



Research Paper

Heterologous expression of rice *chitinase* gene in garden pea (*Pisum sativum* L.) against powdery mildew

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ABSTRACT

Pisum sativum L. (Garden pea) is an annual crop of immense importance. In India it is cultivated in an area of more than 280.0 thousand hectares with annual production of more than 4.0 million tonnes but its yield decline heavily due to powdery mildew a fungal disease caused by *Erysiphe pisi*, *E. polygoni*; *E. cichoracearum* etc. Severe infection reduces plant growth seed weight, seeds per pod and pod number. Pod infection causes seed discoloration leading to downgrading its quality. Management using fungicides; most commonly triadimefon is in regular practice, but they only protect uninfected foliage and have limited systemic activity. Over time, the fungicides accumulate at the leaf margins, leaving other parts of the leaf more open to infection which also has hazardous effect on the environment. One of the effective methods for controlling fungal infection may be the introduction of rice *chitinase* into the sensitive but otherwise high yielding crops. Large number of genetic transformation procedures are available using both direct i.e. biolistic or gene gun or particle bombardment or in-direct i.e. *Agrobacterium* mediated for constitutive expression of introduced *chitinase* gene. Upon standardization of efficient shoot regeneration and transformation system in pea, transgenic plants may be produced against these fungi leading to reduced yield loss in this economically valued crop. The functional validation of *chitinase* in pea will show way for its effective utilization in future for reducing loss caused due to several fungi.

Keywords: *Agrobacterium*, *Chitinase*, *Erysiphe*, *Pisum sativum*, Transformation

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I. INTRODUCTION

Pisum sativum L. is an annual plant, with a life cycle of one year. It is grown in many parts of the world; planting can take place from winter to early summer depending on location (1). The average pea weighs between 0.1 and 0.36 grams. In India, the area under green peas rose continuously from 177.7 thousand hectares in 1991-1992 to 272.6 thousand hectares in 1999-2000. The percentage of area under peas in India to global area under peas has also risen from 3.2 percent in 1991-92 to 4.5 per cent in 1999-2000. The production of green peas has increased from 1.30 million tons in 1991-92 to 3.20 million tons in 2003-04.

A variety of diseases affect peas through a number of pathogens, including insects, viruses, bacteria and fungi (9). Among all these Powdery mildew caused by fungus *E.pisi* has a great economic importance (2&3). Powdery mildew caused by *E.pisi* DC results heavy losses in the yield and quality of pods and seeds of pea crop. Outbreak of this disease is associated with dry weather. The disease affects the crop between February to April. The disease develops late in season and reaches to maximum intensity at the time of pod formation. It attacks leaves first producing faint, slightly discolored specks from which grayish white powdery growth of mycelium and spores spread over leaf, stem and pod (5 &6). The leaves turn yellow and die. The fruits do not either set or remain very small. It causes (5-17) defoliation. Later stages; powdery growth also covers the pod making them unsuitable for markets.

These pathogenic fungi have always been a major problem in agriculture. One of the effective methods for controlling pathogen fungi to date is the introduction of resistance genes into the genome of crops. A rice *chitinase* gene under enhance version of CaMV 35S if introduced into pea (*Pisum sativum* L) through *Agrobacterium* mediation, will express fungal tolerance and crop damage can

be reduced. Putative transgenic shoots will be regenerated (11) and grown on MS medium supplemented with 5 mg/l BAP, 1 mg/l kinetin, and 30 mg/l hygromycin then examined and confirmed through Southern hybridization analysis of the genomic DNA. It has been observed that survival rate of the *in vitro* regenerated plantlets (11) was over 60% and all the plants flowered and set seed normally (6). Transgenic strains (10 & 11) exhibited a higher resistance than the control (non-transgenic plants).

