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Beta glucanase (Bgn13.1) expressed in transgenic *Brassica napus* confers antifungal activity against *Sclerotinia sclerotiorum*

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Abstrcat: *Brassica napus* is an important oilseed crop and the yield loss due to fungal disease stem rot caused by *Sclerotinia sclerotiorum* is a serious problem in cultivation of this crop. The pathogenesis-related (PR) protein, glucanase, hydrolyzes a major cell wall component, glucan, of the pathogenic fungi and acts as a plant defense barrier. In this study, a β-1,3-glucanase (*bgn*13.1) gene was isolated from the biocontrol fungus *Trichoderma virens*-10 (showing the high β-glucanase activity) and cloned in pUC19 cloning vector. The cloned fragment was confirmed by molecular analysis and showed to contain two short introns, 52 and 57 bp and an open reading frame coding 761 amino acids. The *bgn*13.1 gene was over-expressed under the CaMV35S promoter in *B. napus*, R line Hyola 308. Transformation of cotyledonary petioles was achieved by pBIKH1 containing *bgn*13.1 gene via *Agrobacterium tumefaciens* LBA4404. The insertion of transgene was verified by the polymerase chain reaction (PCR) and genomic DNA Southern dot blotting in T0 generation. RT-PCR analysis indicated that the transgenic canola plants were able to transcribe the β-1,3 glucanase gene. Also, we used transgenic over-expression approach in order to investigate antifungal activity of expressed Bgn13.1 on *S. sclerotiorum*. The heterologous expressed Bgn13.1 of line # 7 and line # 10 compared with other lines showed stronger inhibition against hyphal growth of *S. sclerotiorum* with inhibition rate of 35.2% and 32.8%, respectively.

Keywords: *bgn*13.1, *Brassica napus*, glucanase enzyme, *Sclerotinia sclerotiorum*, transgenic plants, *Trichoderma virens,*

Interoduction

Fungal diseases are rated either the most important or the second most important factor contributing to the yield losses in many important crops (Wang *et al.,* 2003). Contribution of the fungal diseases towards the total yield losses in some important crops is about 30% (Adams, 2004). Various fungal cell wall degrading enzymes, including the chitinases and different glucanases or glucosidases are involved in the mycoparasitic process of *Trichoderma* (Chet *et al.,* 1998). Among these hydrolases, β-1, 3-glucanases are attractive molecules because they have strong antifungal activity against a wide range of fungi (Stone and Clarke, 1993; Walsh *et al.,* 2000; Lorito *et al.,* 1994; Lorito, 1998). These hydrolytic enzymes catalyze the degradation of β-1,3-glucan and, since this compound is abundantly present in the cell wall of many filamentous fungi (Wessels and Sietama, 1981), they are thought to be capable of inhibiting fungal growth.

Canola (*Brassica napus* L.) is one of the most important sources of edible vegetable oils, industrial used oil, and protein-rich products in

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the world. Like many other crops, the production of this crop is challenged by phytopathogenic fungi. Extensive use of chemical fungicides, which have drawbacks such as environmental pollution, producing residual poisons to the human beings and animals, and expensive cost, is the conventional method of control of fungal pathogens. Therefore, it is desirable to introduce the foreign fungal-resistant genes into the important crop plants. Theoretically, the foreign genes can be transferred into the genomes of plants without altering any other agro-economically important traits (Chang *et al.,* 2002). Since β-1, 3-glucan is a structural polymer in the fungal cell wall, among fungal resistant genes, the glucanases which are potential antifungal agents through their glucan degradation activity, are excellent candidates for controlling of fungal pathogens development (Lorito *et al.,* 1998).

The aim of this study was to amplify and clone the β-glucanase gene (*bgn*13.1) from *Trichoderma virens*-10 and transform canola (R line Hyola 308) by *bgn*13.1 via *Agrobacterium*-mediated transformation. The inhibitory effect of the expressed Bgn13.1 in transgenic lines on the *Sclerotinia sclerotiorum* growth will be evaluated.

Materials and Methods

Plant material

The rapeseed (*B. napus* L.) R line Hyola 308, was used as receptor, which was kindly provided by the Oilseed and Development Co. Tehran, Iran

Microorganisms, plasmid vector, and culture conditions

Trichoderma virens-10 was identified in our laboratory as a high producer of the beta 1,3 glucanase enzyme (Bahramsari *et al.,* 2005) and used for DNA isolation. The stock culture was stored on agar (1.5%) slants of MY medium (2% malt extract, 0.2% yeast extract,1% maltose). For chromosomal DNA preparation, the spores were inoculated in the liquid MY medium and grown for 2 days at 30 °C with shaking, and the mycelia were harvested by filtration (Takashima *et al.,* 1998). *S. sclerotiorum* was kindly provided by H. Afshari-Azad from Iranian Research Institute of Plant Protection, Tehran, Iran.

