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REVIEW ARTICLE

Rice chitinase gene as a tool to develop fungal resistant plants—A review

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Abstract

Plant diseases are caused by a variety of plant pathogens including fungi and their management requires the use of techniques like transgenic technology, molecular biology and genetics. Genes encoding chitinase can deteriorate fungal cell-wall components by enhancing the pathogenesis related (PR) proteins, so as to develop fungal disease-resistant plants via recombinant DNA technology. Rice chitinase gene is attractive candidate for this approach. Rice chitinase gene has been inserted to a variety of plants and all plants showed fungal disease resistance. In this review, different plants showing fungal disease resistance on insertion of rice chitinase gene through transgenic approach are reviewed

Introduction:

Fungal attacks occur in most of the agricultural and horticultural species; more than 70% of all major crop diseases are caused by fungi (Agrios, 2005). Fungal diseases are rated either the most important or second most important factor contributing to yield losses in major crops like tomato (Lee & Raikel, 1995), wheat (Anand et al., 2003), spring wheat (Smith, 2002), cotton (Cui et al., 2000), groundnut (Mace et al., 2006) and grapevine (Dhekney et al., 2007). Fungal plant diseases are usually managed with the applications of chemical fungicides. For some diseases, chemical control is very effective, but it is often non-specific in its effects, killing beneficial organisms as well as pathogens and may have undesirable health, safety and environmental risks (Manczinger et al., 2002).

A promising method for protecting plants against diseases is constructing and employing pathogen-resistant cultivars. Although a number of resistant cultivars have been developed through breeding programs, but these cultivars become obsolete in a short time due to the rapid evolution of the phytopathogens and the emergence of virulent forms capable to overcome the plant resistance.

The most significant development in the area of varietal development for disease resistance is the use of the techniques of gene isolation and genetic transformation to develop transgenic plants resistant to fungal diseases. Recombinant DNA and genetic transformation technologies can circumvent taxonomic limitations to the gene pool for pathogen resistance even to the extent that plants need not be the only sources for disease resistance traits. Further, molecular biological techniques provide capabilities to engineer host plant resistance that is effective both against specific as well as a broad spectrum of pathogens and are genetically stable. Further, these techniques permit to locate, clone and sequence individual genes (DNA fragments) from a complex DNA sequences (Yun et al., 1997).

Genetic transformation technology promises to overcome the major agronomic problems not yet solved through recombination breeding due to the non-availability of relevant genes within the accessible primary or secondary gene pools. Recent advances in tissue culture and recombinant DNA technology have opened new avenues of transformation of crop plants to produce transgenic plants new genetic properties (Yun et al., 1997), including specific applications for the semi-arid tropics (Sharma & Ortiz, 2000). Rice chitinase gene is being transferred in various crops by recombinant DNA technology and genetic transformation either by direct DNA transfer or by Agrobacterium-mediated gene transfer. More cases of Agrobacterium-mediated gene transfer are being reported.

Plants are equipped with a variety of defence mechanisms to protect themselves against the attack of pathogens. Some of these are constitutive, while others are induced upon the attack by pathogens. The interaction between plants and pathogens induces a variety of defence mechanisms, which includes cell wall strengthening (Bradley et al., 1992), de novo production of antimicrobial compounds (pathogenesis response proteins and secondary metabolites) (Hammerschmidt, 1999, Misra & Gupta., 2009, Gupta et al., 2010), rapid localized cell death, etc (Alverez, 2000). Genes encoding chitinase enhance the production of pathogenesis response proteins, which hydrolyzes chitin (Jeuniaux, 1966) and protect the plant from fungal pathogen. Rice chitinase gene encoding chitinase is transferred in plants by recombinant DNA technology and genetic transformation to develop fungal resistant plants.

The present review provides an overview of current knowledge concerning rice chitinase gene as a tool to develop fungal resistant plants.

