Plant Biotechnology 31, 89–96 (2014)
DOI: 10.5511/plantbiotechnology.13.1209a

Original Paper

Transgenic tobacco and tomato plants expressing *Wasabi defensin* genes driven by root-specific *LjNRT2* and *AtNRT2.1* promoters confer resistance against *Fusarium oxysporum*

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Received October 5, 2013; accepted December 9, 2013 (Edited by Y. Hoshino)

Abstract Antifungal peptides are a potential group of defense molecules that have been utilized to develop resistance to various plant pathogens. *Wasabi defensin* (*WD*) gene (0.5 kb) consists of cysteine-rich peptides that show potent growth inhibition of pathogenic filamentous fungi, such as *Botrytis cinerea*. Under regulation by the root-specific *LjNRT2* or *AtNRT2.1* promoter, *WD* gene was expressed in the roots of transgenic tobacco and tomato plants by *Agrobacterium*-mediated transformation. The regenerated plants showed stable integration of the transgene, with different insertion sites, and the transgene was expressed in the root tissues but not in the leaf tissues. This result confirmed that WD protein accumulated only in the roots of transgenic plants. In a bioassay for resistance to *Fusarium oxysporum*, all transgenic plants showed increased resistance to the fungus as compared to non-transformed plants. Protein extracts from root and leaf tissues were assayed for antifungal activity and the activity was express as the number of colonies formed per cm^2 (CFU cm^{-2}). The CFU values of the root and leaf extracts of control plants did not show significant differences. In contrast, the CFU values of the root extracts of the transgenic plants were significantly lower than those of the leaf extracts and much lower than those of control. These results suggest that *LjNRT2* and *AtNRT2.1* promoters triggered the antifungal gene expression in the roots and conferred increased resistance to the root pathogen *F. oxysporum*. In the view of bio-safety, the root-specific expression of the transgene is desirable because the roots of tomato are not edible.

Key words: Bio-safety, Fusarium wilt, root colonization assay, root-specific promoter, soil-borne disease.

Over the years, various promoters have been characterized and tested for their ability to regulate constitutive and high-level transgene expression in transgenic plants. Constitutive promoters, such as the cauliflower mosaic virus (CaMV) 35S promoter, have been used for the development of stress resistance in transgenic plants. However, the strong constitutive expression of foreign genes and/or transcription factors often confers undesirable phenotypes on transgenic plants. For example, the drought-tolerant transgenic *Arabidopsis* overexpressing transcription factor *DREB1A* gene (*35S:DREB1A*) displayed growth retardation and severe reduction in seed production (Kasuga et al. 1999; Liu et al. 1998). Similar phenomena were observed in tomato overexpression *C-repeated/dehydration response element binding factor 1* (Hsieh et al. 2002a; Hsieh et al. 2002b) and in rice overexpressing *adenine decarboxylase* (Capell et al. 1998). Thus, depending on the purpose, the expression of a foreign gene should be limited to a particular target tissue of the transgenic plants.

Many transgenic cereal and vegetable plants have been developed through genetic transformation. Although the practical application of genetically modified (GM) plants faces many problems, the most plausible concern of GM food is its induction of unexpected allergic response. Therefore, protein products of foreign transgenes should not accumulate in the edible organs of transgenic plants. The roots of most cereal and vegetable plants are not edible to humans, but they are channels of attack by soil-borne pathogenic fungi, such as *Fusarium oxysporum*. If an antifungal gene were expressed only in the roots of such transgenic plants, the plants would develop

Abbreviations: CTAB, cetyl-trimethyl ammonium bromide; DIG, Digoxygenine; GUS, β-glucuronidase; IAA, indole-3-acetic acid; MS, Murashige and Skoog; *npt*II, neomycin phosphotransferase II; PCR; Polymerase Chain Reaction; PVDF, polyvinylidine difluoride; PDA, potato-dextrose agar; SDS, sodium dodecyl sulphate.

This article can be found at http://www.jspcmb.jp/
Published online March 26, 2014

resistance to root pathogens and bio-safety concerns would be reduced.