Escherichia coli DH5α was used in all the molecular biological experiments and *Agrobacterium tumefaciens* LBA4404 was used for plant transformation. Bacteria were grown in the LB medium at the appropriate temperatures (37 °C for *E. coli* and 28 °C for *A. tumefaciens*) with shaking (200 rpm). Plasmid pUC19 (MBI, Fermantas) was used for cloning of PCR fragment containing *bgn*13.1 gene and sequencing and plasmid pBI121 (Novagen) was used as a binary plant expression vector.

Amplification of β-glucanase gene from the genomic DNA

For amplification of the beta 1,3-glucanase (*bgn*13.1) gene from *T. virens*-10, two specific primers were synthesized (Table 1) based on the sequence similarity of the *T. harzianum bgn* gene present in the database (X84085). To facilitate the subsequent cloning of the PCR-derived fragments, the *Xba*I/*Sac*1 restriction sites were added to the 5'-end of Fbgn13.1 and R'bgn13.1 primers, respectively.

Table 1 Oligonucleotides (primers) used in this study.

Name	Oligonucleotides sequence
Fbgn13.1	5'-GCTCTAGAATGTTGAAGCTCACGGCGC-3'
Rbgn13.1	5'-GCGAGCTCTTAAGTAGTATAACGGGCAACG-3'
F2bgn13.1	5'-AGAGCTTTCTTTCGCCGTAGCTATTAAG-3'
R2bgn13.1	5'-CTGAAGTTCTCAGCGATGTGATCCG-3'
virGf	5'-ATGATTGTACATCCTTCACG-3'
virGr	5'-TGCTGTTTTTATCAGTTGAG-3'

Fungal chromosomal DNA was prepared as described by Sun *et al.,* (2002). Amplification of the DNA fragments encoding *T. virens*-10 *bgn*13.1 gene was performed using the polymerase chain reaction (PCR). The PCR reactions contained 2.5 units of Fermentas *Pfu* DNA polymerase, 1X buffer, 200 μM of each deoxynucleotide triphosphate, 2 μM $MgSO_4$ and

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0.5 µM primers. The PCR reaction conditions were as follows: initial denaturation for 5 min at 94 °C and 30 cycles with 94 °C for 1min, 62 °C for 1 min, and 72 °C for 3 min, for followed by a final extension of 10 min. The PCR products were separated by electrophoresis on a 1% agarose gel and the products were purified by High pure PCR product purification Kit (Roche Diagnostics GmbH). The purified fragment digested with the *Xba*1/*Sac*1 and cloned into the same sites in pUC19.

General DNA procedures

Plasmid DNA preparation and electrophoresis of the DNA fragments were performed by routine procedures (Sambrook and Russell 2001). The restriction enzyme analysis of the amplified DNA and the cDNA molecules were carried out as recommended by the manufacturer (Fermentas, Germany). The bacteria were transformed by the heat shock method (Sambrook and Russell, 2001).

Preparation of the explants and bacterial strain for transformation

Seeds were sterilized by being submerged in 70% ethanol for 5 min and then in 0.1% $HgCl_2$ for 8 min. They were then rinsed several times with the sterilized water and plated on the ½MS medium (Murashige and Skoog 1962) under light for 5 days. After germination, the cotyledonary petioles were cut and placed on the MS solid medium with 3.5 mg/l benzylaminopurine (BAP) (CM) for pre-culture. After 2 days, the explants were used for transformation.

Single colonies of the *A. tumefaciens* strain harboring the recombinant plasmid containing the *bgn*13.1 gene were grown in the LB medium supplemented with 50 mg/l kanamycin, and allowed to grow overnight at 27-28 °C with constant shaking (200 rpm) to mid-log phase. The bacterial culture was then transferred to a fresh medium with the amount of 0.1% and cultivated till OD_{600} = 0.4 with liquid medium. The bacterial cells were collected by centrifugation and re-suspended in ½MS medium for use.

Transformation and selection procedure

The explants were immersed in the bacterial suspension for 1.5 min with constant shaking, and then placed onto the sterile filter paper to remove the excessive moisture, and placed on the CM medium in the Petri dishes for co-cultivation at 25 °C for 2 days.

