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Agrobacterium-mediated transformation of indica rice with chitinase gene for enhanced sheath blight resistance

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Abstract

Four rice indica genotypes of local importance were transformed with *RC7*, rice chitinase cDNA clone through *Agrobacterium*-mediated gene transfer method using mature seed derived calli as explants. The putative hygromycin resistant calli showed varied level of regeneration efficiency ranging from 2.0 to 7.6 %. The stable integration and expression of *RC7* was confirmed through polymerase chain reaction (PCR) and Western analysis. Transformation efficiency ranged from 0.9 to 5.2 %. The expression of *RC7* (35 kDa chitinase) in different tissues of transgenic plant (root, sheath and leaf) was proved through Western analysis and in terms of increased chitinase activity. The inheritance of transgene was studied through PCR and Western analysis in transgenic plants of Pusa Basmati 1. Bioassays with transgenic plants of local cultivars exhibited enhanced resistance up to 33.3 % to rice sheath blight pathogen *Rhizoctonia solani* under glasshouse conditions. Enhanced expression or 3- to 4-fold increased activity of chitinase in transgenic plants was correlated with sheath blight resistance.

Additional key words: antifungal activity, *Oryza sativa*, PCR, *Rhizoctonia solani*.

Introduction

Genetic improvement of rice for pathogen resistance is a pressing need, because this important crop is vulnerable to attack by devastating diseases, caused by fungi, bacteria and viruses. Among the fungal diseases, sheath blight, caused by *Rhizoctonia solani* Kühn, is one of the most important, widespread diseases found in all the rice growing countries. Although the extensive use of chemicals remains the main strategy of disease control, management of rice sheath blight could also be achieved through transgenic approaches involving identification, isolation and transfer of genes into rice plants (Lin *et al.* 1995, Datta *et al.* 2000, 2001, Kim *et al.* 2003).

Chitinase is PR3 group of pathogenesis related (PR) protein, that hydrolyse the β-1,4 linkage of the *N*-acetyl glucosamine residue of chitin, a structural polysaccharide of the cell wall of many fungi of all classes except *Oomycetes*. Chitinases, purified from plants, microbes and animal sources showed strong antifungal activity against fungal pathogens *in vitro* (Neuhaus 1999). We previously showed that chitinase preparation from rice plants effectively inhibits mycelial growth of rice sheath blight pathogen (isolate *Rs7*) used in this study (Nandakumar *et al.* 2002, Radjacommare *et al.* 2004). Constitutive or over expression of introduced chitinase genes in several transgenic plants is correlated with increased disease resistance to fungal pathogens (Broglie *et al.* 1991, Howie *et al.* 1994, Lin *et al.* 1995, Lorito *et al.* 1998, Nishizawa *et al.* 1999, Datta *et al.* 2000, 2001, Kim *et al.* 2003).

Rice chitinase genes against sheath blight disease have already been over expressed in rice. Lin *et al.* (1995) introduced a genomic clone of the rice chitinase gene into indica varieties and produced transgenic rice lines with enhanced resistance to the sheath blight pathogen, *R. solani*. Datta *et al*. (2000) subsequently produced several transgenic lines expressing cDNA encoding chitinase through particle bombardment and genomic clone through *Agrobacterium*-mediated

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Abbreviations: *bar* - biolaphos resistance gene; 2,4-D - 2,4-dichlorophenoxy acetic acid; *hph* - hygromycin resistance gene; MS medium - Murashige and Skoog medium; NAA - naphthalene acetic acid (NAA); PCR - polymerase chain reaction; PDI - percent disease index; PR - protein-pathogenesis-related proteins.

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transformation. Transgenic plants with a high level expression of chitinase exhibited similar enhanced resistance to the sheath blight pathogen (Datta *et al.* 2000, 2001). Co-expression of rice basic chitinase gene and ribosome-inactivating protein in rice caused significant reduction in sheath blight development (Kim *et al.* 2003). Hence in this study we attempted to introduce the rice chitinase cDNA clone, specific to sheath blight pathogen into widely grown local indica rice cultivars of South India through *Agrobacterium*-mediated transformation as *Agrobacterium* system is becoming the method of choice for rice transformation due to its low cost, convenience, high transformation efficiency, high probability of single copy integration and high fertility of transgenic plants (Hiei *et al.* 1997).