Plant roots perform many essential functions, such as storage of carbohydrates, absorption of water and minerals, and transportation of phosphorus and nitrogen. The roots have at least two transport systems that are involved in nitrate uptake; 1) low-affinity nitrate transport system (NRT1) and 2) high-affinity nitrate transport system (NRT2) (Crawford and Glass 1998; Mathilde et al. 2002). Although the NRT2 family has several members, AtNRT2.1 is considered the major transporter for the NRT2 system in *Arabidopsis*. Okamoto et al. (2003) conducted an investigation at the mRNA level and found that *NRT2.1* transcript expression predominantly occurred in roots, whereas *NRT1* genes were expressed in both roots and shoots. Forde (2000) identified a potential nitrate transporter gene, *NRT2*, in *Lotus japonicus*. Here, we were interested in isolating two root specific promoters, *AtNRT2.1* and *LjNRT2*, in order to direct antifungal gene expression to the roots of transgenic tobacco and tomato plants.

Higher plants contain thionins, which are highly basic 5 kDa peptides toxic to bacteria and fungi (Florack and Stiekema 1994). Most plant species have various kinds of thionins in their seeds, stems, roots, and leaves. Plant defensin or gamma-thionin is an antimicrobial peptide that has significant structural homology to the defensins of mammals and insects (Terras et al. 1995). *Wasabi defensin* (*WD*) gene, encoding a small plant defensin, was isolated from wasabi (*Wasabia japonica* Matsum.) (GenBank accession no. AB012871). It is a highly basic cysteine-rich peptide apparently ubiquitous throughout the plant kingdom (Thomma et al. 2002). Many authors have reported that transgenic plants expressing the defensin gene show growth inhibition of various phytopathogenic microbes (Hoshikawa et al. 2012b; Kanzaki et al. 2002; Khan et al. 2006; Lay et al. 2003; Ntui et al. 2011).

Tomato (*Lycopersicon esculentum* Mill.) is a popular plant widely cultivated all over the world. Besides having outstanding processing qualities, its fruits are an excellent source of vitamins and minerals (Christopher et al. 2010). Tomato plants are often affected by several fungal pathogens. Soil-borne *F. oxysporum* f. sp. *lycopersici*, which causes fusarium wilt, is one of the most serious pathogens responsible for significant yield losses (Chan et al. 2005), because there are no commercially available cultivars that show resistance to *F. oxysporum* (Larkin and Fravel 1998). Most root pathogens are necrotrophic, that is, the pathogen first invades plant roots and kills root tissues with toxins or lytic enzymes. Therefore, directing the expression of antifungal genes to the roots by mean of a root-specific promoter is a promising method for engineering resistance to root pathogens in plants.

In this study, the objective was to investigate whether the expression of *WD* gene under the regulation of the root-specific *LjNRT2* or *AtNRT2.1* promoter could confer high levels of resistance to the root pathogen, *F. oxysporum* f. sp. *Lycopersici*, in transgenic tobacco and tomato plants. Our results demonstrated that WD protein was produced predominantly in the roots of the transgenic plants, thereby significantly enhancing resistance to fusarium wilt caused by *F. oxysporum*.

Materials and methods

Binary vector constructions

DNA fragments containing *LjNRT2* and *AtNRT2.1* promoter sequences were cloned by PCR (Kong et al. 2013). The *LjNRT2* (1.0 kb) or *AtNRT2.1* (1.1 kb) promoter was linked to *WD* gene at *Hin*dIII/*Bam*HI or *Hin*dIII/*Bgl*II restriction sites, respectively. The resulting two chimeric constructs were introduced to the plant transformation vector pEKH2 (Ikuo Nakamura unpublished) at *Hin*dIII site between, kanamycin (*nptII*) and hygromycin (*hpt*) selection marker cassettes (Figure 1). The final constructs, pEKH2-*LjNTR2::WD* and pEKH2-*AtNTR2.1::WD* were independently mobilized into

