Enhancement of resistance against fungal pathogens in peanut (Arachis hypogaea L.) cultivar L14 by heterologous expression of gene encoding chitinase 42 kDa from Trichoderma asperellum SH16

Phung Thi Bich Hoa  
Hue University  https://orcid.org/0000-0002-9989-7713

Nguyen Hoang Tue  
Hue University  https://orcid.org/0000-0003-4646-7426

Huynh Thi Quynh Trang  
Hue University

Hoang Anh Thu  
Hue University

Le Ngoc Huyen Nhung  
Hue University

Nguyen Ngoc Luong  
Hue University  https://orcid.org/0000-0002-6123-7437

Nguyen Xuan Huy  
Hue University  https://orcid.org/0000-0002-8744-0927

Nguyen Quang Duc Tien  
Hue University  https://orcid.org/0000-0002-7330-3139

Nguyen Hoang Loc (nhloc@hueuni.edu.vn)  
Hue University  https://orcid.org/0000-0002-6387-0359

Research Article

Keywords: Agrobacterium tumefaciens, Arachis hypogaea, Chi42, chitinase 42 kDa, root-specific promoter, Trichoderma asperellum

Posted Date: March 25th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1487302/v1

License: © This work is licensed under a Creative Commons Attribution 4.0 International License.  Read Full License
Abstract

This study reports the expression of the 42 kDa chitinase genes from Trichoderma asperellum SH16 in peanut (Arachis hypogaea) roots under the regulation of tissue-specific Asy promoter through Agrobacterium tumefaciens-mediated transformation. The 42 kDa chitinase genes, including one wild-type sequence (Chi42) and two synthetic sequences (syncodChi42-1 and syncodChi42-2) which were optimized for codon usage for plant expression, were incorporated into the peanut genome and successfully expressed in their roots. The investigation revealed that chitinase from two synthetic genes had higher activity than that from the wild-type gene, about 901 U/mg (140 U/mL) and 1124 U/mg (197 U/mL) vs about 739 U/mg (105 U/mL), respectively. Transgenic peanut roots also exhibited extracellular chitinase activity which was driven by signal peptide of rice amylase 3D gene against the pathogenic fungus Sclerotium rolfsii under in vitro conditions. The higher chitinase activity of two synthetic genes in peanut roots promises potential applications in the field of transgenic crops against phytopathogenic fungi.

Introduction

Chitinases (EC 3.2.2.14) are enzymes that break down the linear polymer of N-acetyl-D-glucosamine monomers known as chitin (Wang and Yang 2007). Chitinases may be found in a wide range of organisms, including bacteria, fungi, animals, and plants (Ramos and Malcata 2011; Zarei et al. 2011; Hamid et al. 2013; Veliz et al. 2017). Because of their propensity to secrete extracellular chitinases, Trichoderma species are widely utilized as biocontrol agents of phytopathogenic fungi with chitin in cell walls (Abdel-lateif 2017; Poveda 2021).

Peanut (Arachis hypogaea L.), which belongs to the family Fabaceae (or Leguminosae), is a high-value grain legume crop derived from Central Brazil and widely cultivated in tropical and subtropical regions for its edible oil and seeds (Pal et al. 2014; Singh et al. 2021). It is, however, one of the crops sensitive to serious diseases like stem rot, root rot, and pod rot caused by a variety of soilborne pathogens such Rhizoctonia solani, Aspergillus niger, and Sclerotium rolfsii (Ismail et al. 2007; Gour et al. 2012; Thiessen et al. 2012; Xu et al. 2015).

The method of using Agrobacterium tumefaciens to transfer a gene of interest into plant cells, resulting in transgenic plants, is known as Agrobacterium-mediated transformation (Gelvin 2003). Because of the advantages of transferring pieces of DNA with defined ends and minimal rearrangement, transferring relatively large segments of DNA, integrating small numbers of copies of genes into plant chromosomes, and the high quality and fertility of transgenic plants, Agrobacterium-mediated transformation has become the most widely used method for transferring genes into plants (De La Riva et al. 1998; Pratiwi and Surya 2020).

