi1)</td>
<td>Rhizopus oligosporus</td>
<td>ABM</td>
<td>pBI121CH</td>
<td>Tobacco</td>
<td>Terakawa et al. (1997)</td>
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<td>Chitinase (RCC2)</td>
<td>Rice</td>
<td>ABM</td>
<td>pBI121-EN4-RCC2</td>
<td>Chrysanthemum</td>
<td>Takatsu et al. (1999)</td>
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<td>Glucanase (SN1)</td>
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<td>ABM</td>
<td>pBI101.1</td>
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<td>Cheong et al. (2000)</td>
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<td>pBI121-RCC2</td>
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<td>pC1301-HY</td>
<td>Rice</td>
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<td>p’YOT175G</td>
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<td>pBI121-pBTex</td>
<td>Peanut</td>
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<td>pGL2RC7</td>
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<td>BioIastic</td>
<td>KLYX71-pHS2JS101</td>
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<tr>
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<td>ABM</td>
<td>pGlucChit</td>
<td>Potato</td>
<td>Chang et al. (2002)</td>
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<td>pART27-CT5</td>
<td>Tobacco</td>
<td>Carstens et al. (2003)</td>
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<td>Ribosome-inactivating protein (MOD1); Chitinase (RCH10)</td>
<td>MOD1 from Maize; RCH 0 from Rice</td>
<td>BioIastic</td>
<td>pZRC72</td>
<td>Rice</td>
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<td>Cationic peptide (msa3)</td>
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<td>pGAglubsens</td>
<td>Flax</td>
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<td>Trichoderma atroviride</td>
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<td>pCAMBIA (different vectors for each gene)</td>
<td>Rice</td>
<td>Mei et al. (2004)</td>
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<td>Chitinase (cbl); Ribosome in activating protein(Rip)</td>
<td>Chi from bean; rip from barley</td>
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<td>pBRC; pARIP; pBchE</td>
<td>Soya bean</td>
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<td>pCambia1300:ubi::nat::alp::nos; pCambia1300:ubi::synt::alp::nos</td>
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<td>BioIastic</td>
<td>pPin35S; pBar35S</td>
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</table>
Several complex mechanisms have been identified in plants which evolved in response to pathogens. Many genes have also been elucidated that are involved in the various pathways and immune response when fungus pathogen infestation occur (Islam 2006). These genes that play an important role in defense response have been used in production of transgenic plants that were fungal resistant (Grover and Gowthaman 2003). Glucanases, chitinases and other antifungal genes have been used for this purpose. In Table 1 described the genes name, delivery methods, source and host organism and gene transformation method used to develop transgenic plants.

Saprophytic fungi (that parasitizes other fungi as their food source) are potential natural sources to control the infectious plant pathogens. Trichoderma harzianum is one of these fungi that produces enzymes having the ability to

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<th>(AFP-PIN) preparations</th>
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<td>pHCI; pHGLU;</td>
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<td>ABM</td>
<td>pCubi::Ap-CecA:nos;</td>
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<td>pBinGB</td>
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<td>pCAMBIA2300</td>
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<td>pNSP3</td>
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<td>pCAMBIA 1300:ubi-383; pCAMBIA 1300:ubi-638; pCAMBIA 1300:ubi-POCl</td>
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*ABM=Agrobacterium-mediated.
hydrolyze the cell wall of other fungi resulting in their destruction (Hendrix and Stewart 2002). *Trichoderma* spp. is one of the biocontrol agents successfully employed for the biological control of many economically essential pathogens (Steyaert et al. 2004). *Trichoderma* spp. show biocontrol activity and have ability to degrade chitins and glucans by chitinolytic and glucanolytic enzymatic activity. Glucanases are therefore one of the key groups of enzymes involved in mycoparasitism (Balasubramanian et al. 2012). Plant transformation plays an important role in improvement of agronomically significant traits. To develop transgenic plant selectable marker genes are used for analysis of successful transformation. Recently many selectable marker genes have been identified that are environmentally friendly and safe. (Wei et al. 2012; Razzaq et al. 2021b).

2. Conclusion

Fungal pathogens are one of the serious disastrous agents. Previously disease resistant varieties, application of appropriate fungicides, the use of biocontrol agents, plant-based extracts and induction of innate host resistance were developed to prevent fungal diseases. Resistance development against the fungal pathogens inspires scientists to explore modern technologies for the development of plants having broad spectrum resistance against plant pathogens. Transgenic technology has great potential against fungal pathogens.

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