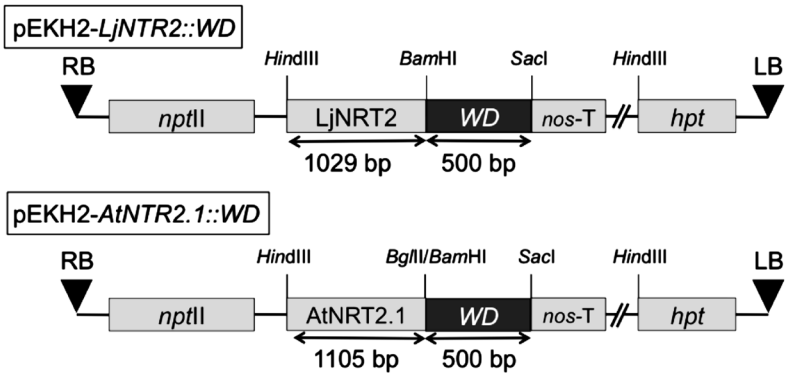

Figure 1. Schematic representation of the T-DNA region of pEKH2-*LjNRT2::WD* or pEKH2-*AtNRT2.1::WD* binary vector. *nptII*: *neomycin phosphotransferase* cassette, *LjNRT2* and *AtNRT2.1:* promoters of nitrate transporter genes from *Lotus japonicus* and *Arabidopsis thaliana*, respectively, *WD: wasabi defensin* gene, *hpt*: *hycromycin phosphotransferase* cassette, *nos*T: *nopaline synthase* terminator, LB: left border, RB: right border.

A. tumefaciens stain EHA101 by triparental mating.

Regeneration of transgenic plant

Tomato (*Lycopersicon esculentum* Mill.) seeds of line CL5915 were obtained from Dr. Peter Hanson of Asian Vegetable Research and Development Center (AVRDC), Taiwan. Seeds were surface sterilized and cultured on half-strength MS medium. Leaf explants of ten-days-old seedlings of tomato and tobacco (*Nicotiana tabacum* 'Petite Havana' SR1) were used for *Agrobacterium*-mediated transformation as previously described by Hoshikawa et al. (2012a).

PCR and Southern blot analysis of transgenic plants

To confirm transgene insertion, genomic DNA was extracted from each of the transformed and non-transformed plants using cetyltrimethylammonium bromide (CTAB) (Rogers and Bendichl 1985). PCR was carried out in 25 μl reaction mixture containing genomic DNA as template to amplify 500 bp fragment of *WD* gene in transgenic tobacco and tomato lines using a pair of primers as shown in Table 1. For Southern blot, 15 μg of genomic DNA was digested with *Xba*I, separated on 0.8% agarose gel and subsequently blotted onto nylon membrane (Immobilon-Ny+Transfer Membrane; Millipore Co.). PCR DIG-label probe of *WD* gene was used for hybridization at 41°C for 16 h. Hybridized membrane was incubated with anti-digoxigenin-AP and signals were detected with chemiluminescent substrate CDP-Star (Roche Molecular Biochemicals, Mannheim, Germany) and exposed to Hyperfilm TM-MP X-ray film (Amersham Pharmacia Biotech).

Protein extraction and western blot analysis

Protein extracts were prepared from root and leaf tissues of transgenic and non-transformed plants. Fifty milligrams plant material was ground in liquid nitrogen and homogenized in sample buffer (62.5 mM Tris-HCl (pH 6.8), 2% SDS, 10% glycerol and 0.2% 2-mercaptoethanol). Homogenized samples were boiled for 3 min; incubated in ice for 1 min and subsequently centrifuged (10,000 *g*) at 4°C for 5 min. Protein concentrations were determined according to Bradford (1976). Thirty micrograms of total protein extracts from the root and leaf tissue were separated respectively on 15% SDS-PAGE and electro-blotted onto a polyvinylidene difluoride (PVDF) membrane (Amersham BioScience, USA). Detection was performed using polyclonal antisera (1 : 1,250 v/v) raised against synthetic peptide of WD protein as primary antibody and goat-anti-rabbit IgG conjugated to horseradish peroxidase (HRP) as secondary antibody.

