Scientia Horticulturae 234 (2018) 81–86

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Increased resistance against storage rot in transgenic carrots expressing chitinase *chit42* from *Trichoderma harzianum*

Seyedmohammadreza Ojaghian[a,b,*], Ling Wang[c], Guan-Lin Xie[d], Jing-Ze Zhang[d]

[a] Department for Management of Science and Technology Development, Ton Duc Thang University, Ho Chi Minh City, Vietnam
[b] Faculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City, Vietnam
[c] College of Biosystems Engineering and Food Science, Zhejiang University, Hangzhou 310058, China
[d] Ministry of Agriculture, Key Lab of Molecular Biology of Crop Pathogens and Insects, Institute of Biotechnology, Zhejiang University, Hangzhou 310058, China

ARTICLE INFO

Keywords:
Chitinase
Isoform
Plasmid
Southern blot

ABSTRACT

White rot caused by *Sclerotinia sclerotiorum* is a cosmopolitan disease which causes economically important losses in carrot-producing areas during storage and distant transportation. This study was conducted to introduce chimeric *chit42* from *Trichoderma harzianum* to carrots through Agrobacterium-mediated transformation to evaluate the resistance of transgenic carrot roots to *S. sclerotiorum*. Using polymerase chain reaction (PCR), the transgene was detected in twenty-seven transformants with 5.8% transformation efficiency. Four randomly chosen transgenic lines were assessed for the copy number of the transgene via southern blot hybridisation. One, two and one plants carried three, two and one copies of the transgene, respectively. After protein extraction, the chitinase activity of four plants was evaluated. The level of endochitinase was markedly increased in all tested lines. In all four tested lines, levels of three isoforms were significantly increased compared with controls. In addition, disease severity was measured in transgenic carrots. The results showed that introducing the gene *chit42* to carrots was able to significantly decrease ($P < 0.05$) severity of carrot rot caused by three isolates of *S. sclerotiorum* after three, six and 12 d.

1. Introduction

Carrot (*Daucus carota* L.) rot is a cosmopolitan disease which causes major losses in many carrot-producing areas during storage and distant transportation. The causal agent, *Sclerotinia sclerotiorum* (Lib.) de Bary, is an ascomycetous fungus which can attack a great number of dicotyledonous plants all over the world (Boland and Hall, 1994). The disease usually has no symptom in the filed and major damage occurs during storage (Kora et al., 2005). Although no carrot cultivar has been *S. sclerotiorum*, some non chemical methods *in vivo* and *in vitro*. Application of fungicides in the field can decrease disease severity during storage (Huang and Erickson, 2007). However, due to emergence of fungicide-resistant isolates (Gossen and Rimmer, 2001) and environmental concerns, researchers have been motivated to search for alternative control methods.

Chitinases are extracellular pathogenesis-related (PR) proteins with low-molecular weight which are involved in plant defense against fungal pathogens (Neuhaus, 1999). Chitin constituting 3–60% of fungal mycelial walls (Collinge et al., 1993) is a linear homopolymer of β-1,4 linked N-acetylglucosamine (Sahai and Manocha, 1993). The chitinases catalyse the hydrolysis of β -1,4 linkages of N-acetyl-glucosamine resulting in cell wall degradation of phytopathogenic fungi. The chitinases can be found in fungi, bacteria, higher plants and insects (Kramer and Muthukrishnan 1997).

There are many studies showing that chitinase-encoding genes inserted into genome of plants can increase resistance against numerous fungal phytopathogens. In previous studies, different plants such as tobacco (Broglie et al., 1991; Patil and Widholm 1997), tomato (Tabaeizadeh, 1997), carrot (Punja and Raharjo, 1996), rice (Lin et al., 1995) and rose (Marchant et al. 1998) transformed with chitinase-encoding genes from other plants have developed higher resistance against a broad range of fungal pathogens.