Materials and methods

Plants, plasmid and pathogen: Mature seed-derived calli of four indica rice (*Oryza sativa* L.) genotypes (Pusa Basmati 1, Co43, White Ponni and ADT38) grown on MS (Murashige and Skoog 1962) callus induction medium, supplemented with 2.5 mg dm^{-3} 2,4-dichlorophenoxyacetic acid (2,4-D), were used for transformation. *Agrobacterium tumefaciens* strain, LBA4404 harbouring the binary plasmid pCAMBAR.*RC7* (kindly provided by Dr. Muthukrishnan, Kansas State University, USA) was employed in the study. The binary vector contained the 1.1 kbp rice chitinase gene (*RC7*) driven by a 2.0 kbp ubiquitin promoter, hygromycin resistance (*hph*) and biolaphos resistance (*bar*) genes in its T-DNA region. The sheath blight pathogen, *Rhizoctonia solani,* isolate *Rs7*, classified as a highly virulent, was used for screening the transgenic plants (Sriram *et al.* 1997).

Co-cultivation, selection and regeneration: Co-cultivation was carried out as described by Rashid *et al.* (1996). Briefly, four-weeks-old white friable calli, subcultured 3 d before cocultivation, were immersed in the bacterial suspension (absorbance A_{600} - 1.0) containing 100 µM acetosyringone for 10 min. The calli were blot dried and cocultivated for 3 d, in the dark, at 25 °C on MS co-cultivation medium (MS medium containing 2.5 mg dm^{-3} 2,4-D, 10 g dm^{-3} glucose, 200 mg dm^{-3} casamino acid, 500 mg dm^{-3} proline, 100 µM acetosyringone). A total of six independent co-cultivation experiments were carried out on Pusa Basmati 1, three for White Ponni and two each on ADT38 and Co43. Three days after cocultivation, calli were washed with sterile distilled water containing the antibiotic cefotaxime (250 mg dm^{-3}), to kill the *Agrobacterium,* and selected on MS medium containing 50 mg dm^{-3} hygromycin and 250 mg dm^{-3} cefotaxime. After three rounds of selection, at 3 weeks intervals, the putative hygromycin resistant calli were transferred onto regeneration medium (MS medium, 3 mg dm^{-3} kinetin, 2 mg dm^{-3} naphthalene acetic acid (NAA), 250 mg dm^{-3} cefotaxime, 50 mg dm^{-3} hygromycin, 4 g dm^{-3} phytagel) and cultured at 25 °C under a 16-h photoperiod (irradiance of 50 µmol m^{-2} s^{-1} provide by white fluorescent tubes). Regenerated calli with emerging shoot buds were transferred to a rooting medium (half-strength MS medium, 30 mg dm^{-3} hygromycin) and rooted plants were transferred to soil in cups, subsequently to glasshouse for further studies.

Polymerase chain reaction (PCR) for *hph* gene: PCR was carried out as the first method to confirm the transgenic nature of the regenerated plants as described by Sambrook *et al.* (1989). PCR analysis was performed using 100 ng of genomic DNA (for plasmid DNA 5 ng) in a 0.025 cm^3 reaction mixture with *hph* specific primers (Forward 5'GATCTCCAATCTGCGGGATC3' and reverse primer 5'ACTCACCGCGACGTCTGTCG3' to yield 957 bp fragment). The hygromycin sequence in total DNA was amplified in a *PTC-100 Minicycler* (*MJ Research*, Watertown, MA, USA) with following temperature conditions: pre-incubation period at 94 °C for 3 min, leading to 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and synthesis at 72 °C for 1 min, followed by extension at 72 °C for 5 min. The amplified PCR product (0.010 cm^3) was subjected to electrophoresis on a 1 % agarose gel and visualised under UV radiation.