Even though certain chitinase genes from Trichoderma or other species, such as rice and tobacco, have been incorporated into various crops to aid them against fungal infections (Nishizawa et al. 1999; Takahashi et al. 2005; Gentile et al. 2007; Baranski et al. 2008; Zaripanjeh et al. 2016; Ojaghian et al. 2018; Ojaghian et al. 2020). To date, there has been no record of chitinase gene transfer from Trichoderma, especially T. asperellum, into peanuts.

As a consequence, the present study might be the first to employ Agrobacterium-mediated transformation to transfer the chitinase gene from Trichoderma to peanuts in order to increase their antifungal activity. Different chitinase genes were employed in this study, including one wild-type gene, Chi42, from T. asperellum SH16, which encodes chitinase 42 kDa, and two synthetic genes (syncodChi42-1 and syncodChi42-2) generated from
the Chi42 gene by optimizing codon usage for plant expression (Luong et al. 2021). The goal of this study is to evaluate the level of expression of the wild-type gene and the two synthetic genes regulated by the tissue-specific promoter (pAsy) in peanut roots, as well as their antifungal efficacy. The high chitinase activity in transgenic peanut roots holds promise for resistance to phytopathogenic fungi.

**Materials And Methods**

*Plant materials*

Peanut (*A. hypogaea* L.) cultivar L14 from the Vietnam Academy of Agricultural Sciences’ Field Crops Research Institute was utilized in this study (Hoa et al. 2021). The seed coat was removed after being soaked in 70% EtOH for 1 min and sterilized with 65% NaClO/100 µL Tween 20 solution for 10 min. The embryonal and de-embryonal cotyledons were separated from the seeds longitudinally for use as explants in *Agrobacterium* transformation.

*Plant expression binary vector*

*Agrobacterium tumefaciens* strain LBA 4404 containing the pNHL20 vector harboring one of three chitinase genes (*Chi42, syncodChi42-1,* and *syncodChi42-2*) expressing 42 kDa chitinase was used in the present study (Tue et al. 2021). *Chi42* (HM191683.1) is a wild-type gene from *T. asperellum* SH16 (Loc et al. 2011). Both *syncodChi42-1* (MT083802.1) and *syncodChi42-2* (MT083803.1) are synthetic genes derived from the *Chi42* gene optimized for codon usage for plant expression (Luong et al. 2021). Chitinase genes were driven by the root-specific Asy promoter from peanut (Geng et al. 2014). A signal peptide of amylase 3D gene from rice (Jung et al. 2016) was situated at the 5’ end of the genes guides newly synthesized chitinase to the periplasm for extracellular secretion (Fig. 1).

Briefly, the COOL program was used to optimize the *chi42* gene for plant expression (Chin et al. 2014). The optimization algorithm included maximizing the codon context, ignoring individual codon usage, destabilizing the mRNA secondary structure at 5’ termini, and limiting the presence of restriction enzyme sequences. Two sequences with high codon context scores (named *syncodChi42-1* and *syncodChi42-2*) were randomly selected for synthesis (PHUSA Biochem Co., Can Tho, Vietnam).