Antifungal activity against Fusarium oxysporum

Fusarium oxysporum was cultivated on PDA medium at room temperature (RT) in low light. Conidia were collected aseptically according to Khan et al. (2011) and adjusted to 2×10^6 spores ml^{-1} using sterilized water. Ten micrograms of protein extracts from the root and leaf of transgenic and non-transformed plants were determined (Bradford 1976) and the supernatant of protein extract was mixed with a protein inhibitor cocktail (P9599; Sigma-Adrich). *F. oxysporum* conidial suspension was mixed 1 : 9 with the protein extract, plated and incubated at RT in low light. Antifungal activity was determined as the number of germinated conidia CFU cm^{-2} (colony forming unit) after 48 h and 72 h post incubation (hpi) (Ntui et al. 2011; Yevtushenko et al. 2005). The experiment was set up as randomized complete block design with 3 replications, data collected were subjected to analysis of variance and graphed using Sigma Plot program.

Fungal root colonization assay and disease resistance bioassay using in vitro whole plant

To determine the level of fungal colonization within the roots, transgenic and non-transformed plants were inoculated by dipping the roots in the fungal suspension (2×10^6 spores ml^{-1}) for 10 min and the inoculated plants were grown in sterile soil. After 5 days, the roots were collected, washed to remove all adhering materials, surface sterilized, and cultured on PDA medium containing 200 mgl^{-1} streptomycin at RT (25±1). The presence or absence of *F. oxysporum* was scored as described by Ntui et al. (2011). The level of fungal colonization of individual plants was quantified as the percentage of roots with the pathogen.

Resistance of transgenic plants to *F. oxysporum* f. sp. *lycopersici* was tested using in vitro whole plant bioassay. Three rooted plantlets of transgenic tobacco and tomato, and non-transformed control were cultured on antibiotic-free MS medium. One ml of spore suspension of *F. oxysporum* was injected into medium so that the fungus could be able to attack the roots (Khan et al. 2011; Ntui et al. 2011). The inoculated plants were incubated at RT for 4 weeks. Disease developments were observed daily and photographs were taken at 21 days post inoculation (dpi).

Table 1. Specific pairs of primers used for PCR analysis.

Code	Gene	Sequences	Size (bp)
LjNRT2	*NRT2*	F: 5′-TAAGCTTGAATGATCATGATTAGAAA GCAAG-3′ R: 5′-CGGATGCAGTGTGAAGGATGAAGCAAGGAGT-3′	1029
AtNRT2.1	*NRT2.1*	F: 5′-TAAGCTTCGCTAGCTACTACGAAAATCTAAATG-3′ R: 5′-ATAGATCTCTGAATATTAATCACACGATGG-3′	1100
	WD	F: 5′-TGTTTCTTTTGT CGATGCTCACCCTGTTGTTTGGT-3′ R: 5′-GATTGAATC CTGT-TGCCGGTCTTGCGATGATTATC-3′	500

Results

Vector construction and PCR analysis of WD transgenic plants

In order to express the *WD* gene only in the roots of transgenic plants, the *WD* gene was linked to either the *LjNRT2* or *AtNRT2.1* root-specific promoter via *Hin*dIII/*Bam*HI and *Hin*dIII/*Bgl*II sites. Each construct was then introduced into the plant expression binary vector pEKH2 at the *Hin*dIII restriction site, giving rise to pEKH2-*LjNRT2::WD* and pEKH2-*AtNRT2.1::WD* (Figure 1). The transgenes, *LjNRT2::WD* and *AtNRT2.1::WD*, were introduced into tobacco SR1 and tomato CL5915 lines by *Agrobacterium*-mediated transformation respectively. Ten tobacco and five tomato transgenic plants were selected for PCR to confirm the integration of the *WD* gene into their genomes. All the transgenic tobacco and tomato plants showed amplified fragments of 500 bp from the *WD* transgene (Figure 2), indicating that the *WD* gene was integrated into the plant genome. No amplified fragment was observed in the non-transformed plants (Figure 2).

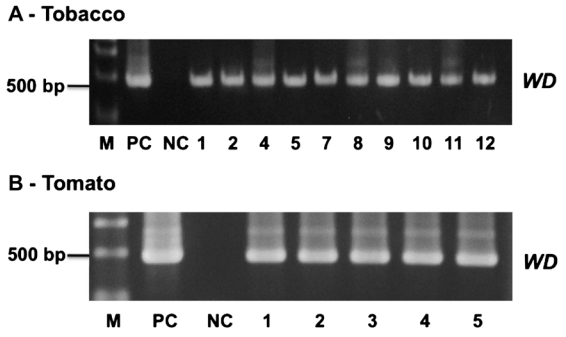

Figure 2. PCR amplification of DNA fragment (500 bp) from *WD* transgene in transgenic plants. Lane M: molecular marker, PC: positive control, NC: negative control, A) lane 1–12: *LjNRT2::WD* transgenic tobacco lines, B) 1–5: *AtNRT2.1::WD* transgenic tomato lines.