Western analysis: Total protein was extracted from leaf samples of both putative transformants and non-transformed plants and used as crude enzyme source for Western analysis and for assaying of chitinase activity. The protein content of the sample was determined by the Bradford method (Bradford 1976). Protein samples (50 µg) were fractionated on an SDS-PAGE as described by Laemmli (1970). The crude enzyme extracted from IR50 rice plants after inoculation with *R. solani* (contains 35- and 28-kDa chitinase isoforms) was used as a positive control. After SDS-PAGE, the proteins were electro-blotted onto 0.45 µm nitrocellulose membranes (*Sigma*, St. Louis, MO, USA) using a *Biorad* (Hercules, CA, USA) semi-dry blot transfer cell in accordance with manufacturer's instructions. Western analysis was carried out as described by Gallagher *et al.* (1995) with barley 28-kDa chitinase anti-rabbit antibody (kindly provided by Dr. S. Muthukrishnan, Kansas State University, USA) and affinity purified goat anti-rabbit immunoglobulin (IgG) conjugated with alkaline phosphatase (*Genei*, Bangalore, India) as secondary antibody. Immunological reactions were visualized in an alkaline phosphatase reagent containing nitroblue tetrazolium and 5-bromo-4-chloro-3-indolyl-phosphate (*Sigma*).

Transformation efficiency, progeny analysis: Transformation efficiency (TE) of a given rice genotype

was calculated as the ratio between the number of explants used for co-cultivation and number of callus lines which proved positive to the putative 35-kDa chitinase polypeptide in Western analysis. Progeny analysis of transgenic plants was carried out in 10 T_1 plants of Pusa Basmati 1 through PCR and Western analysis for both *hph* and *RC7*, respectively.

Chitinase assay procedure: Chitinase activity in transgenic plants was determined using colloidal chitin as substrate. The release of *N*-acetylglucosamine (GlcNAc) was determined colorimetrically at 585 nm using GlcNAc as a standard (Boller and Mauch 1988). The enzyme activity was expressed as nmol(GlcNAc equivalents) g^{-1}(f.m.) min^{-1}.

Estimation of chitinase expression in different parts of transgenic plants: Chitinase activity was estimated in different parts of putative transgenic and non-transformed rice plants (root, sheath and leaf) consequent to inoculation with *Rs7* or otherwise. A 6 mm mycelial disc of *R. solani* was placed in between a sheath and the stem of a rice plant, the inoculated portion was covered with absorbent cotton and tied with parafilm. It was then moistened with sterile distilled water regularly to maintain high humidity. Seven days after inoculation, the root, sheath and leaf portions of both inoculated and uninoculated plants were collected from putative transgenic and control plants. Enzyme was extracted from different plant parts and used for Western analysis and chitinase enzyme activity.

Bioassay of transgenic plants against rice sheath blight: The bioassay of transgenic plants expressing *RC7* was carried out in putative transgenic and selected T_1 transgenic plants by inoculating with the virulent isolate of *R. solani Rs7*, under glasshouse conditions, as described earlier. Bioassays were performed at 40 d after planting. Respective non-transformed plants were used as controls. Development of symptoms was observed and recorded 7 d after inoculation as grades 0 to 5 (Sriram *et al.* 1997). Based on the grades, the percent disease index (PDI) was calculated using the following formula: PDI = (sum of individual grades/total number of sheaths) × (100/maximum grade).

Results and discussion

Transformation of indica genotypes: Transformation of indica rice genotype with disease resistant genes and regeneration of fertile transgenic plants are limited by its recalcitrant nature, tissue culture techniques and or difficulties encountered in distinguishing transformed calli from non-transformed calli during selection (Zhang *et al.* 1998, Lin and Zhang 2005). Despite these difficulties, transgenic plants were obtained in 4 elite indica genotypes Pusa Basmati 1, White Ponni, ADT38 and Co43 with regeneration efficiency ranging from 2.0 % to 7.6 %. Transformation efficiency in intro-gressing *RC7* into rice genotypes varied between 1.79 and 5.17 % in Pusa Basmati 1, 1.22 and 2.43 % in White Ponni, 0.86 and 2.33 % in ADT38, 0.0 and 1.02 % in Co43. However, high and varied transformation efficiency like 23 % (Chan *et al.* 1993), 27 % (Aldemita and Hodges 1996), 3.8 to 33 % (Nishizawa *et al.* 1999) for japonica genotypes, 22 % for indica genotype (Rashid *et al.* 1996) were reported. The lesser transformation efficiency in the present study could be attributed to the recalcitrant nature and poor tissue culture response posed by the local genotypes used. Further, the high transformation efficiency reported by other groups was based on the regeneration or the presence of marker genes. In contrast, the present study transformation efficiency was calculated based on the expression of 3 kDa chitinase in transgenic plants. This difference could also be one among the reasons for the lower transformation efficiency.