Chitinase

Chitin is a polysaccharide found in the outer skeleton of insects, crabs, shrimps and lobsters, and in the internal structures of other invertebrates. It also constitutes 3-60% of cell wall in various fungi (Bartnicki-García, 1968). Chitin is composed of ß-(1,4) linked units of the amino sugar, N-acetyl-glucosamine. Chitinase attacks on chitin molecules and catalyzes the hydrolysis of the ß-(1,4) linkages of the N-acetyl-D glucosamine polymer chitin.

A wide variety of biochemical constituents, including small molecules, peptides and sugar polymers, contribute to the interactions between plant and fungal or Oomycete pathogens. Molecules of microbe or plant origin are critical for the pathogen recognition and further triggering of plant defence responses. Moreover, defence molecules of a broad range of biological activity are produced by plants either before fungal attack (preformed) or in response to infection (induced). During the course of their host adaptation, fungal pathogens develop sophisticated molecular tools to overcome plant biochemical weapons. These tools include enzymes, which are able to metabolise plant bioactive molecules as well as own toxins interfering with plant defence reactions and chitinase is one of them.

Pathogenesis-related Proteins

Pathogenesis-related (PR) proteins are a class of novel proteins that are synthesized de novo and accumulated in plant tissues after pathogen infection. PR proteins synthesise hydrolytic enzyme chitinase, which can hydrolysis major component of fungal cell wall, i.e., chitin. Hydrolysis of these fungal cell wall leads to the inhibition of the growth of several fungi in vitro (Punja, 2006).

Rice Chitinase Gene

Recombinant DNA technology allows the enhancement of inherent plant responses against a pathogen by either using single dominant resistance genes not normally present in the susceptible plant (Keen, 1999) or by choosing plant genes that intensify or trigger the expressions of existing defense mechanisms (Bent and Yu, 1999, Rommens and Kishore, 2000). The availability of techniques in molecular biology now permits the isolation of specific genes and their reintroduction into plants, providing a powerfull tool to elucidate the roles of specific enzymes in plants (Muthukrishnan et al., 2001) and rice chitinase gene is one of them.

Recently it is reported that various transgenic plants expressing rice chitinase gene showed resistance to different fungal diseases. It is observed that class-I chitinase gene, Cht-2 or Cht-3, showed significant resistance against two races of Magnaporthe grisea (Nishizawa et al., 1999). Similarly on insertion of rice chitinase or rice thaumatin-like protein, transgenic plants showed enhanced resistance against Rhizoctonia solani (Lin et al., 1995; Datta et al., 1999; Datta et al., 2000; Datta et al., 2001) and R. Solani (Datta et al., 2001). Likewise, transgenic rice expressing rice endochitinase gene showed enhanced resistance to sheath blight (Lin et al., 1995), transgenic tobacco expressed resistance against powdery mildew (Erysiphe cichoraccrum) (Nishizawa et al., 1993) and transgenic cucumber plants showed resistance to grey mold (Bortytis cinnerra) (Tabei et al., 1998) . Rice plants induced with chitinases and ß-1,3-glucanase resulted in moderate field resistance to sheath blight caused by R. solani (Anuratha et al., 1996). Therefore, chitin metabolism forms an excellent target for selective pest control strategies (Kramer & Koga, 1986; Cohen, 1993; Kramer et al., 1997).

Transfer of rice chitinase gene via recombinant DNA technology

Two ways of transfer of rice chitinase gene via recombinant DNA technology are reported till date:

Direct DNA transfer

Physical as well as chemical methods have been developed to facilitate DNA delivery across the plasma membrane, which lead to both stable and transient gene expression. Microinjection, sonication, elctroporation and biolistic mediated transfer are the main procedures to let the desired DNA molecules enter any living cell; plant, animal, or

microbial. Microinjection involves immobilization of protoplasts and micro injecting DNA directly into the nucleus. Electroporation can also be used to introduce exogenous DNA to plant protoplasts (dicot and monocot). Whereas in the process of sonication; low frequency ultra sonic waves ranging between 1.O MHz-1.5 MHZ have the capacity to produce small pores in the cell which facilitate the entry of a plasmid containing the desired gene (Zhmg et al., 1997). While biolistic method involves bombardment of particles carrying DNA of interest onto target cells using high velocity transfer mechanism.