Southern blot hybridization of WD transgenic plants

Southern blot hybridization showed that one to four copies of the *WD* transgene were differentially integrated

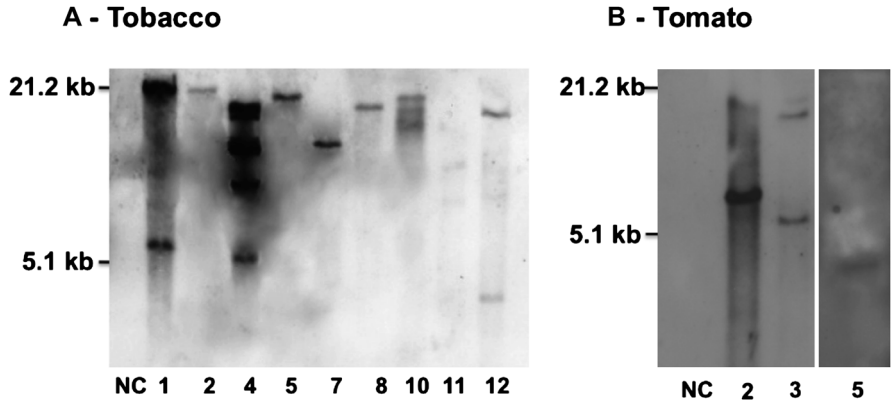

Figure 3. Stable integration of *WD* transgene into genome of transgenic plants. Genomic DNA's were digested with *Xba*I and subject to Southern blot analysis. A: *LjNRT2::WD* transgenic tobacco (9 lines), B: *AtNRT2.1::WD* transgenic tomato (3 lines), NC: non-transformed control plant.

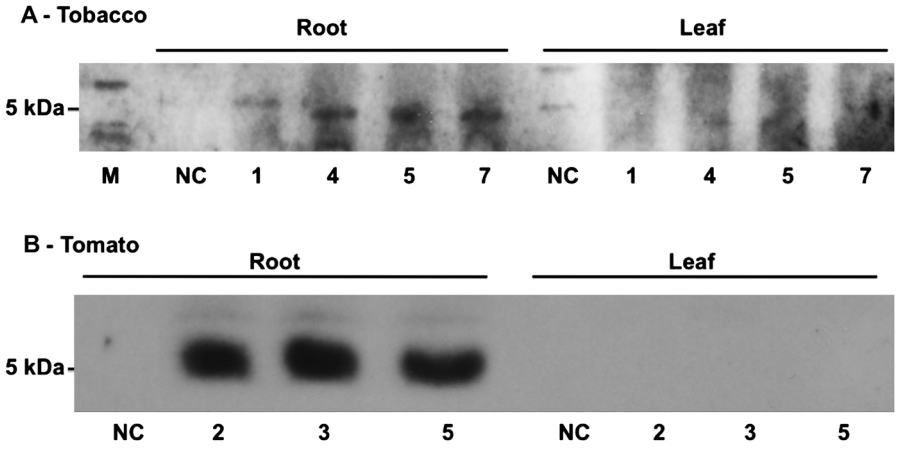

Figure 4. Root-specific accumulation of WD protein (5 kDa) in transgenic plants. Protein extracts from leaves and roots of transgenic and non-transformed plants were analyzed by western blot analysis using a rabbit polyclonal antiserum against WD Protein. A: *LjNRT2::WD* transgenic tobacco (4 lines), B: *AtNRT2.1::WD* transgenic tomato (3 lines), NC: non-transformed control plants. Artificial bands, bigger than 5 kDa, were found in transgenic tobacco line 1 (root) and NC (root and leaf).

into the genomes of nine PCR-positive transgenic tobacco plants (Figure 3A). Of the three PCR-positive transgenic tomato plants produced, two lines had one copy, whereas one line had two copies of the WD transgene (Figure 3B). No transgene insertion was detected in the non-transformed tobacco and tomato plants. The unique band pattern of the *WD* gene in lanes containing the *Xba*I-digested genomic DNA of transgenic tobacco and tomato plants indicated that these transgenic lines originated from independent transformation events.