PCR analysis: Before confirming the expression of *RC7* in transgenic plants, integration of the selectable marker gene *hph* was confirmed by amplifying an *hph* sequence in the putative transgenic plants. Out of 53 putative transgenic lines regenerated, of all genotypes, 32 lines proved PCR positive to *hph* (Fig. 1). Some of the

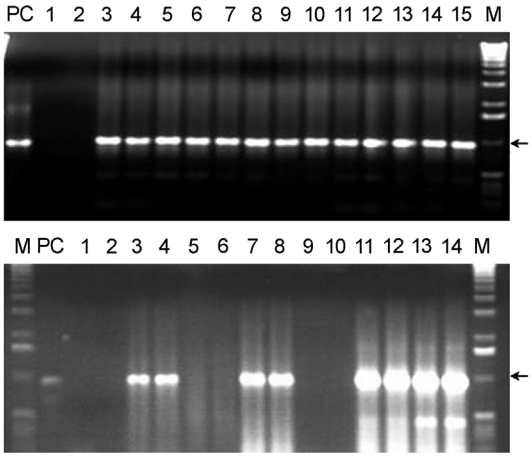

Fig. 1. PCR amplification of *hph* in putative transgenic lines of Pusa Basmati 1 (PB1) (*top*) and White Ponni (WP) and ADT38 (*bottom*). *Top*: M - 1 kb ladder, PC - positive control (pCAMBAR.RC7), *lane 1* - water control, *lane 2* - negative control (non-transgenic PB1), *lanes 3 - 15* - putative transgenic plants of Pusa Basmati 1. *Bottom*: M: 1 kb ladder, PC - positive control (pCAMBAR.RC7); *Lane 1*: water control, *lane 2* - negative control (WP); *lanes 3 - 9* - putative transgenic plants of WP, *lane 10* - negative control (ADT38), *lanes 11 - 14* - putative transgenic lines of ADT38. *Arrow* indicates the 957 bp fragment of *hph.*

hygromycin regenerated calli lines were not positive to *hph,* this could have been due to suppression or cross protection of the transformed tissues by non-transformed tissues, which could result in continual growth and regeneration of non-transgenic tissue on selection medium (Zhang *et al.* 1998). Similar kinds of escapes for *hph* in indica rice cultivar also reported in rice plants transformed with rice chitinase genes (Datta *et al.* 2000).

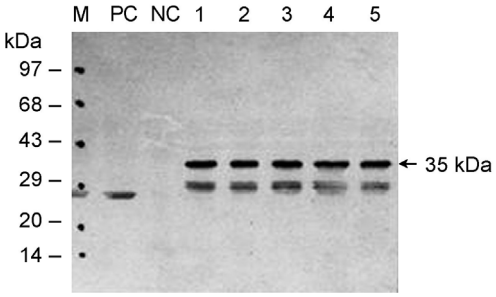

Fig. 2. Western analysis for the expression of *RC7* chitinase in putative T_0 transgenic lines of Pusa Basmati (PB1). M - marker, PC - positive control (purified 28-kDa rice chitinase), NC - negative control (non-transgenic PB1 plant), *lanes 1 - 5* - putative transgenic lines of Pusa Basmati 1, PB1-5.1.2, PB1-5.2.2, PB1-5.3.3, PB1-5.4.1, 5:PB1-5.5.1, respectively. *Arrow* indicates the expected 35-kDa chitinase.

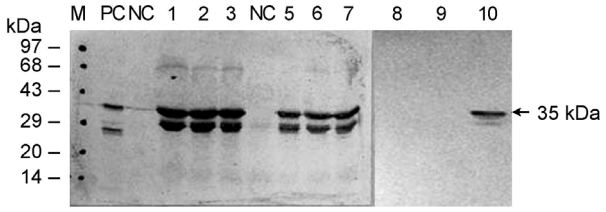

Fig. 3. Western analysis of *RC7* chitinase expression in putative T_0 transgenic lines of White Ponni, ADT38 and Co43. M - marker, PC - positive control (*R. solani* infected IR50 plants, 35- and 28-kDa), NC - negative control (White Ponni), *lane 1* -WP2-1.1, *lane 2* - WP2.4.1, *lane 3* - WP2.5.1, NC - negative control (ADT38), *lane 5* - ADT38-1-1.2, *lane 6* - ADT38-1-2.1, *lane 7* - ADT38-1-2.2, *lane 8* - negative control (Co43), *lane 9* - Co43-1, *lane 10* - Co43-1.1. *Arrow* indicates the expected 35-kDa chitinase.