*Agrobacterium transformation*

Explants were infected for 20 min with *A. tumefaciens* LBA4404 carrying the pNHL20 vector, then co-cultured on TDT medium supplemented with 15 mg/L benzylaminopurine (BAP) for de-embryonal cotyledon or 15 mg/L BAP and 2 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) for embryonal cotyledon at 25±2°C for 3 days in the dark. The *Agrobacterium* suspension used for transformation had an OD600 of 1. TDT medium was made up of 200 µg/mL acetosyringone, 3% sucrose, 0.8% agar, and MS basal medium (Murashige and Skoog 1962). After co-culture, explants were subcultured on the same medium with 100 mg/L kanamycin and 250 mg/L cefotaxime for screening transformants. Shoots were isolated from the screening culture to multiply on MS basal medium supplemented with 3% sucrose, 0.8% agar, 2 mg/L BAP, 0.1 mg/L indoleacetic acid (IAA), and 100 mg/L kanamycin. Finally, single shoots from the shoot cluster were grown into whole plants on MS basal medium with 1 mg/L naphthaleneacetic acid (NAA) and no antibiotics. Except for *Agrobacterium* treatment, all *in vitro* cultures were maintained at 25±2°C for 4 weeks at a light intensity of 2000-3000 lux and 16 h of daylight.

*PCR amplification*
The cetyltrimethylammonium bromide (CTAB) method was used to extract genomic DNA from *in vitro* transgenic peanut leaves (Clarke 2019). PCR amplification was carried out using specific primers for DNA segments that served as chitinase gene indicators (Table 1). The reaction is made up of 20 ng of genomic DNA as a template, 10 pmol of each primer, 1 µL of Master Mix (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and water added to a final volume of 12 µL. The PCR settings were as follows: 15 min of genomic denaturation at 95°C; then 30 cycles of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min; followed by a final extension at 72°C for 10 min.

**SDS-PAGE and Western blot analysis**

The total soluble protein (TSP) from *in vitro* transgenic peanut roots was extracted with phosphate buffer (pH 7) and quantified using Bradford's assay (1976). Fifty micrograms of TSP of each sample were denatured for 10 min at 95°C before being fractionated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). After the fractionation, one twin gel was stained with Coomassie Brilliant Blue while another twin gel was blotted onto a nitrocellulose membrane (Novex™-Thermo Fisher Scientific, Waltham, MA, USA). The non-specific binding on the blot was blocked by 5% skim milk (Sigma-Aldrich, St. Louis, MO, USA). The primary antibody was a mouse anti-Ta-CHI42 polyclonal antibody diluted 1:2000 in Tris-buffered saline with Tween 20 (TBST), Ta-CHI42 is recombinant chitinase 42 kDa derived from fungus *T. asperellum* SH16 (Luong et al. 2021). The secondary antibody was a 1:5000 dilution of goat anti-mouse IgG antibody conjugated with alkaline phosphatase (AbD Serotec-currently Bio-Rad Antibodies, Bio-Rad Laboratories, Hercules, CA, USA). Western signals on the blot were developed with 5-bromo, 4-chloro, 3-indolyl phosphate (BCIP)/ nitro-blue tetrazolium (NBT)ctab solution (Sigma-Aldrich, St. Louis, MO, USA) for 10 min in the dark and then their density was measured by the ImageJ program (v. 1.49).

**Chitinase assay**

The plate assay method was used to *preliminarily* evaluate the chitinolytic activity of plant chitinase. The pre-punched holes on the assay plate containing 1.5% agar and 1.2% colloidal chitin were filled with 50 µg TSP (crude chitinase). Colloidal chitin was made according to Murthy and Bleakley's method (2012). The assay plate was incubated at 4°C for 8 h for diffusing enzyme, and then at 28°C for 6 h for chitinolysis. The plate was colored with 0.1% Lugol's solution after incubation to detect chitin degradation (Calissendorff and Falhammar 2017). Bacterial purified chitinase 42 kDa (10 U/mL) was used as a positive control. Fifty micrograms of TSP of the extract from the non-transgenic root were used as a negative control.