After co-cultivation, the explants were washed with the sterile water containing 200 mg/l cefotaxime to inhibit the growth of *A. tumefaciens* attached to the explants and then transferred to the MS solid medium with 3.5 mg/l BAP, 15 mg/l kanamycin, and 200 mg/l cefotaxime. After shoot initiation, the explants were transferred to MS solid medium with 25 mg/l kanamycin, and 200 mg/l cefotaxime. The regenerating shoots (about 3 cm in length) were excised from the explants and transferred to the MS solid medium with 2 mg/l 3-Indolebutyric acid (IBA), 25 mg/l kanamycin, and 200 mg/l cefotaxime for rooting and recovering the complete plants. All the above media contained 3% (w/v) sucrose with pH 5.8, and all the explants, were cultured in a growth chamber under 23-25 °C and 16 h of day time with light intensity of 2000 Lux.

Molecular analysis of the transgenic canola

The leaf material from the transgenic and non transgenic canola was harvested, lyophilized and grinded into a fine powder for extraction of genomic DNA by the method of Doyle and Doyle (1990). PCR amplification was used for initial evidence of the transgene presence in the putative transgenic plants. The DNA fragment containing the *bgn*13.1 gene was amplified by PCR using the genomic DNA and specific primers.

Expression analysis

Specific mRNAs of the transgene were checked using reverse transcriptase (RT)-PCR. Total RNA was isolated from the leaves of the transgenic and non-transgenic canola plants using an RNA isolation kit (Roche Applied Science Gmbh, Germany). The first strand cDNA was generated using the *bgn*13.1 specific primer (R'bgn13.1). PCR amplification of the

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above gene was achieved by using the first strand synthesis as template with the specific primers as described for PCR amplification of *bgn*13.1 gene.

Southern dot blot analysis

The genomic DNA was extracted from the fresh leaves of the putative transgenic plants and untransformed control plant with the Cetyl Trimethyl Ammonium Bromide (CTAB) method (Doyle and Doyle 1990). The genomic DNA (15 µg) was denatured for 10 min in boiling water and chilled on ice. The denatured genomic DNAs were spotted on a nylon membrane (Hybond N+, Amersham), and hybridized to Dig-dUTP labeled *bgn*13.1 probe. A fragment (1200 bp in size) was obtained from PCR amplification of the *bgn*13.1 gene using the F2bgn13.1/R2bgn13.1 primers and plasmid pBIKH1 containing the *bgn*13.1 as template and subjected to the DIG DNA labeling (Roche Applied Science Gmbh, Germany) and used as a probe in hybridization experiments.

Bioassay of the transgenic canola plants

The antifungal activity of the crude extracts from transgenic plants was tested using a modification of the radial diffusion assay as described by Broglie *et al.,* (1991). Canola leaf material (3 g) was grinded to a fine powder in liquid nitrogen using a mortar and pestle. Two volumes of 1 M NaCl in 20 mM NaOAc (pH 4.7) were added to the leaf material. The extracts were then shaken for 1 h at 4 °C and subsequently centrifuged at 13000g for 20 min at 4 °C. The resulting supernatants were then stored at -20 °C. The protein content was determined against BSA using the Bradford assay (Bradford, 1976). An agar disc (5 mm in diameter) containing *S. sclerotiorum*, which was derived from the fungus in an actively growing state, previously cultured on PDA, was placed at the center of Petri dishes containing PDA. The plates were then incubated at 28 ºC. After incubation for 48 h, 50 µg of the crude protein was added at different position at the front edge of the fungal colony. Crude protein from non transgenic canola plant was used as a control. The plates were incubated for a further 48 h after which the inhibition of hyphal extension around the crude protein was observed. The inhibition rate (%) was calculated as follows: [(the distance between the control hyphal edge to the center–the distance between the treated hyphal edge to the center)/the distance between the hyphal edge to the center in control] × 100. The experiments were conducted three times.

Results

In this study, we isolated β-glucanase gene (*bgn*13.1) from *T. virens*-10 which had previously exhibited the highest β-glucanase activity. To isolate the *bgn*13.1 gene, the oligonucleotide primers were designed based on the related DNA sequences available in the GenBank database. PCR amplification was performed on the genomic DNA generating the specific band of approximately 2.4 Kb which was cloned into a cloning vector and confirmed by PCR. Based on *T. harzianum* β-glucanase gene sequence, the cloned gene was confirmed by restriction pattern analysis using *Pst*I and *Bgl*I enzymes and sequencing (data not shown). This sequence was submitted to the NCBI under accession number of EF426721.

Comparison of the cloned DNA sequence with that of the *bgn*13.1 cDNA (Raoufzadeh *et al.,* 2008) demonstrated that this gene is interrupted by two short introns, 52 and 57 bp in length. This gene contains an open reading frame encoding a protein of 761 amino acids, with the calculated molecular mass of 80.7 kDa. The consensus sequences, GT on the 5' end and AG on the 3' end of the introns of the *bgn*13.1 gene are also observed (data not shown).