Expression of *RC7:* Expression of introduced chitinase gene was confirmed by Western analysis. Polyclonal barley chitinase antibody detected constitutive expression of a 35-kDa chitinase in putative transgenic (T_0) plants of all genotypes tested. A total of 12 positive lines were obtained for Pusa Basmati 1 (Fig. 2) and 4, 3 and 1 lines for White Ponni, ADT38 and Co43, respectively. Though equal amount of protein (50 µg per well) was loaded for Western analysis for all genotypes, high level of expression in terms of band intensity was observed in White Ponni and ADT38 (Fig. 3). Varied levels of expression of chitinase genes in T_0 plants of rice and grapevine have been reported (Lin *et al.* 1995, Yamamoto *et al.* 2000, Datta *et al.* 2001). In addition to the expected 35-kDa chitinase band, the polyclonal antibody also detected other protein, possibly chitinase, of molecular mass of 30 kDa, this could have been a result of proteolytic degradation of 35-kDa protein, as reported previously in rice plants transformed with a genomic clone of a rice chitinase, *Chi*11 (Lin *et al.* 1995, Datta *et al.* 2000) and a cDNA chitinase clone, *RC7* (Datta *et al.* 2001). The introduced *RC7* integrated and was expressed in subsequent progeny. Out of 10 T_1 plants of Pusa Basmati 1 line (PB1-5.1, which proved positive to *RC7* in Western analyses in T_0 generation) used for progeny analysis, nine plants expressed the 35-kDa polypeptide (Fig. 4).

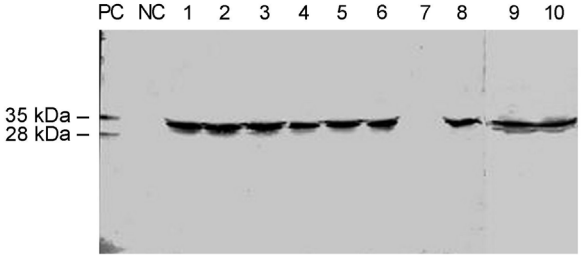

Fig. 4. Inheritance and expression of *RC7* chitinase in T_1 transgenic lines of PB1. PC - positive control *(R. solani* infected IR50 plants), NC - negative control (non-transformed T_1 Pusa Basmati 1), *lanes 1 - 10* - transgenic plants of Pusa Basmati 1 single line PB1-5.1.

Chitinase activity: Plant's resistance to pathogens involves the activation and expression of defense proteins during active defense mechanism, but these mechanisms are too weak or appear too late to be effective for preventing the establishment of pathogens. Hence increased constitutive expression of introduced chitinase is necessary for arresting the early development of the fungi. Several reports on intensified chitinase activity are available in studies on transgenic plants expressing introduced chitinase genes (Lin *et al.* 1995, Grison *et al.* 1996, Marchant *et al.* 1998, Datta *et al.* 2000). Lin *et al.* (1995) and Marchant *et al.* (1998) reported up to 14 and 4 - 5 times greater chitinase activity in transgenic rice and rose plants, respectively. In the present study, an average of 2- to 3-fold increase in activity of *RC7* was recorded in 10 putative T_1 Pusa Basmati 1 transgenic plants of the line, T_1-PB1-5.1 and 3 plants in each of T_0 lines of White Ponni (WP-2.1) and ADT38 (ADT38-2.1), as compared to respective non-transgenic control plants, respectively (Table 1).