Trichoderma species, as another source of chitinase transgenes, are soilborne imperfect fungi used as biocontrol agents against a great number of phytopathogens. Lorito, 1998 showed that chitinase-encoding genes from *Trichoderma* spp. have higher antifungal potential than the corresponding plant genes. In previous researches, antifungal efficacy of different chitinase genes from *Trichoderma harzianum* such as *chit33* against *R. solani* (Limón et al., 1995; de las Mercedes Dana et al., 2006) and *chit42* against *Alternaria alternata*, *A. solani*, *Botrytis cinerea* and *R. solani* (Lorito, 1998; de las Mercedes Dana et al., 2006) has been shown in transformed plants.

* Corresponding author.
E-mail address: Seyedmohammadreza.Ojaghian@tdt.edu.vn (S. Ojaghian).

https://doi.org/10.1016/j.scienta.2018.02.025
Received 28 December 2017; Received in revised form 9 February 2018; Accepted 10 February 2018
0304-4238/ © 2018 Elsevier B.V. All rights reserved.

The objective of this study was to introduce the gene *chit42* from *T. harzianum* to carrots to evaluate the resistance of transgenic carrot roots against *S. sclerotiorum*.

2. Materials and methods

2.1. Source of carrot roots andS. sclerotiorum

Three isolates of *S. sclerotiorum* including 44-2, 7-3 and SSCL1 were kindly provided by Institute of Biotechnology, Zhejiang University, and were designated as 1, 2 and 3, respectively. The fungi were cultured on carrot dextrose agar (CDA, Ojaghian et al., 2013) and maintained in refrigerator (4 °C) until used. In addition, carrot seeds (cv. Carson) were provided by Institute of Agronomy, Zhejiang University. All chemicals, culture media and antibiotics were purchased from Merck (Darmstadt, Germany), unless stated otherwise.

2.2. Plasmid construction

Escherichia coli DH5α was used in all molecular experiments and *A. tumefaciens* strain HANG287 was used for the plant transformation procedure. The binary plasmid pGisPEC1 (Fig. 1) was designed from pBik39 and contained, within the T-DNA region, the neomycin phosphotransferase II (*nptII*) and *chit42* genes. The *nptII*, as a selectable marker and kanamycin-resistant gene for plant selection, was regulated by the nopaline synthase (NOS) promoter and terminator. The chimeric *chit42* gene was regulated by the cauliflower mosaic virus 35S promoters (CaMV 35S) and terminated by the NOS terminator. Using freeze–thaw method, the recombinant plasmid was then mobilized into *A. tumefaciens* (Wise et al., 2006).

2.3. Transformation procedure

2.3.1. Explants and bacterial preparation

Carrot seeds were disinfected firstly in ethanol (10%) and then in 0.1% (v/v) sodium hypochlorite each for 3 min, and washed three times with sterile deionized water. The seeds were then germinated on MS medium (Murashige and Skoog, 1962) and incubated in the presence of light for 6 days. The cotyledonary petioles were cut off from germinated plants and located on the MS solid medium with 4 mg/L benzylaminopurine (BAP) for pre-culture. After 4 days, the explants were utilized for transformation. The colonies of the *Agrobacterium*-harbouring strain were grown in the LB medium amended with 55 mg/L kanamycin and grew overnight at 27 °C on a shaker (170 rpm) to mid-log phase. The culture of bacteria was then transferred to a new medium and grown until OD600 = 0.5 with liquid medium. The bacterial cells were collected after centrifuging (6000 g for 6 min), and re-suspended in MS medium for the next inoculation step.

2.3.2. Carrot transformation

The explants were submerged in the bacterial suspension for 6 min with constant shaking and located on autoclaved filter paper to eliminate the moisture, and then located on the MS solid medium with 4 mg/L BAP in Petri plates for co-cultivation at 26 °C for 4 days under dark condition. In next step, the explants were rinsed with the sterile deionized water including 300 mg/L cephatoxime to prevent the growth of *A. tumefaciens* joined to the explants and then transferred to the MS solid medium with 4 mg/L BAP, 250 mg/L cephatoxime and 9 mg/L kanamycin. After initiation of shoot, the explants were translocated to MS solid medium with 250 mg/L of cephatoxime and 16 mg/L of kanamycin. The regenerated shoots (2.5–3 cm long) were cut from the explants and translocated to MS solid medium with 3 mg/L of 3-indolebutyric acid (IBA) and 250 mg/L of cephatoxime to get ready for rooting. All above-mentioned media had 10 g/L agar and 4% (w/v) sucrose. The media pH was 5.5–5.9 before adding agar and autoclaving. The explants were cultured at 27 ± 2 °C and 17 h photoperiod. The non-transformed controls were obtained using the same procedure with the exception that sterile deionized water was used instead of *A. tumefaciens* suspension. The regenerated seedlings at the two-true-leaf stage were grown in mineral soil in a growth chamber (25–27 °C, 90% humid relativity and 17 h photoperiod) and mature carrots were harvested with a diameter of 30–40 mm.