II. MATERIAL AND METHOD:

1. Pea seeds will be collected from Bihar Agricultural University, Sabour.
2. Pea seeds will be sterilized and germinated *in vitro* and leaf explants will be collected *in vitro* condition.
3. These explants will be infected with *Agrobacterium tumefaciens*' strain 4404 having Rice chitinase gene solution and cocultivated in dark chamber.
4. After 24 hours these explants will be placed into MS modified medium against kanamycin antibiotics under Slandered Tissue culture condition i.e. Light condition 16 h l and 25+2 0C .
5. After two weeks Callus formation will start.
6. From this callus Under organogenic regeration firstly shoot buds will be organised.
7. These shoot buds will be separated and transferred into rooting medium.
8. Under rooting medium root will be formed.
9. Within two weeks a larger number of putatively transformed pea plants will be formed.
10. On Kanamycin antibiotics those which have NPTII genes are there they will detoxify kanamycin and grow into Kanamycin antibiotics..
11. Those putatively trannsformed plants will be collected and will be analysed by four methods. I. .PRR.

2. Southern hybridisation, Nothern hybridization and Western hybridization
Chitinase

Chitin is a poly sachharide found in the outer skellton of insects crabs, shrimps and constitutes enternal structure of other invertebrates.

3-60% cell wall is made up of fungi. Chitin is composed of β – (1,4) linked units of the amino sugar, N- acetyl glucosamine.

Chitinase attacks on chitin molecules and catalyzes the hydrolysis of the β – (1,4) linkages of the N- acetyl glucosamine polymer chitin.

Many variety of biochemical constituents, including peptides, sugar polymers and small molecules help in the interactions plant and fungal.

Plants defence response triggers further and are very critical for the plants for the pathogen recognition.

Defence are done by plant to induced infection i.e. plant produced molecules of different wide range for biological activity before fungal attack. As a plant biochemical weapons are developed.

By the fungal pathogens develop molecular tools to overcome. These tools include enzymes, which are able to metabolism plant bioactive molecule as well as its own toxins which interfere with plant defence reaction.among them chitinase is one of them.

Pathogenesis-related proteins:-

PR proteins are a class of novel proteins that are synthesized *de novo* and accumulated in plant tissues after pathogen infections. PR proteins synthesis hydrolytic enzyme chitinase, which hydrolyse major component of fungal cell wall i.e. chitin.

Rice-chitinase gene:-

Recombinant DNA technology allows the enhancement of inherent plant responses against pathogen by either using single dominant resistances genes not normally present in the susceptible plant (Keen 1999) or by choosing plant genes that intensify or trigger the expressions of existing defence mechanism (Bent and Yu 1999, Rommens and Kishore 2000).

The availability of tools and techniques in molecular biology now allows the isolation of specific genes and their reintroduction into plants, giving a helpful tools to show the roles of specific enzymes in plants (Muthu Krishnan et al 2001) and rice chitinase is one of them.

Recently it is reported that various transgenic plants expressing rice chitinase gene showed resistance to different fungal disease.

Similarly on insertion of rice chitinase like protein, transgenic plant showed enhanced resistance against *Rhizoctonia Solani* (Lin et al. 1995 Datta et al. 1999 Datta et al. 2000, Datta et al 2001), transgenic tobacco expressed resistance against powdery mildew (*Erysiphe cichoraccum* Nishizawa et al.1993) and transgenic cucumber plants showed resistance to grey mold (Tabei et al 1998).

Therefore chitin metabolism forms an excellent target for selective pest control strategies (Kramer and Korga 1986, Cohen 1993, Kramer 1996).

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 x ananassa)	Rice chitinase gene (RCC2 Pbi 121)	Sphaerotheca humuli	Asao et al., 1997
2	Bread wheat (Triticum sativum)	Rice chitinase gene (chil)	Fungal disease	Chen et al., 1998
3	Cucumber (Cucumis sativus L)	RCC2	Gray mold resistance	Tabei et al., 1998
4	Japonica rice(Oryza sativa)	Class-I chitinase (Cht-2,Cht-3)	Magnaporthe grisea	Nishizawa et al., 1999
5	Cucumber (Cucumis sativus L)	RCC2	Gray mold (Botrytis)	Tabei et al., 1999

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