Chitinase activity and expression of *RC7* in different plant parts: Expression of *RC7* was studied in root, leaf and sheath tissues of transgenic (T_1) Pusa Basmati 1 after inoculation with *R. solani.* Sheath blight pathogen mainly attacks the sheath tissues first and, as the disease progress, it spreads towards roots and leaves. Hence analysis of expression of the introduced gene in the different plant parts has practical significance in arresting the pathogen spread with in the plant. The expression of

35-kDa chitinase did appear in the sheath, where the infection starts and develops and leaves and root tissues of uninfected transgenic plants (Fig. 5). Pathogen-inducibility of *RC7* was established as there was increased expression of putative 35-kDa chitinase in infected transgenic plants. The isolation and cloning of such infection-related PR-genes in to plants could be more useful in generating greater resistance to pathogenic fungi (Datta *et al.* 2001). Constitutive expression of a 42 kDa endogenous chitinase was detected in root tissues irrespective of inoculation with the pathogen or otherwise (Fig. 5). This result is in agreement with the report of Lin *et al.* (1995). Protein extracts from root, sheath and leaf of a single plant in Pusa Basmati 1 T_1 line (T_1-PB1-5.2) exhibited 2 - 3 times higher chitinase activity than the non-transformed control. An enhanced chitinase activity (41.1, 57.4 and 37.2 % increase over non-transformed control) was detected in transgenic lines upon *R. solani* inoculation in transgenic plants but not in control plants (Table 2). Inoculation with *R. solani* induced two chitinase isoforms with molecular masses of 35- and 28-kDa in wild type indica rice cultivars of Chinsurah Boro II and IR58 (Lin *et al.* 1995, Anuratha *et al.* 1996) and IR50 (Nandakumar *et al.* 2002). However, in the present study, no such chitinase isoforms were noticed in the wild type Pusa Basmati 1 indicating the need for incorporating chitinase gene for sheath blight management.

Table 1. Chitinase activity [nmol(GlcNAc equivalents) g^{-1}(f.m.) min^{-1}] in transgenic plant lines transformed with rice chitinase gene Values are mean of three replications. In a column, means followed by a common letter are not significantly different by DMRT (P = 0.05 %).

Transgenic plants/lines	Chitinase activity
T_1-PB5.1.1	20.16f^{g}
T_1-PB5.1.2	23.55^{a-f}
T_1-PB5.1.3	20.96^{efg}
T_1-PB5.1.4	24.02^{a-e}
T_1-PB5.1.5	21.019^{d-g}
T_1-PB5.1.6	18.15^{g}
T_1-PB5.1.7	5.88^{i}
T_1-PB5.1.8	21.4^{c-f}
T_1-PB5.1.9	21.29^{c-g}
T_1-PB5.1.10	21.90^{b-f}
Untransformed plants (PB)	5.36^{i}
T_0-WP2.1.1	24.98^{ab}
T_0-WP2.1.2	22.61^{a-f}
T_0-WP2.1.3	25.44^{a}
Untransformed plants (WP)	7.96^{i}
T_0-ADT38-2.1.1	24.26^{a-d}
T_0-ADT38-2.1.2	24.50^{abc}
T_0-ADT38-2.1.3	21.42^{c-f}
Untransformed plants (ADT38)	6.81^{i}

Bioassay of transgenic plants against sheath blight disease: Bioassays conducted with 7 T_1 plants of a Pusa Basmati 1 line (PB1-5.1), 3 T_0 plants of a White Ponni line (WP-2.1) and 2 T_0 plants of a ADT38 line (ADT38-2.1), after inoculation with *Rs7* revealed that there was an enhanced resistance to sheath blight in the

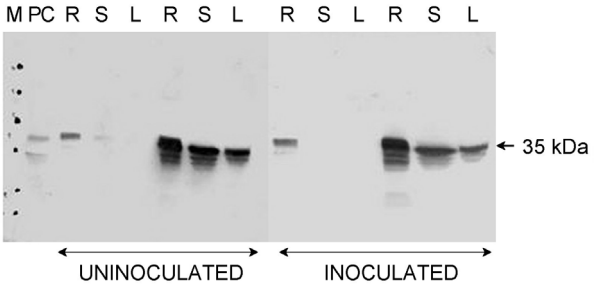

Fig. 5. Expression of 35-kDa chitinase in different plant parts of rice tissues of T_1 plant of PB1. PC - positive control, R - root, S - sheath, L - leaf.

Table 2. Chitinase activity [nmol(GlcNAc equivalents) g^{-1}(f.m.) min^{-1}] in different plant parts of T_1 transgenic Pusa Basmati 1 plant without or with inoculation of *R. solani*. Values are mean of three replications. In a column, means followed by a common letter are not significantly different by DMRT (P = 0.05%).