For cloning of the *bgn*13.1 gene in plant binary expression vector pBI121 for transformation of *B. napus,* R line Hyola 308, the fragment containing the *bgn*13.1 gene was subcloned into *Xba*1/ *Sac*1 sites of pBI121 and confirmed by PCR and restriction patterns (Fig. 1).

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The open reading frame of *bgn*13.1 gene in pBI121 (pBIKH1 construct) which is between the CaMV 35S promoter and nopaline synthase terminator was confirmed by DNA sequencing (data not shown).

The constructed pBIKH1 mobilised into the *A. tumefaciens* (LBA4404) and subsequently used for 5 days old cotyledonary petioles transformation of the *B. napus*, R line Hyola 308. The shoots were regenerated from the cotyledonary petioles 1-2 weeks after planting. Independent transgenic canola lines were successfully rooted (about 75%) on the kanamycin-containing selection media and then transferred to the greenhouse (Fig. 2). Eight independent putative transgenic lines (1, 2, 4, 5, 7, 9, 10 and 12) showed to contain the *bgn*13.1 transgene (a fragment corresponding to the size "2.4 Kb" of the *bgn*13.1 gene in the lines tested) using PCR (Fig. 3). The *bgn*13.1-specific primers did not amplify corresponding *bgn*13.1 fragment in the untransformed sample.

A set of *vir*G primers (virGf/virGr) used for detection of *Agrobacterium* contamination if any that might have escaped the selection. The PCR detection under various conditions showed no detectable band using the transgenic plants DNA as tempetale. Using *vir*G primers an expected 738 bp band was detected when the *Agrobacterium* DNA was used as control (data not shown).

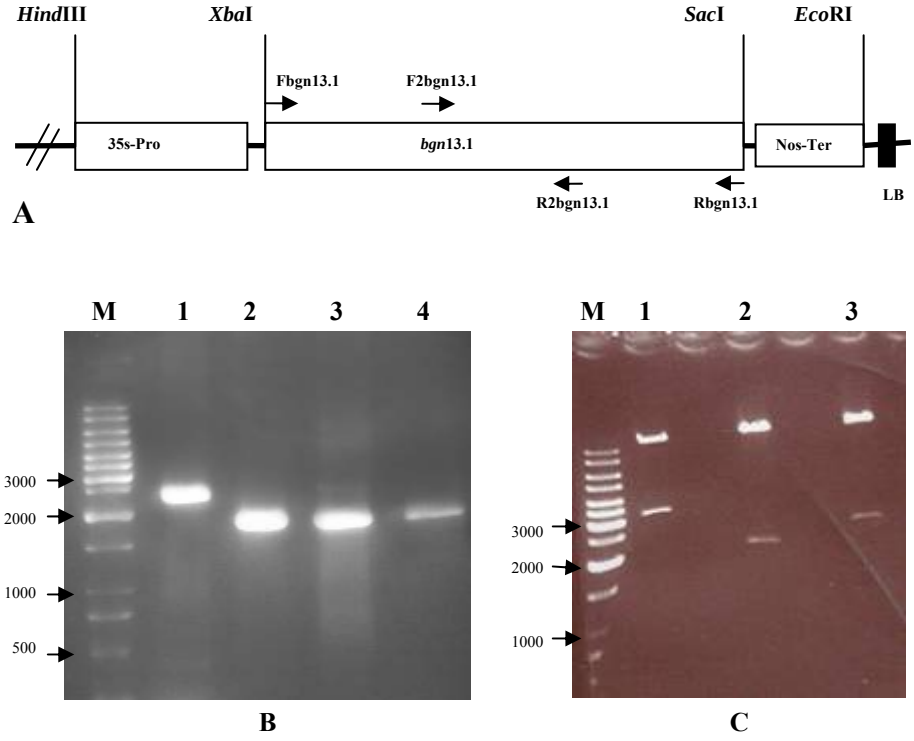

Figure 1 Confirmation of pBIKH1construct containing *bgn*13.1 gene by PCR and digestion pattern.
A) Schematic representation of the *bgn*13.1 cassette with expected position of different primers and restriction sites used for PCR and digestion patterns confirmation, respectively.
B) line 1, PCR pattern using the Fbgn13.1/Rbgn13.1 as primers; line 2 and 3, PCR product using the Fbgn13.1/R2bgn13.1 as primers; line 4, PCR product using the F2bgn13.1/Rbgn13.1 as primers.
C) Line 1, 2 and 3 digestion patterns using *Eco*RI/*Hind*III, *Xba*I/*Sac*I, and *Xba*I/*Eco*RI enzymes, respectively; M, 1 Kb DNA ladder.