Table 1
Primers used for polymerase chain reaction (PCR) confirmation of the transgenic carrots.

Gene	Sequence of primer (5′–3′)	Reference
nptII	gAggCTATTCggCTATgACT AATCTCgTgATggCAggTTg	Yao et al. (1995)
chit42	CGTTCCCGCAAGCAAGATCG GTGAAGCTTCCCGATCTAGTAACAT	Zarinpanjeh et al. (2016)
virG	ATGATTGTACATCCTTCACG TGCTGTTTTTATCAGTTGAG	Zarinpanjeh et al. (2016)

2.4. PCR analysis of transgenic carrots

Polymerase chain reaction (PCR) analysis was used for initial evidence of the presence of transgene in transgenic carrots using specific primers (Table 1) for *nptII* and *chit42* genes. The DNA was extracted from leaf material of transgenic and non-transgenic control carrots as described by Deng et al. (1995). The PCR were performed in a PTC-200 DNA Engine Cycler (MJ Research, Waltham, MA, USA). PCR was carried out as follows: an initial denaturation at 95 °C for 14 min, followed by 45 cycles of denaturation at 95 °C for 1 min, annealing at 59 °C for 1 min, extension at 73 °C for 1 min and a final extension at 73 °C for 12 min. PCR products were loaded on 1.5% agarose gels in TAE buffer and visualized under UV light. This experiment was carried out twice with two replicates for each sample.

2.5. Southern blot analysis

Four PCR-positive and control plants were tested by Southern blot analysis to corroborate the integration of the introduced genes. Genomic DNA was extracted from leaf material of transgenic and non-transgenic control carrots and digested with *HindIII*. After electrophoresis on 1% (w/v) agarose gels, the digested DNAs were blotted to a nylon membrane (Amersham Hybond NTM + , Amersham, UK). The probe was obtained by PCR amplification with specific primers for *chit42* of the plasmid pGisPEC1 and purified from gel with QIAEX II gel extraction kit (Qiagen, Valencia, USA). The purified PCR product was labelled by DIG DNA Probe Kit (Roche Applied Science GmbH, Mannheim, Germany). The hybridization was carried out at 43 °C and the detection was fulfilled by using DIG-High Prime DNA Labelling and Detection Starter Kit II according to the manufacturer's instructions

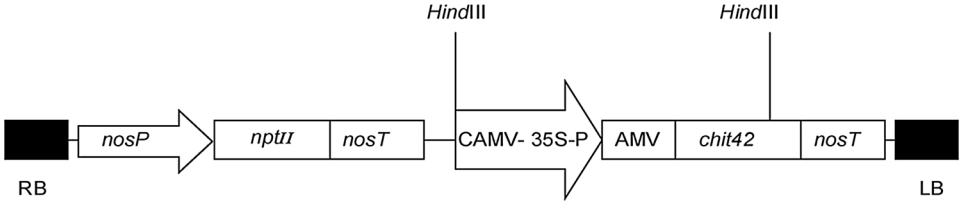

Fig. 1. Schematic drawing of the T-DNA region of plasmid pGisPEC1. RB: right border; LB: left border; *nosP* and *nosT*: nopaline synthase promoter (320 bp) and terminator (280 bp), respectively; *nptII*: neomycin phosphotransferase II-encoding gene (812 bp); CaMV-35S-P: cauliflower mosaic virus 35S promoter (842 bp); AMV: alfalfa mosaic virus leader sequence (53 bp).

Download English Version:

https://daneshyari.com/en/article/8892744

Download Persian Version:

https://daneshyari.com/article/8892744

Daneshyari.com