The chitinase activity was determined by measuring the absorbance of *p*-nitrophenol at 420 nm (Tsujibo et al. 1998). A reaction mixture containing 50 µg TSP and 15 µL of 2.5 mM 4-nitrophenyl-N-acetyl-β-D-glucosaminide (pNpGlcNAc) (Merck, Kenilworth, NJ, USA) as substrate was incubated at 45°C for 10 min. After that, the hydrolysis was stopped using 1 mM of 0.2 M sodium carbonate. The amount of chitinase required to release 1 µmol of *p*-nitrophenol from pNpGlcNAc per minute was defined as one activity unit. A *p*-nitrophenol standard purchased from Merck (Kenilworth, NJ, USA) was used to make the calibration curve. The specific activity (U/mg protein) of an enzyme is determined by dividing its total activity (U/mL) by the TSP content (mg/mL).

**In vitro** antifungal activity
In vitro roots of chitinase transgenic peanuts were transferred in 1/2 PDA (potato dextrose agar) medium containing $10^4$ S. rolfsii spore for evaluating their antifungal activity based on mycelium growth inhibition. Incubation was performed at 28°C for 96 h. Twenty microliters (10 U/mL) of bacterial purified chitinase 42 kDa was used as a positive control, sterile distilled water and non-transgenic peanut roots were used as negative controls.

**Results And Discussion**

**Agrobacterium transformation**

Table 2 shows that after 4 weeks of culture, 303 surviving shoots were obtained from two types of transformed explants on a selective medium containing kanamycin and cefotaxime (Fig. 2). Among them, 207 shoots were from embryonal explants (68 for Chi42, 74 for syncodChi42-1, and 65 for syncodChi42-2) and the rest were from de-embryonal explants (33 for Chi42, 36 for syncodChi42-1, and 27 for syncodChi42-2). However, PCR amplification revealed that only about 50% of the total number of surviving shoots contained the chitinase gene. In embryonal cotyledons, it was just 48.5% for Chi42, 52.7% for syncodChi42-1, and 40% for syncodChi42-2, whereas in de-embryonal cotyledons, it was 54.5% for Chi42, 61.1% for syncodChi42-1, and 55.6% for syncodChi42-2 (Fig. 3).

A study by Iqbal et al (2012) obtained 70% of surviving plantlets on the selective medium from cotyledonal nodes of peanut and 40% of which were putatively chitinase-3 transgenic plants. Prasad et al (2013) also obtained a total of 65 regenerated shoots from peanut mature cotyledons which were transferred rice Rchit gene encoding chitinase. While Sharma and Anjaiah (2000) achieved a significant percentage (55%) of transgenic peanuts cultivar JL-24 from de-embryonal cotyledons through *Agrobacterium* transformation. These data seem to suggest that genotype and explant type had an impact on transformation efficiency. Although the ratio of shoot regeneration on the selective medium is important, the number of transgenic plants and their gene expression level are the determining factors. Of fact, *Agrobacterium*-mediated transformation is possible with other types of peanut explants. Iqbal et al (2012) obtained an efficiency of 42% for cotyledonary node explants. Whereas it is around 63 and 62% for embryo cotyledons or around 72 and 77% for mesocotyl-derived explants of two peanut cultivars Huayu 20 and 26 (Chen et al. 2015).

**Expression of chitinase 42 kDa**

The expression of three genes encoding chitinase 42 kDa in transgenic peanuts was examined using SDS-PAGE and Western blot analysis. Figure 4A illustrates several protein bands with a molecular weight of 42 kDa, as expected for *T. asperellum* SH16 chitinase, from various samples on the polyacrylamide gel. Transgenic peanut lines with putative recombinant chitinase were chosen for Western blot analysis. Immune signals were observed in the majority tested transgenic peanut lines and positive control, except for the non-transgenic negative control and a few others (Fig. 4B). In this study, the gene syncodChi42-2 (line S2A-12) had the strongest signal with a density of about $4 \times 10^6$; while the syncodChi42-1 (line S1A-15) and Chi42 (line WTA-2) genes only reached about $2.3 \times 10^6$ and $8.2 \times 10^5$, respectively (Fig. 5). These findings imply that chitinase 42 kDa genes were successfully expressed in transgenic peanuts. However, some transgenic individuals were unable to produce or only produced a small amount of chitinase 42 kDa due to the position effect (Betts et al. 2019; Pérez-González and Caro 2019), resulting in no or weak signals on the blot (Data not shown). To our knowledge, until now no studies have
employed antigen-antibody interaction, such as Western blot, to determine chitinase gene expression in transgenic peanuts. The majority of studies have focussed on transcriptional expression or chitinase activity in transgenic peanuts, as well as their antifungal effectiveness (Sharma and Anjaiah 2000; Iqbal et al. 2012; Prasad et al. 2013; Chen et al. 2015; ul Hassan et al. 2016).