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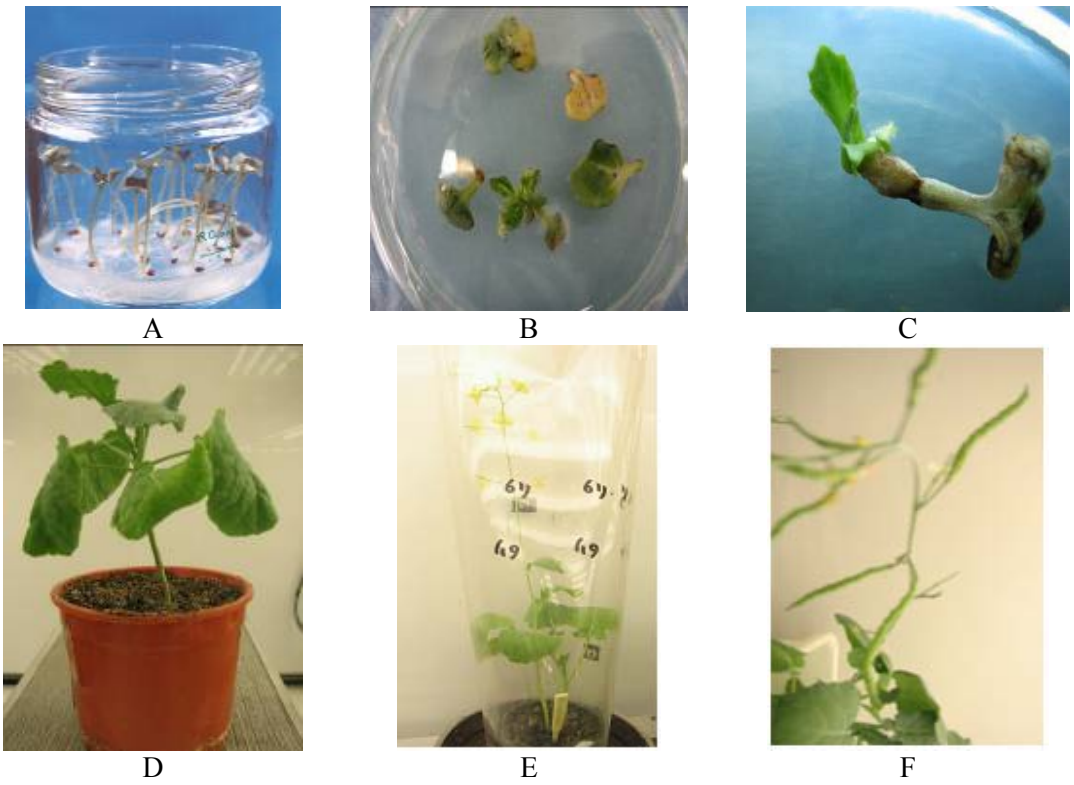

Figure 2 Transformation and regeneration of transgenic canola plants.
A) Germination of the R line Hyola 308 seeds.
B) Shoot regeneration from cotyledonary petioles after transformation (1-2 weeks).
C) regeneration shoots in growth medium.
D) regeneration plantlet in the pot and acclimated to the non-aseptic environment; E and F, transgenic canola plant flowers and pods in the greenhouse.

Southern dot blot analyses on the PCR positive transgenic plants were performed and the results verified the integration of the exogenous gene into the genomes of all eight putative transgenic canola plants (Fig. 4). No hybridization signal occurred in the non-transgenic control plants.

Expression of the specific mRNA transgene (*bgn*13.1) in the transformed canola lines was proven by means of RT-PCR. The RNA was isolated from the leaf tissues of these putative transgenic canola lines for cDNA generation. The expected size (about 2.3 Kb) of the amplified cDNA fragment was detected in five transformed lines (2, 5, 7, 10 and 12) (Fig. 5). Non-transformed plant was used as negative control and no transcript was detected. To ensure that the amplified fragment from the transgenic plants is the spliced form of the transgene (*bgn*13.1), restriction patterns of amplified cDNA was compared with those of *bgn*13.1 genomic DNA. Digestion pattern using the *Pst*I and *Bgl*I enzymes indicated that the amplified band from transgenic lines is derived from *bgn*13.1 mRNA (Fig. 6).