Transgenic sorghum plants constitutively expressing gene encoding class I rice chitinase (chrll) were generated by biolistic transformation of scutellum-derived embryogenic calli with a plasmid DNA carrying the bar gene as the selectable marker. The rice chitinase gene under control of a cauliflower mosaic virus 35S promoter showed increased resistance to the stalk rot causing fungus, Fusarium thapsinum (Krishnaveni et al., 2001). Transgenic rice was developed from both calli and immature embryos of popular cultivar 'Swarna' by transfer of rice chitinase gene (chill) by particle gun bombardment (Baisakh et al., 2001), whereas wheat was biolistically transformed with a vector DNA containing a rice chitinase gene under the control of CaMV 35S promoter and the bar gene under the control of ubiquitin promoter as a selectable marker (Chen et al., 1998). A chitinase gene (RCH8) in plasmid vector pCAMBIA1308 was also delivered into 3 wheat cultivars (Yangmai 158, Wan 9210, Wanmai 32) by low energy Ar+ beam mediated method (Lifang et al., 2001). Co-bombardment, integration and expression of rice chitinase and thaumatin-like protein genes in barley (Hordeum vulgare cv. Conlon) was also attempted (Tobias et al., 2007).

Agrobacterium mediated DNA transfer

Agrobacterium mediated DNA transfers is the most common and widely used method for transformation of dicotyledonous plants. The first transgenic Nicotiana tabaccum plants were produced via Agrobacterium mediated transformation (Barton et al., 1987). Although members of Agrobacterium have a wide host range, the ability of the bacterium to produce a compatible reaction varies widely with the host plant species and even with genotypes within a species. Further discoveries in this field indicated that even in cereals and non-host species Agrobacterium mediated DNA transfer system could work with the aid of phenolic compound called acetosyringone.

Different strains of Agrobacterium were reported in various crops during Agrobacterium mediated rice chitinase gene transfer. Transgenic cotton (Gossypium hirsutum cv. SVPR2) plants were produced by pCambia-bar-Chi II (13.8 kb) under the control of the CaMV 35S promoter, harboured in strain LBA4404 by using shoot tip explants (Ganesan et al., 2009), whereas Agrobacterium tumefaciens strain LB4404 harbouring the binary vector (pB1333-EN4-RCG3) and containing the chitinase (chit) in transgenic peanut for fungal pathogen resistance (Iqbal et al., 2012). A. tumefaciens strain EHA105 harboring plasmid pBI121/ricchi11 and containing rice chitinase gene (ricchi11) was used for transformation in taro (Colocasia esculenta (L.) Schott) (He et al., 2008).

To enhance the antifungal response of litchi (Litchi chinensis Sonn.) (cv. Bedana), transgenic plants were generated by transferring rice chitinase gene driven by a maize-ubiquitin promoter along with its first intron into the zygotic embryos via Agrobacterium tumefaciens-mediated transformation (Das & Rahman, 2012). The rice chitinase gene (CHI), the alfalfa defensin gene (alfAFP) and their bivalent gene (CHI-AFP) were introduced into tomato via Agrobacterium-mediated gene transfer method and transgenic tomato showed enhanced resistance to Botrytis cinerea (Chen et al., 2009). Whereas transgenic cucumber plants (Cucumis sativus L.) resistant to gray mold (Botrytis cinerea) were also developed by Agrobacterium mediated gene transfer by the expression of rice chitinase cDNA and its environmental risk assessment was done (Tabei et al., 1999).