**Chitinase activity assay**

Colloidal chitin hydrolysis activity of chitinase 42 kDa from transgenic peanuts was determined using the agar plate assay. The transgenic peanut lines with the highest chitinase expression are shown in Figure 6. The largest $D-d$ difference (about 2.4 cm) was found in $\text{syncodChi42-2}$ transgenic peanuts (line S2A-12), which was about 1.2- and 1.4-fold greater than $\text{syncodChi42-1}$ (line S1A-15) and $\text{Chi42}$ (line WTA-2) transgenic peanuts, respectively. Where $D$ denotes the clear zone diameter and $d$ denotes the diameter of the pre-punched hole for loading enzyme. Whereas in the non-transgenic control, hydrolysis was negligible (Fig. 6). These findings suggest that chitinase 42 kDa was present in transgenic peanuts in an active form.

Line S2A-12 also displayed the highest chitinase specific activity of 1124 U/mg (197 U/mL) among transgenic peanuts regenerated from embryonal cotyledon, 1.3 (1.4) and 1.5 (1.9) times higher than line S1A-15 and line WTA-2, respectively. The non-transgenic control had the lowest chitinase activity, only about 54 U/mg (31 U/mL) (Fig. 7). Overall, the chitinase expression levels in the transgenic peanuts and the tested genes were different. In every case, the synthetic genes $\text{syncodChi42-1}$ and $\text{syncodChi42-2}$ were expressed stronger than the wild-type $\text{Chi42}$ gene. Recently, Tien et al (2021) also reported that transient expression of $\text{syncodChi42-1}$ and $\text{syncodChi42-2}$ synthetic genes in $\text{N. benthamiana}$ was 1.7 and 2.6 times greater than wild-type $\text{Chi42}$ gene, respectively. These results demonstrate that the methods employed to optimize codon use for plant expression in previous work (Luong et al. 2021) were successful in peanuts.

Plants do not or only produce minimal quantities of chitinase unless they are triggered by a fungal disease, according to several studies (Collinge et al. 1993; Grover 2012; Punja and Zhang 1993). In the present study, the root extract from in vitro non-transgenic and chitinase transgenic peanuts without fungal disease infection was used to determine chitinolytic activity on the colloidal chitin plate and chitinase activity. Although non-transgenic peanuts also produced chitinase, it was only in negligible amounts, so the chitinase activity was low and the hydrolysis region was small. Prasad et al (2012) and Iqbal et al (2012) improved the chitinase activity of peanuts up to 2-14 times and 1.8-6.5 times higher than non-transgenic controls, respectively, by transferring the $\text{Rchit}$ and chitinase genes from rice.