In order to test the antifungal activity of the expressed *Bgn*13.1 from transgenic plants on the actively growing phytopathogenic fungus *S. sclerotiorum,* total protein extracts from leaves of transgenic and non-transgenic plants were tested using the radial diffusion assay. Antifungal activity was detected in extracts from five RT-PCR positive transgenic lines. The crude protein from line # 7 and line # 10 showed stronger inhibition against hyphal growth of *S. sclerotiorum* with inhibition rate of 35.2% and 32.8%, respectively, while transgenic line # 12, 5 and 2 exhibited weaker inhibition with inhibition rate of 19.8%, 18.1% and 14.7%, respectively, (Table 2). Crude protein from non transgenic plant, as control, did not inhibit hyphal growth of the fungal pathogen tested.

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Table 2 Characterization of putative canola transgenic plants using PCR, Dot blot, Rt-PCR and fungal inhibition rate.

Plant No.	PCR	Dot blot	RT-PCR	Inhibition rate (%)
1	+	+	-	-
2	+	+	+	14.7
4	+	+	-	-
5	+	+	+	18.1
7	+	+	+	35.2
9	+	+	-	-
10	+	+	+	32.8
12	+	+	+	19.8
WT	-	-	-	00.0

The transgenic lines were phenotypically analyzed and compared to the untransformed controls and did not show any abnormalities with regards to the growth, size or reproduction.

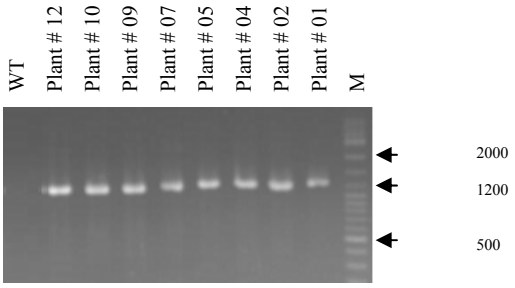

Figure 3 PCR analysis of putative transgenic canola: fragment (about 1200 bp) was amplified using the specific primers (F2bgn13.1/R2bgn13.1) and DNA of the putative transgenic plants as template (1, 2, 4, 5, 7, 9, 10 and 12); WT = wild type; M, 1 Kb DNA ladder.

Discussion

With regard to the importance of yield losses due to the contribution of fungal diseases, some research has been conducted to develop transgenic crop plants that have increased expression levels of pathogen related (PR) protein genes in hopes of producing fungal disease resistant varieties (Lawrence *et al*., 2000; Vleeshouwers *et al*., 2000; Gau *et al*, 2004).

Our previous study has shown that an Iranian isolate of *T. virence*-10 is a high glucanase enzyme activity source (Bahramsari *et al*., 2005), so in this study its glucanase gene, *bgn*13.1, was amplified and used for transformation of canola to confer resistance against *S. sclerotiorum* the causal agent of stem rot in canola.

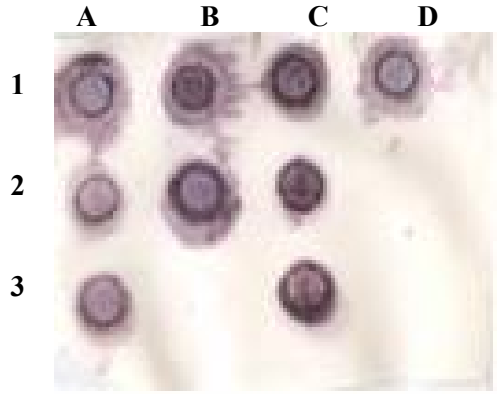

Figure 4 Genomic DNA dot blot analysis of putative transgenic canola with the total DNA loaded onto hybond N + membrane hybridized with a digoxigenin-labeled *bgn* 13.1 probe. A1 (plant # 1), A2 (plant # 2), A3 (plant # 4), B1 (plant # 5), B2 (plant # 7), C1 (plant # 9), C2 (plant # 10), and D1 (plant # 12); C3, DNA from pBIKH1 plasmid as positive control; B3, DNA from non transgenic plant as negative control

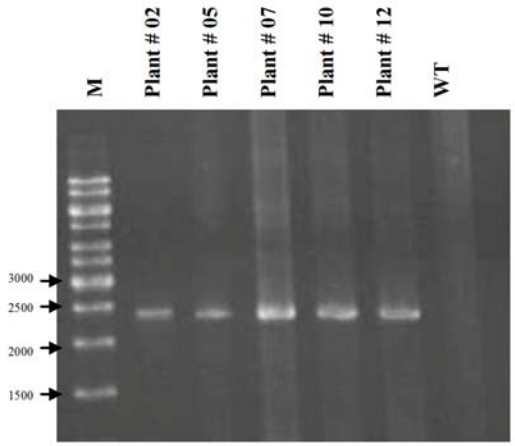

Figure 5 RT-PCR analysis of transgenic canola expressing *bgn*13.1 gene in plant No. 2, 5, 7, 10 and 12. WT, RT-PCR of the non transgenic canola plant as the negative control; M, 1 Kb DNA ladder.