Root-specific accumulation of WD protein

To confirm that the root-specific promoters, *LjNRT2* and *AtNRT2.1*, triggered *WD* gene expression in the roots of transgenic plants, total protein extracts from either the roots or leaves of transgenic and non-transformed plants were subjected to western blot analysis (Figure 4). A 5 kDa band corresponding to WD protein was found in the roots of *LjNRT2::WD* tobacco (lines 4, 5, and 7) and *AtNRT2.1::WD* tomato (lines 2, 3, and 5) plants. This band, however, was conspicuously absent in the leaves of the transgenic lines, and in the roots and leaves of the non-transformed plants (Figure 4). An artificial band, larger than the WD protein band, was found in the roots of the transgenic tobacco plant (line 1) as well as in the root and leaf tissues of the non-transformed tobacco plant. These results indicated that the root-specific promoters directed the production of *WD* protein in a root-specific manner.

Antifungal activity of protein extract from WD transgenic plants

Crude protein extracts from the roots and leaves of transgenic tobacco lines 4, 5, and 7, and tomato lines 2 and 3 were added to *F. oxysporum* spore suspensions to test for antifungal activity. Obviously, at 48 hpi, all protein extracts from the roots of the transgenic lines producing WD protein significantly inhibited the growth of *F. oxysporum*, resulting in very low values of 2.6 and 2.4 CFU cm^{-2} in transgenic tobacco line 4 and tomato line 3, respectively (Figure 5A). In contrast, the protein extracts from the roots and leaves of the non-transformed plants showed high CFU values. Interestingly, the protein extracts from the leaves of the transgenic plants inhibited fungal growth strongly than those from the non-transformed plants. Similar results were also obtained at 72 hpi (Figure 5B).

Assay for fungal root colonization in WD transgenic plants

The roots of transgenic tobacco and tomato plants expressing the *WD* gene were artificially inoculated with *F. oxysporum* spore suspensions to determine their inhibitory effects on fungal root colonization. The result

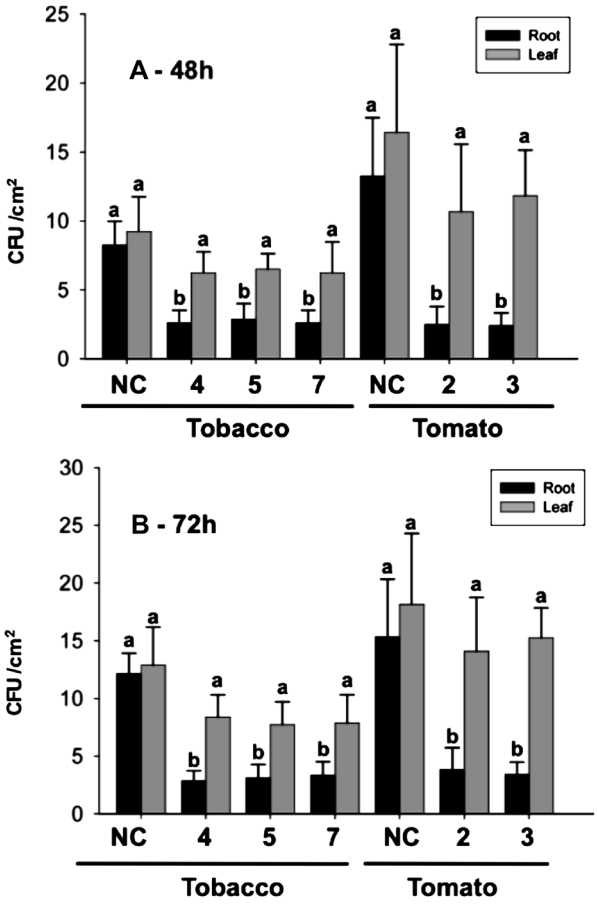

Figure 5. Antifungal activities of protein extracts from roots and leaves of *WD* transgenic and non-transformed control plants. Number of *F. oxysporum* colony was counted at 48 (A) and 72 (B) hpi on media containing extracts from roots and leaves of *LjNRT2::WD* transgenic tobacco (3 lines) and *AtNRT2.1::WD* tomato (2 lines) plants. CFU: colony forming unit, NC: non-transformed control plants. Vertical lines represent the standard error of the mean, and different letters indicate statistically significant differences (LSD test, $p \leq 0.05$).