**In vitro antifungal activity of chitinase**

To assess antifungal effectiveness, WTA-2, S1A-15, and S2A-12 transgenic peanut lines with the highest chitinase activity were chosen. After 96 h of treatment, the transgenic roots of all three peanut lines completely inhibited the growth of $\text{S. rolfsii}$, which causes white mold wilt disease. The positive control also showed a similar finding. While $\text{S. rolfsii}$ grew strongly in treatments for negative controls (Fig. 8). This investigation revealed that chitinase had significant antifungal activity against $\text{S. rolfsii}$ in all three transgenic peanut lines examined, with no differences found. Our results also demonstrated that the signal peptide of the rice amylase 3D gene was effectively active in chitinase transgenic peanut roots. Peanut recombinant chitinase was extracellularly secreted against $\text{S. rolfsii}$. Compared with Figure 8B, Figure 8C shows that it seems that non-transgenic peanut chitinase was also induced but not enough to completely inhibit fungal growth.
According to studies conducted by Rohini et al (2001) and Iqbal et al (2012), chitinase transgenic peanuts outperformed non-transgenic controls in terms of resistance to *Cercospora arachidicola*, an ascomycete that causes early leaf spots in peanuts. Prasad et al (2012) discovered that only 0-10% of *Ritch* transgenic peanut seeds were infected with *Aspergillus flavus* under *in vitro* seed inoculation tests.

**Conclusion**

The chitinase 42 kDa genes from *T. asperellum* SH16, which are driven by the root-specific Asy promoter, were successfully heterologously expressed in peanut cultivar L14. All three genes, one wild-type (*Chi42*) and two synthetic genes (*syncodChi42-1* and *syncodChi42-2*) were secreted extracellularly by the guidance of a signal peptide of the rice amylase 3D gene. Chitinase activity of two synthetic genes outperformed the wild-type gene but all of them exhibited a strong antifungal activity for *S. rolfsii*. These findings suggest that the *Chi42*-derived optimized chitinase genes might aid peanuts to fight the phytopathogenic fungus *S. rolfsii*.

**Declarations**

**Acknowledgments**

The authors would like to thank the partial support of Hue University under the Core Research Program, Grant No. NCM.DHH.2022.13. The authors would like to thank the partial support of Hue University under the Core Research Program, Grant No. NCM.DHH.2022.13. Phung Thi Bich Hoa was awarded a scholarship from the PhD Scholarship Program and Nguyen Hoang Tue was awarded a scholarship from the Master Scholarship Program of Vingroup Innovation Fund (VINIF) and Vingroup Big Data Institute (VINBIGDATA) with codes VINIF.2020.TS.111 and VINIF.2021.ThS.46, respectively.

**Author Contribution Statement**

NHL designed this study. NQDT, NXH, and NHT designed plant expression vectors. NHT performed the triparental mating experiment. PTBH, NHT, HTQT, HAT, and LNHN performed *in vitro* culture and transformation experiments. NHL, PTBH, and NNL performed gene expression analyses. NHL prepared the manuscript. All authors have read and approved the manuscript.

**Funding Information**

This work was supported by National Foundation for Science and Technology Development (NAFOSTED), Vietnam (Grant number 106.02-2017.346). The authors would also like to thank Hue University, Vietnam for facilitating this study.

**Compliance with Ethical Standards**

**Competing Interests**

The authors declare that they have no competing interest.

**References**


Tue NH, Tuong TGC, Trang PTH, Chung ND, Tien NQD, Loc NH (2021) Cloning the root-specific Asy promoter and genes encoding chitinase 42 kDa of *Trichoderma asperellum* into the plant expression vector. J Appl Biol Biotechnol. Online First


### Tables

#### Table 1. Oligonucleotide sequence of specific primers for indicators of chitinase genes.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Directions</th>
<th>Sequences (5’-3’)</th>
<th>PCR products (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Chi42</em></td>
<td>Forward</td>
<td>TGGTACTATGCAGCTTGACCT</td>
<td>505</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>GTACTCCCAGTGCACGTCAA</td>
<td></td>
</tr>
<tr>
<td><em>SyncodChi42-1</em></td>
<td>Forward</td>
<td>TGGTACTATGCAGCTTGACCT</td>
<td>689</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>CGTAATCCAAGACTTGACCAG</td>
<td></td>
</tr>
<tr>
<td><em>SyncodChi42-2</em></td>
<td>Forward</td>
<td>TGGTACTATGCAGCTTGACCT</td>
<td>931</td>
</tr>
<tr>
<td></td>
<td>Reverse</td>
<td>AGAACCGGAACCAATACCATT</td>
<td></td>
</tr>
</tbody>
</table>

Note: TGGTACTATGCAGCTTGACCT sequence of all three forward primers was located in signal peptide segment of *rice amylase 3D* gene that flanked 5’ end of chitinase genes (data not shown). The signal peptide was used for
the extracellular expression of the enzyme chitinase.