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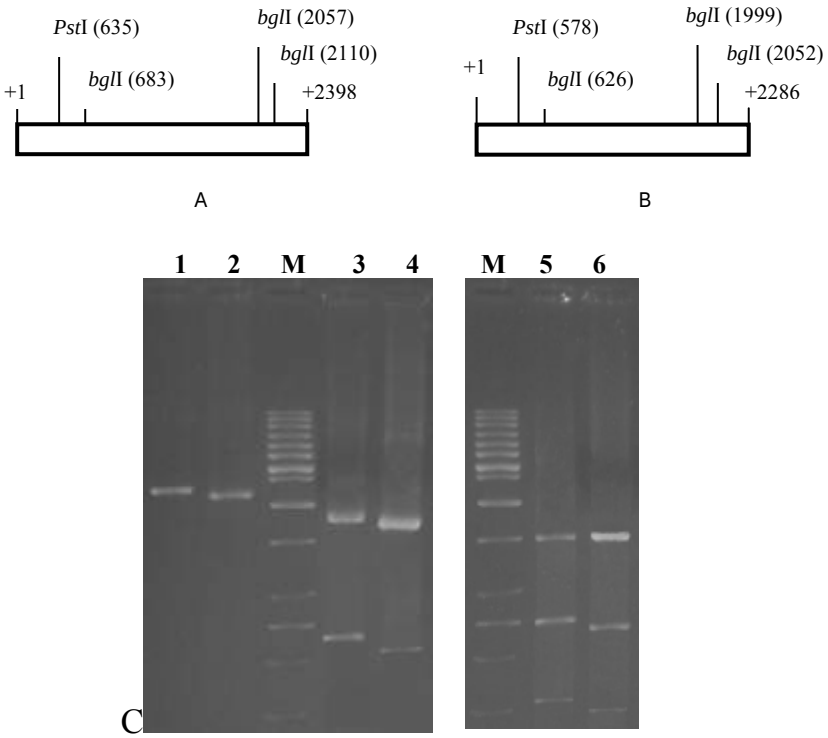

Figure 6 Confirmation of *bgn*13.1 cDNA from T0 transgenic plants in compared with the genomic DNA:
A and B, Schematic representation of genomic and cDNA restriction map, respectively;
C, line 1, PCR amplification of the *bgn*13.1 from genomic DNA using the specific primers (Fbgn13.1/Rbgn13.1), approximately 2.4 Kb; line 2, *bgn*13.1 cDNA amplification using the specific primers (2.3 Kb); line 3 and 4, confirmation of PCR product of *bgn*13.1 genomic DNA, (1763 and 635 bp) and cDNA (1708 and 578 bp) by restriction enzyme digestion using the *Pst*I, respectively; line 5 and 6, digestion of PCR product of *bgn*13.1 genomic DNA (1374 and 288 bp) and cDNA (1374 and 233 bp) by *Bgl*I enzyme, respectively; M, 1 Kb DNA ladder.

To study the *bgn*13.1 gene, in this paper, we report the sequence deposition in the NCBI and analysis of genomic DNA clone of the *bgn*13.1 gene, encoding beta 1,3-glucanase, from *T. virens*-10. Analysis of the *bgn*13.1 DNA sequence demonstrated that it contains two short introns which have also been reported in other β-glucanases from *Trichoderma* species (DeLaCrus *et al.,* 1995; Kim *et al.,* 2002; Gao *et al.,* 2006) while Donzelli *et al.,* (2001) reported a beta 1,3-glucanase gene from *T. atroviride* containing no introns.

There are several reports indicating transgenic plants expressing *bgn13.1* gene have shown enhanced fungal disease resistance in different species including apple (Bolar *et al*., 2000, 20001), creeping bentgrass (wang *et al.,* 2003), and rice (Liu *et al*., 2004).

In our study, under experimental conditions, a similar improvement in resistance to stem rot disease was demonstrated. The ability of the introduced *bgn*13.1 gene to enhance the antifungal potential of transgenic canola plants was studied by the agarose diffusion assay. The transgenic lines were able to exhibit antifungal activity against fungal pathogen *S. sclerotiorum,* in the *in vitro* assay. No inhibition was detected in the presence of non transgenic proteins.

Transformation of R line Hyola 308 of *B. napus* was mediated by *Agrobacterium* and the cut surfaces of cotyledoneary petioles containing the target cells. Results showed that this target is a vigorous source of new material leading to very rapid shoot development. The origin of these shoots has been shown by

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Sharma (1987) to be cells located around the cut end of the petioles.