Agrobacterium strain LBA4404 helped in transformation of the tropical forage legume Stylosanthes guianensis with a rice-chitinase gene which confered resistance to Rhizoctonia foliar blight disease (Kelemu et al., 2005), similarly transgenic strawberries were developed resistant against pathogenic fungus Sphaerotheca humuli on insertion of rice chitinase gene under the control of cauliflower mosaic virus (CaMV) 35S promoter using Agrobacterium tumefaciens strain LBA4404 carrying a pB1121-RCC2 (Asao et al., 1997). Rice chitinase gene-RCC2 with vector pB1333-EN4 was introduced under the control of enhanced CaMV 35S promoter via Agrobacterium strain EHA101 in Basmati 385 and fungal disease resistant variety was developed (Asghar et al., 2007). Transgenic grapevine (Vitis vinifera L.) were also generated by transferring rice chitinase gene under a maize-ubiquitin promoter along with its first intron into the leaf disc-induced somatic embryos via Agrobacterium mediated transformation with strain LBA4404 (Nirala et al., 2010) while factors affecting Agrobacterium mediated gene transformation in tomato (Lycopersicon esculentum Mill.) cv. Riogrande using rice chitinase (CHT-3) gene was also studied (Jabeen et al., 2009).

Some very important crops like pigeonpea (Cajanus cajan (L.) Millsp.) was made fungal disease resistant on insertion of rice chitinase gene harboured in the plasmid pCAMBIA 1302:RChit delivered via Agrobacterium (Kumar et al., 2004), similarly transgenic finger millet (Eleusine coracana (L.) Gaertn.) was developed resistant to leaf blast disease on insertion of rice chitinase (chi11) gene through Agrobacterium-mediated transformation

(Ignacimuthu & Antony Ceasar, 2012). Transgenic groundnut (Arachis hypogea) were developed fungal resistant using disarmed Agrobacterium strains harbouring pCAMBIA 1302:Rchit plasmid (Ramu, 2001) . But in sorghum plants Agrobacterium strains used were LBA4404 harbouring pcambia-ubi-chi11 (rice chitinase), EHA105 harbouring pcambia-ubi RC7 (rice chitinase) with bar gene and EHA105 harbouring pMKURF2 (rice chitinase gene) having hph gene were used (Arulselvi et al., 2010).

Likewise, rice chitinase gene (RCC2) was transferred in banana cultivar, Rastali (AAB) via Agrobacterium strain (EHA 101) into single buds with plasmid pBI333-EN4-RCC2 (Sreeramanan et al., 2009). Genetic transformation in potato was carried out using Agrobacterium tumefaciens strain ERA 101 harboring binary plasmid vector pBI333-EN4-RCG3 (Iqbal, 2007), while genetic transformation in rice (cv. Chainat 1) was mediated by Agrobacterium tumefaciens strain LBA4404 which harbored the plasmid pCAMBIA 1305.1 (Maneewan et al., 2005). The chitinase gene HCH1 deriving from the powdery mildew resistant hop cultivar 'Zenith' was inserted into the genomes of two powdery mildew susceptible hop cultivars by Agrobacterium-mediated transformation (Miehle & Seigner). A. tumefaciens strain LBA4404 carrying pBI121 plasmid containing chitinase gene under control of CaMV35S was used for genetic transformation in Rosa damascene cv. Ghamsar (Pourhosseini et al., 2012) and Agrobacterium tumefacien strain LBA4404 harboring plasmid pB1333-EN4-RCG3 having chitinase gene (RCG3) was used for genetic transformation in potato (Ahmad et al., 2012) .

A rice chitinase cDNA (RCC2) was introduced into Grapevine (Vitis vinifera) (Yamamoto et al., 2000) and groundnut (Sharma et al., 2006) through Agrobacterium mediation whereas rice chitinase gene (chi11) isolated from Oryza sativa was introduced into tomato (Lycopersicon esculentum Mill.) through Agrobacterium mediated transformation using ubiquitin promoter (Kalaiarasan et al., 2008). A. tumefaciens strain LBA4404 harbouring the transforming vector pCAMBAR chi11 containing chitinase gene was used in transformation studies using leaf as an explant in apple rootstock MM106 (Sharma et al., 2012), Safflower (Carthamus tinctorius L.) also showed fungal resisance similarly (Kumar et al., 2009) while Agrobacterium-mediated genetic transformation was also opted for transformation in chrysanthemum (Dendranthema grandiflora Tzelev) cv. 'Snow Ball' using internode explants (Sen et al., 2011) but Agrobacterium strain, EHA 105 harboring plasmid pMKU-RF2 was used for genetic transformation in rice (Kumar et al., 2003) .