Table 2. Chitinase transgenic efficiency in peanuts through embryonal and de-embryonal cotyledon.

<table>
<thead>
<tr>
<th>Explant type</th>
<th>Gene</th>
<th>Number of samples</th>
<th>Number of shoot regeneration samples</th>
<th>Number of shoot/sample</th>
<th>Number of surviving shoots</th>
<th>Number of shoots with positive PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Embryonal cotyledon</td>
<td>Chi42</td>
<td>200</td>
<td>200</td>
<td>4.26</td>
<td>68</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>SyncodChi42-1</td>
<td>200</td>
<td>200</td>
<td>4.07</td>
<td>74</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>SyncodChi42-2</td>
<td>200</td>
<td>200</td>
<td>4.01</td>
<td>65</td>
<td>26</td>
</tr>
<tr>
<td>De-embryonal cotyledon</td>
<td>Chi42</td>
<td>200</td>
<td>35</td>
<td>7.29</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>SyncodChi42-1</td>
<td>200</td>
<td>36</td>
<td>7.05</td>
<td>36</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>SyncodChi42-2</td>
<td>200</td>
<td>30</td>
<td>6.23</td>
<td>27</td>
<td>15</td>
</tr>
</tbody>
</table>

Figures

Figure 1

Figure 2

Chitinase gene transfer into peanut by *Agrobacterium*-mediated transformation. A: de-embryonal and embryonal cotyledons were used as explants to transfer chitinase genes, B: shoot regeneration of transgenic peanuts on selective medium, C: transgenic shoot multiplication, D: whole transgenic peanuts.

Figure 3

Figure 4

SDS-PAGE (A) and Western blot (B) analysis of some chitinase transgenic peanut lines have positive PCR. M: 180-10 kDa PageRuler™ Prestained Protein Ladder (Thermo Fisher Scientific, Waltham, Massachusetts, USA); PC: bacterial purified chitinase 42 kDa as a positive control; NC: non-transgenic peanut as a negative control; 1-2
(Chi42): WTA-2 and WTA-4; 3-4-7 (syncodChi42-2): S2A-13, S2A-14 and S2A-12; 5-6 (syncodChi42-1): S1A-9 and S1A-15.

**Figure 5**

The density of immune (Western) signals of some chitinase transgenic peanut lines was measured by the ImageJ software.
Figure 6

Colloidal chitin plate assay for chitinolytic activity from some transgenic peanut lines. PC: bacterial purified chitinase 42 kDa as a positive control; NC: non-transgenic peanut as a negative control; S2A-12, S2A-13 and S2A-14: syncodChi42-2; S1A-9 and S1A-15: syncodChi42-1; WTA-2 and WTA-4: Chi42.
Figure 7

The total and specific activity of chitinase from some transgenic peanut lines. NC: non-transgenic peanut as a negative control; S2A-12, S2A-13 and S2A-14: syncodChi42-2; S1A-9 and S1A-15: syncodChi42-1; WTA-2 and WTA-4: Chi42.
**Figure 8**

*In vitro* *S. rolfsii* antifungal activity of chitinase transgenic peanut roots on the agar plate. A: bacterial purified chitinase 42 kDa as positive control, B: medium without chitinase as 1st negative control, C: non-transgenic peanut roots as 2nd negative control, D: *syncodChi*42-1 (line S1A-15), E: *syncodChi*42-2 (line S2A-12), and F: *Chi42* (line WTA-2).