Plant parts	Chitinase activity	
	without inoculation	with inoculation
Non-transformed root	10.16^{a}	13.20^{a}
Non-transformed sheath	4.40^{b}	6.15^{b}
Non-transformed leaf	6.12^{b}	6.58^{b}
Transformed root	23.55^{a}	31.56^{a}
Transformed sheath	17.21^{c}	27.09^{b}
Transformed leaf	20.25^{b}	27.79^{b}

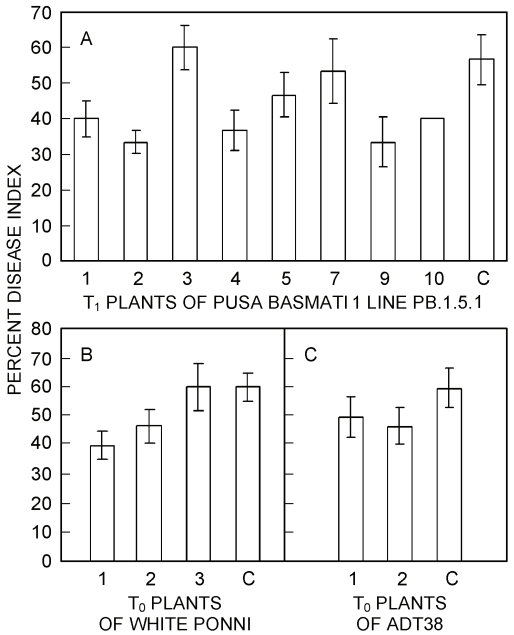

Fig. 6. Bioassay of transgenic plant lines inoculated with rice sheath blight pathogen *R. solani*. Values are mean of three replications (sheath). *Error bars* indicate ± SE

transgenic lines as compared to the non-transformed controls. The transgenic plants of Pusa Basmati 1 registered a PDI which ranged from 33.3 (PB1-5.1-2) to 60.0 % (PB1-5.1.3), while non-transgenic controls registered PDI 55.0. For other genotypes it ranged between 33.3 and 60.0 % (T_0 line of White Ponni, WP2.1), 46.7 and 50.0 % (T_0 line of ADT38, ADT38-2.1) against 60.0 and 66.7 % of their respective non-transgenic plants (Fig. 6). The reduction of 39.4, 33.3, and 30.0 % sheath blight disease over wild type was observed in three tested cultivars, respectively. Similar instances of engineered resistance against *R. solani* occurred in indica genotypes Chinsurah Boro II, Pusa Basmati 122, Vaidehi and Tulsi (Lin *et al.* 1995, Datta *et al.* 2000). Datta *et al.* (2001) reported that such an enhanced resistance to sheath blight conferred by *RC7* was more than 50 % in two transgenic homozygous lines of IR72 and 11.1 % in IR64. In the present study, the transgenic lines expressing enhanced resistance produced fewer lesions registering sheath blight grades of 0 to 3, while it was 4 to 5 in the non-transgenic lines inducing larger lesions. These observations were similar to the results of previous reports (Lin *et al.* 1995, Datta *et al.* 2001). Besides, several reports on the increased resistance to fungal pathogens conferred by chitinase are available in the literature (Grison *et al.* 1996, Asao *et al.* 1997, Tabei *et al.* 1998, Takatsu *et al.* 1999, Yamamoto *et al.* 2000, Rohini and Rao 2001).

In all these above studies, disease resistance was correlated with over expression of chitinase genes in transgenic plants. In the present study also, three T_1 plants of Pusa Basmati 1 (plant number 2, 5 and 9) registering the lowest blight (PDI 33.33 %) and one T_0 plant of White Ponni (plant number 1), recorded a 3-fold increased chitinase activity as compared to the non-transformed plants. In other plants, though all the plants tested showed increased chitinase activity, they differed in their resistance level to *R. solani* supporting the earlier reports of over expression of chitinase not necessarily result enhanced resistance (Data *et al.* 2001). In this point, the environmental factors, complexity of pathogen infection behaviour, genotype and time of expression of chitinase gene would have played an important role, rendering the plants susceptible. However, all the transgenic plants showed reduced infection than non-transgenic plants. Thus the expression of cDNA clone of rice chitinase gene and enhanced resistance, confirms the antimicrobial property of chitinase against the sheath blight pathogen in transgenic plants.

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