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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. α-1, 3-glucanases, β-1, 3-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

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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-

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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. *Trichpderma* 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-glucans 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, actinomycetes, 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, proteinase 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 *Trichpderma* 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 *Trichpderma* as biological control agent of

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plant diseases and purified enzymes in the form of expressed genes in transgenic plants. The mechanisms involved in the antagonistic effect of *Trichpderma* 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; Zafar et al. 2022).

Table 1: Described the plasmid, genes and their gene delivery method for developing transgenic plants resistant to fungus

Name of the Gene	Source	*Method of Transformation	Vector	Host plant	References
Chitinase (chi1)	*Rhizopus oligosporus*	ABM	pBI121CH	Tobacco	Terakawa et al. (1997)
Chitinase (RCC2)	Rice	ABM	pBI121-EN4-RCC2	Chrysanthemum	Takatsu et al. (1999)
Glucanase (SGN1)	Soybean	ABM	pBI101.1	Tobacco	Cheong et al. (2000)
Chitinase (RCC2)	Rice	ABM	pBI121-RCC2	Grapevine	Yamamoto et al. (2000)
Trichosanthin (TCS)	Snake gourd	ABM	pC1301-HY	Rice	Xiaotian et al. (2000)
Chitinase (Oschia)	Rice pistils	ABM	pYOT175G	Rice	Takakura et al. (2000)
Chitinase (Chi)	tobacco	ABM	pBI121-pBTex	Peanut	Rohini and Rao. (2001)
Chitinase (RC7)	Rice	Biolistic and PEG-mediated	pGL2RC7	Rice	Datta et al. (2001)
Chitinase (RCC2)	Rice	ABM	pBI121_/RCC2	Cucumber	Kishimoto et al. (2002)
Chitinase like cDNA (Chs2)	American elm	Biolistic	KYLX71-pHS2;JS101	Creeping bent grass	Chai et al. (2002)
b-1,3 glucanase and chitinase genes	Pea	ABM	pGlu;pChit	Potato	Chang et al. (2002)
Chitinase (CTS1-2)	*Saccharoyces cerevisiae*	ABM	pART27:CTS	Tobacco	Carstens et al. (2003)
Ribosome-inactivating protein (MOD1); Chitinase (RCH10)	*MOD1* from Maize; *RCH 0* from Rice	Biolistic	pZRC72	Rice	Kim et al. (2003)
Stress-inducible β-Glucanase (Gns1)	Rice	ABM	pBI333-35S-Gns1	Rice	Nishizawa et al. (2003)
Chitinase (RCH10); Glucanase (ALG)	*RCH10* from rice; *ALG* from alfalfa	Biolistic	pABT127; pZ100	Creeping bent grass	Wang et al. (2003)
Chitinase (chi11)	Rice	ABM	pMKU-RF2	Rice	Kumar et al. (2003)
Cationic peptide (msrA3)	Synthetic preparation	ABM	pDMSRA3-1217	Potato	Osusky et al. (2004)
Glucanase (Bglu)	Potato	ABM	pGAglubsens	Flax	Wro ´bel Kwiatkowska et al. (2004)
Chitinase (ech42); Chitinase (nag70); Glucanase (gluc78)	*Trichoderma atroviride*	ABM	pCAMBIA (different vectors for each gene)	Rice	Mei et al. (2004)
Chitinase(Chi); Ribosome in activating protein(Rip)	*Chi* from bean; *rip* from barley	ABM	pBRC; pARIP; pBchE	Soya bean	Li et al. (2004)
Chitinase (CHIT); Glucanase (GLUC)	*CHIT* from cucumber; *GLUC* from tobacco	ABM	pIL12	Potato	Moravčíková et al. (2004)
Glucanase (OsGLN2)	Rice	ABM	pGST-OsGLN2	Rice	Akiyama et al. (2004)
Antifungal protein (Afp)	*Aspergillus giganteus* (chemically synthesized)	ABM	pCambia1300:ubi::nat-afp::nos; pCambia1300:ubi::synt-afp::nos	Rice	Coca et al. (2004)
Chitinase (BjCHI1); Glucanase (HbGLU)	*HbGLU* from rubber tree; *BjCHI1* from mustard	ABM	pBj17; pBj47; HEV43	Potato	Chye et al. (2005)
Antifungal protein	Prawn (Synthetic	Biolistic	pPin35S; pBar35S	Finger miller	Latha et al. (2005)

