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Heterologous Expression of Rice *Chitinase* Gene in Garden Pea (*Pisum sativum* L.) against Powdery Mildew

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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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 hactares with annual production of more than 4.0 million tonnes but its yield declines heavily due to powdery mildew a fungal disease caused by *Erysiphae pisi, E. polygoni; E. cichoracearum* etc*.* Severe infection reduces plant growth seed weight, seeds per pod and pod number. Pod infection causes seed discolouration leading to downgrading its quality. Management using fungicides; most commonly triadimefon is in regular practice, but they only protect uninfected foliage. Over time, the fungicides accumulate at the leaf margins, leaving other parts of the leaf more open to infection which also has a 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 standardisation of efficient shoot regeneration and transformation system in pea, transgenic plants may produce 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 utilisation in future for reducing loss caused due to several fungi.

Keywords: Agrobacterium; chitinase; erysiphae; Pisum sativum; transformation.

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1. 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; 1000 hectares in 1991-1992 to 272.6 thousand hectares in 1999-2000 [1]. The percentage of area under peas in India to the global area under peas has also risen from 3.2 percent in 1991-1992 to 4.5 per cent in 1999-2000. The production of green peas has increased from 1.30 million tons in 1991-1992 to 3.20 million tons in 2003-2004.

A variety of diseases affect peas through a number of pathogens, including insects, viruses, bacteria and fungi [2]. Among all these Powdery mildew caused by fungus *E. pisi* has a great economic importance [3 and 4]. 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 the season and reaches to maximum intensity at the time of pod formation. Firstly, it attacks in leaves producing faint, slightly discoloured specks from which greyish 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 tiny. It causes defoliation [5,6,7,8,2,9-16]. 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 enhancing 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 [10] 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 recorded that the survival rate of the in vitro regenerated plantlets [10] was over 60% and all the plants flowered and set seed normally [6]. Transgenic strains [9 and 10] exhibited a higher resistance than the control (non-transgenic plants).

2. MATERIALS AND METHODS

1. Pea seeds were collected from Bihar Agricultural University, Sabour.
2. Pea seeds were sterilised and germinated *in vitro* and leaf explants will be collected *in vitro* condition.
3. These explants were infected with *Agrobacterium tumefactions'* strain 4404 having Rice chitinase gene solution and cultivated in dark chamber.
4. After 24 hours these explants were 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 were start.
6. From this callus under organogenic regeneration firstly shoot buds was organised.
7. These shoot buds were separated and transferred into rooting medium.
8. Under rooting medium root were formed.
9. Within two weeks a larger number of putatively transformed pea plants was formed.
10. On Kanamycin antibiotics those which have NPTII genes are there they were detoxify kanamycin and grow into Kanamycin antibiotics.
11. Those putatively transformed plants were collected and analysed by four methods. I. .PRR. 2. Southern hybridisation, Nothern hybridization and Western hybridisation

3. RESULTS AND DISCUSSION

Chitin is a poly saccharide found in the outer skeleton of insects crabs, shrimps and constitutes internal 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 the

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

S. No.	Plant	Rice chitinase gene type	Fungal pathogen/disease resistance	References
1	Strwberry (*Fragariax ananssa*)	Rice chitinase gene (RCC2 Pbi 121)	*Sphaerothera 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

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 metabolise plant bioactive molecule as well as its own toxins which interfere with plant defence reaction among which chitinase is one of them.

3.1 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.

3.2 Rice-chitinase Gene

The recombinant DNA technology allows the enhancement of inherent plant responses against 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 defence mechanism (Bent and yu 1999, rommens and Kishore 2000). The available tools and techniques in molecular biology allows the isolation of specific genes and their reintroduction into plants, giving helpful tools to show the roles of specific enzymes in plants (Muthu Krishnan etal 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 [17]. 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 rxpressed resistance against powdery miledew (Erysiphe cichoraccum Nishizawa et al.1993) and transgenic cucumber plants showed resistance to grey mold (Tabei et al 1998). Therefore chitin metabolism froms an excellent target for selective pest control strategies (Kramer and Korga 1986, Cohen 1993, Kramer 1996).

4. CONCLUSION

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 standardisation of efficient shoot regeneration and transformation system in pea, transgenic plants may produce 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 utilisation in future for reducing loss caused due to several fungi.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Anonymous. District-wise estimated production of peas in Punjab. Punjab. Horticulture Department; 2002.
2. Rana JC, Banyal DK, Sharma KD, Manish K, Sharma SK. Gupta, Satish K. Yadav. Screening of pea germplasm for resistance to powdery mildew.

3. Bean SJ, Gooding PS, Mullineaux PM, Davies DR. A simple system for pea transformation. Plant Cell Rep. 1997;16: 513–519.
4. Di Carpenter: Pulse Development Officer, NSW Agriculture, Wagga, Wagga.
5. Filippone E, Lurquin PF. Stable transformation of pea tissues after co-cultivation with two *Agrobacterium tumefaciens* strains. Pisum Newslett. 1989;21:16-18.
6. Hussey G, Gunn HV. Plant production in pea (*Pisum sativum* L. cvs Puget and Upton) from long-term callus with superficial meristems. Plant Sci Lett. 1984;37:143-148.
7. Jackson JA, Hobbs SLA. Rapid multiple shoot production from cotyledonary node explants of pea (*Pisum sativum* L.). In vitro Cell Dev Fio. 1990;126:835-838.
8. Jacobsen HJ, Kysely W. Induction of somatic embryos in pea, *Pisum sativum* L. Plant Cell Tissue Organ Cult. 1984;3319-324.
9. Tiwari KR, Penner GA, Warkentin TD, Rashid KY. Canadian Journal of Plant Pathology. Volume 19, Issue 3, 1997. Erysiphepisi, the causal organism of powdery mildew of pea.
10. Lulsdorf MM, Rempel H, Jackson JA, Baliski DS, Hobbs SLA. Optimizing the production of transformed pea (*Pisum sativum* L.) callus using disarmed *Agrobacterium tumefaciens* strains. Plant Cell Rep. 1991;9479-483.
11. Malmberg RL. Regeneration of whole plants from callus of diverse genetic lines of *Pisum sativum* L. Planta. 1979;146:243-244.
12. McDonnell RE, Clark RD, Smith WA, Hinchee MA. A simplified method for the detection of neomycin phosphor-transferase-I1 activity in transformed plant tissues. Plant Mol Biol Rep. 1987;5(380-386).
13. Mroginski LA, Kartha KK. Regeneration of pea (*Pisum sativum* L. cv. Century) plants by in vitro culture of immature leaflets. Plant Cell Rep. 1981;1:64-66.
14. Cavallini A. Regeneration of pea (*Pisum sativum* L.) plantlets by in vitro culture of immature embryos. Plant Breeding; 1987.
15. Nauerby B, Madsen M, Christiansen J, Wyndaele R. A rapid and efficient regeneration system for pea (*Pisum sativum*), suitable for transformation. Plant Cell Rep 1991;9:676-679.
16. Nielsen SVS, Poulsen GB, Larsen ME. Regeneration of shoots from pea (*Pisum sativum*) hypocotyl explants. Physiol Plant. 1991;82:99-102P.
17. David R, Itzhaki H, Ginzberg I, Gafni Y, Galili G, Kapulnik Y. Suppression of tobacco basic chitinase gene expression in response to colonization by the arbuscular mycorrhizal fungus Glomus intraradices. Molecular Plant-microbe Interactions. 1998;*11*(6):489-497.

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