(Ramat.) Kitamura variety of chrysanthemum (Dendranthema grandiflorum) was attempted for transformation using Agrobacterium tumefaciens strain C58 and MP90 harboring rice chitinase gene (RCC2) (Takatsu et al., 1999). Cotton cultivar Coker was also transformed with recombinant pBI121-chi via Agrobacterium tumefaciens carrying the chitinase (chi) gene from bean under the control of the CaMV35S promoter in transgenic offspring of cotton (Gossypium hirsutum) (Tohidfar et al., 2009). The class I chitinase cDNA (RCC2) of rice driven by the CaMV 35S promoter was introduced into cucumber by Agrobacterium-mediated transformation (Kishimoto et al., 2002).

Transformation in trifoliate orange (Mitani et al., 2006), Iranian rice (Oriza Saiva L.) (Sheidai et al., 2009) and Indica rice (Oryza sativa) with rice chitinase gene (Sridevi et al., 2003) was also attempted as reported in Table

Conclusion

After discussing the transfer of rice chitinase gene in various crop varieties either by direct gene transfer or via Agrobacterium mediated gene transfer, all the crop varieties showed resistant to various fungal pathogens. Moreover, the subsequent transgenic generations also retained the fungal resistant property. These transgenic plants with enhanced disease resistance can become a valuable component of a disease management program in the future and rice chitinase gene as a efficient tool for its development. It was also observed that Agrobacterium mediated gene transfer is most suitable for transfer of rice chitinase gene. At last, it is advisable to attempt to transfer rice chitinase gene to other crops also so as to develop more fungal resistant crop varieties.

Table 1. Rice chitinase gene transformed plants to fungal disease resistant

S. No.	Plant	Rice chitinase gene type	Fungal pathogen/disease resistance	References
1.	Strawberry (Fragaria × ananassa)	Rice chitinase gene (RCC2 Pbi121)	Sphaerothera humuli	Asao et al., 1997
2.	Bread wheat (Triticum sativum)	Rice chitinase gene (chi1)	Fungal disease	Chen et al., 1998
3.	Cucumber (Cucumis sativus L)	RCC2	Gray mold resistance	Tabei et al., 1998
4.	Japonica rice	Class-I chitinase (Cht-2,	Magnaporthe grisea	Nishizawa et al., 1999