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(AFP-PIN)	preparations)				
Chitinase (Chi)	Bean	ABM	pBI121-BCH	Cotton	Tohidfar et al. (2005)
Chitinase (RCC2)	rice	ABM	pBI121	Trifoliate orange	Mitani et al. (2006)
Chitinase (ch5B); Glucanase (gln2); Antifungalprotein(ap24)	*ch5B* from Beans (*Phaseolus vulgar is*); *gln2* and *ap24* from tobacco	ABM	pHCHI; pHGLU; pHAP24; pHCA35; pHGA37, pHGC39	Strawberry	Vellicce et al. (2006)
Chitinase; Glucanase	Barley	ABM	pKitGluk:I	Oilseed rape	Melander et al. (2006)
Glucanase (GLU); Antifungal protein (alfAFP); Glucanase (GLU-AFP)	*GLU* from tobacco; *alfAFP* from Alfalfa Synthetically prepared	ABM	pEAFP; pEGlu; pAFP-Glu	Tomato	Chen et al. (2006)
ER-CecA;Ap-CecA(CecropinA)	Synthetically prepared	ABM	pCubi::Ap-CecA::nos; pCubi::ER-CecA::nos	Rice	Coca et al. (2006)
Antifungal protein (Afp)	Aspergillus giganteus	Biolistic	pubi2afp; p35SAcS	Pearl millet	Girgi et al. (2006)
Antifunga lprotein (AFP-PIN)	*Prawn* (Synthetic preparations)	Biolistic	pPin35S; pBar35S	Pearl millet	Latha et al. (2006)
a-I-purothionin;tlp-1gene;b-1,3-glucanase gene	*a-1- purothionin* from wheat; *tlp-1* &b-1,3-glucanase from barley	Biolistic	pKM1; pUBKBarGluc-3; pAHCBarPR5; pAHC25	Wheat	Mackintosh et al. (2006)
Chitinase (CHIT); Glucanase (GLUC)	*CHIT* from cucumber; *GLUC* from tobacco	ABM	pJL06	Potato	Mackintosh et al. (2006)
Chitinases (RCH10&RAC22); Glucanase (β-Glu); Ribosomeinactivatingprotein (B-RIP)	*RCH10* and *RAC2 2* from rice; *b-Glu* from alfalfa; *B-RIP* from barley	Biolistic	pRAS1300	Rice	Zhu et al. (2007)
Chitinase(chi11); Thaumatin-likeprotein (tlp)	Rice	Biolistic	pAHG11; pAHRC-tlp	Barley	Tobias et al. (2007)
Glucanase	Tomato	ABM	PBinGB	Indian mustard	Mondal et al. (2007)
Chitinase (ricchi11)	Rice	ABM	pBI121/ricchi11	Taro	He et al. (2008)
Chitinase (ChiC)	Streptomyces griseus	ABM	pEKHGCO1A	Potato	Raham et al. (2008)
Mustard defencin (BjD)	Mustard	ABM	pCAMBIA2300	Tobacco, peanut	Anuradha et al. (2008)
Chitinase (chi11); Glucanase (gluc)	*chi11* from rice; *gluc* from Tobacco	ABM	pNSP3	Rice	Sridevi et al. (2008)
Chitinase 383; Glucanase 638; Cationic peroxidase (POC1)	Chitinase and Glucanase from Wheat; *POC1* from Rice	ABM	pCambia1300:ubi-383; pCambia1300:ubi-638; pCambia1300:ubi-POC1	Carrot	Wally et al. (2009)
Chitinase (Chit30)	*Streptomyces olivaceoviridis*	ABM	pGreenII0229	Pea	Hassan et al. (2009)
β -1,6- glucan (neg1)	*Penicillium islandi cum, Agaricus brasiliensis*		pCold I DNA		Yamanaka et al. (2020)
licA	*Orpinomyces sp. GMLF 18*	Electroporation	pIL253	*L. lactis.*	Uğur ÇÖMLEKCİOĞLU et al. (2011)
β-1,3-glucanase	*oomycete Phytophthora spp.*	ABM	pCAMBIA 1381Z TDNA vector	*Hevea brasiliensis*	Radhakrishnan et al. (2021)
FfGS6	*Flammulina filiformis*	ABM	pBHg-Egfp	Fujian Edible Fungi	Liu, Yuanyuan et al. (2022)

*ABM=Agrobacterium-mediated.

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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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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