The success of *Agrobacterium*-mediated plant transformation can be a function of the genotype of the species to be transformed, the strain (virulence) of *Agrobacterium*, the selectable marker, the regeneration capacity of the target cells and the accessibility of the bacterium to the regeneration cells. Also, CaMV 35S promoter used to ensure high levels of gene expression in all tissues. *Agrobacterium*-mediated transformation and use of CaMV 35S promoter have been reported by several research groups as effective parameters (Liu *et al.,* 2010; Kahrizi *et al*., 2007; Cardoza and Stewart, 2003).

Since the β-1,3 glucanse gene contains two introns. The results of fungal inhibition indicated that the *Trichoderma* mRNA was processed in transgenic canola. This result shows similar splice junction consensus sequences are present in the T*richoderma* β-1,3 glucanse gene. Thus, our results indicated that canola was able to recognize and splice out the *Trichoderma* introns.

All the lines that gave positive results in the PCR analysis were further confirmed by the use of *vir*G primers, which showed that PCR product, resulted from stable T-DNA integration into the canola genome and not from *Agrobacterium* contamination.

This work demonstrates a successful application of a single glucanase gene from *T. virens* for controlling the causal agent of canola stem rot disease. Meanwhile, the expression of this gene had no deleterious phenotypic effect on the transgenic plants. In the current study, it was demonstrated that the specific product of the *bgn*13.1 gene inhibited the growth of *S. sclerotiorum*, an economically important pathogen of canola plant, which is a first essential step in disease control strategies.

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فعالیت ضدقارچی گیاهان کلزای تراریخت بیان‌کننده بتاگلوکاناز در مقابل قارچ *Sclerotinia sclerotiorum*

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چکیده: کلزا (*Brassica napus*) یکی از مهم‌ترین گیاهان زراعی روغنی می‌باشد. یکی از بیماریهایی که باعث کاهش محصول کلزا می‌گردد بیماری پوسیدگی ساقه می‌باشد که توسط قارچ *Sclerotinia sclerotiorum* ایجاد می‌شود. آنزیم گلوکاناز که یکی از پروتئین‌های مرتبط با بیماریزایی (PR) می‌باشد توانایی هیدرولیز گلوکان که یکی از اجزاء اصلی تشکیل‌دهنده دیواره سلولی قارچها است را دارا می‌باشد. آنزیم‌های گلوکانازی نشان داده‌اند که دارای فعالیت ضدقارچی علیه طیف وسیعی از پاتوژن‌های قارچی می‌باشند. در این مطالعه ژن *bgn*13.1 جدا شده از قارچ *Trichoderma virens*-10 به گیاه کلزا منتقل شده است. قطعه تکثیر شده شناسایی و توسط الگوی هضم آنزیمی مورد تأیید قرار گرفته و به وکتور pUC19 منتقل گردید. مقایسه قطعه کلون شده با ترادفهای DNA موجود نشان داد که این ژن دارای دو اینترون کوتاه به طول ۵۲ و ۵۷ جفت باز بوده و پروتئینی با۷۶۱ اسید آمینه را کد می‌نماید. برای بررسی فعالیت ضدقارچی این آنزیم، از بیان این پروتئین در گیاه تراریخت کلزا استفاده گردید. بدین-منظور سازه حاوی ژن *bgn*13.1 (pBIKH1) که تحت کنترل پروموتر CaMV35S قرار داشت به *Agrobacterium tumefaciens* منتقل و با استفاده از الگوی PCR تأیید و به ریزنمونه‌های کوتیلدونی گیاه کلزا واریته R-line Hylola 308 منتقل گردید. گیاهان تراریخت به‌دست آمده با استفاده از الگوی PCR، dot blot و RT-PCR مورد تأیید قرار گرفتند. جهت بررسی مقاومت این گیاهان به قارچ *S. sclerotiorum* از آزمون radial diffussion استفاده گردید. نتایج به‌دست آمده در این آزمون نشان داد که عصاره گیاهان تراریخت شماره ۷ و ۱۰ در مقایسه با دیگر گیاهان تراریخت توانایی ممانعت‌کنندگی بیشتری در مقابل رشد میسلیوم قارچ *S. sclerotiorum* را دارند. میزان این بازدارندگی برای این دو گیاه تراریخت به‌ترتیب برابر ۳۵/۲ و ۳۲/۸ درصد می‌باشد.

واژگان کلیدی: *bgn*13.1، *Brassica napus*، *Trichoderma virens*، قارچ *Sclerotinia sclerotiorum*، آنزیم گلوکاناز، گیاهان تراریخت

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