	(Oryza sativa)	Cht-3)		
5.	Cucumber (Cucumis sativus L)	RCC2	Gray mold (Botrytis cinerea)	Tabei et al., 1999
6.	Chrysanthemum (Dendranthema grandiflorum (Ramat.) Kitamura)	RCC2	gray mold (Botrytis cinerea)	Takatsu et al., 1999
7.	Indica rice (Oryza sativa)	Class-I chitinase (Chi11)	Rhizoctonia solani	Datta et al., 2000
8.	Grapevine (Vitis vinifera)	RCC2	Fungal disease	Yamamoto et al., 2000
9.	Rice (cv. Swarna) (Oryza sativa)	Rice chitinase gene (chi1)	Sheath blight disease	Baisakh et al., 2001
10.	Rice (Oryza sativa)	RC7chitinase PR-3	Rhizoctonia solani	Datta et al., 2001
11.	Wheat (Triticum sativum)	Rice chitinase gene (RCH8)	Scab resistance	Lifang et al., 2001
12.	Groundnut (Arachis hypogaea)	Rice chitinase gene	Rhizoctonia solani	Ramu D Vijaya., 2001
13.	Cucumber	RCC2	gray mold (Botrytis cinerea),	Kishimoto et al., 2002
14.	Rice	rice chitinase gene	Rhizoctonia solani	Kumar et al., 2003
15.	Indica rice (Oryza sativa)	Class-I chitinase (Chi11)	Rhizoctonia solani	Sridevi et al., 2003
16.	Pigeonpea (Cajanus cajan)	Rchit	Fusarium oxysporum	Kumar et al., 2004
17.	Stylo (Stylosanthes guianensis)	Rice chitinase gene	Foliar blight disease	Kelemu et al., 2005
18.	Rice (Oryza sativa L.) cv. Chainat	Rice chitinase gene (chi11)	Fungal disease	Maneewan et al., 2005
19.	Trifoliate orange (Poncirus trifoliata Raf.)	RCC2	Fungal disease	Mitani et al., 2006
20.	Groundnut	rice chitinase gene	Aspergillus flavus	Sharma et al., 2006
21.	Basmati Rice (Oryza sativa)	RCC2	Uncinula necator	Asghar et al., 2007
22.	Potato	Rice chitinase gene (RCG3)	Fungal disease	Iqbal Hussain., 2007
23.	Barley (Hordeum vulgare L.) cv Conlon	chitinase gene (chi11)	Fungal disease	Tobias et al., 2007
24.	Taro (Colocasia esculenta)	Rice chitinase gene (chi11)	Sclerotium rolfsii	He et al., 2008
25.	Tomato (Lycopersicon esculentum Mill.)	rice chitinase gene (chi11)	root-knot nematode	Kalaiarasan et al., 2008
26.	Tomato (Solanum lycopersicum)	Rice chitinase gene (chi1)	Botrytis cinerea	Chen et al., 2009
27.	Cotton (Gossypium hirsute)	Rice chitinase gene (chi11)	Fusarium oxysporum and Alternaria macrospora	Ganesan et al.,2009
28.	Tomato	Rice chitinase (CHT-3)	Fungal disease	Jabeen et al., 2009

	(Lycopersicon esculentum Mill.) cv. Riogrande	gene		
29.	Safflower (Carthamus tictorius L.)	rice chitinase gene	Alternaria Leaf Spot Disease	Kumar et al., 2009
30.	Iranian Rice (Oriza Saiva L.)	Rice chitinase gene (RICCH-1, RICCH-2 and RICCH-3)	Sheath blight disease	Sheidai et al., 2009
31.	Banana (Musa babisiana)	Rice chitinase gene (RCC2)	Fungal disease	Sreeramanan et al., 2009
32.	Cotton (Gossypium hirsutum)	Rice chitinase gene (chi11)	Fusarium oxysporum and Alternaria macrospora	Tohidfar et al., 2009
33.	Sorghum (Sorghum bicolour)	Rice chitinase gene	Fungal disease	Arulselvi et al., 2010
34.	Grapevine (Vitis vinifera)	Class1 chitinase RCC2	Uninula necator	Nirala et al., 2010
35.	Chrysanthemum cv. 'Snow Ball'	Rice Chitinase (chiII)	Fungal disease	Sen et al ., 2011
36.	Potato (Solanum tuberosum L.)	Rice chitinase gene (RCG3)	Fungal disease	Ahmad et al., 2012
37.	Litchi (Litchi chinensis Sonn.)	Rice chitinase gene	Leaf spot disease	Das & Rahman., 2012
38.	Finger millet (Elcusine coracocna)	Rice chitinase gene	Leaf blast disease	Ignacimuthu & Antony Ceasar., 2012
39.	Peanut (Arachis hypogaea)	Rice chitinase-3	Leaf spot disease	Iqbal et al., 2012
40.	Rosa damascena cv. Ghamsar	Rice chitinase gene	powdery mildew resistant	Pourhosseini et al., 2012
41.	Apple (Malus x domestica Borkh.)	rice chitinase gene (chi11)	Fungal disease	Sharma et al., 2012

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