Table 2. Effect of transgenic plants on root colonization of *F. oxysporum*.

Line	No. of root	Infected root	Percentage	Resistant rate
Tobacco				
LjWD4	27	4	14.8±0.6	Resistant
LjWD5	29	5	17.2±1.2	Resistant
NC	24	19	79.2±1.0	Susceptible
Tomato				
AtWD2	24	4	16.3±0.6	Resistant
AtWD3	30	4	13.3±0.6	Resistant
NC	22	18	82.3±1.0	Susceptible

denoted that 14.8% and 17.2% of the roots of transgenic tobacco lines 4 and 5, respectively, were colonized by the fungus (Table 2). In transgenic tomato lines 2 and 3, 16.3% and 13.3% of the roots were colonized by the fungus, respectively. In contrast, approximately 79.2% and 82.3% of the roots of tobacco and tomato control plants, respectively, were severely colonized by the fungus (Table 2). These results indicated that the WD protein

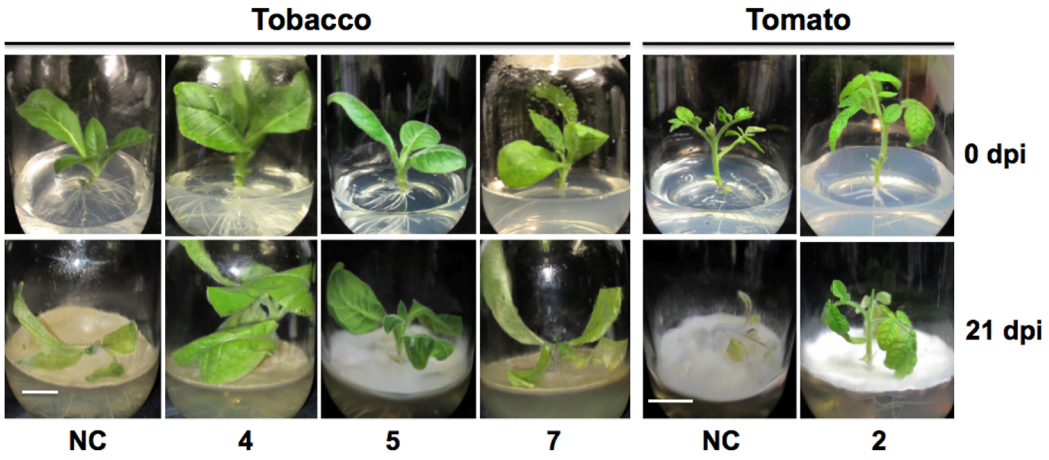

Figure 6. Whole plant bioassay of transgenic plants expressing *WD* gene resistance to *F. oxysporum*. NC: negative control, line 4, 5 and 7: *LjNRT2::WD* transgenic tobacco plant, line 2: *AtNRT2.1::WD* transgenic tomato plant. Upper lane: 0 dpi, lower lane: 21 dpi, bar: 1 cm.

that accumulated in the roots of the transgenic plants probably inhibited root colonization by the fungus.

Bioassay for disease resistance using in vitro whole plants

In vitro whole plantlets of transgenic and non-transformed plants were challenged with *F. oxysporum* spore suspensions. Non-transformed tobacco and tomato plants wilted and died after 10 dpi, whereas transgenic tobacco (lines 4, 5, and 7) and tomato (line 2) plants expressing the *WD* gene showed resistance to growth and proliferation of the fungal hyphae. Moreover, the transgenic plants remained green and grew beyond 21 dpi without severe fungal symptoms (Figure 6). The results suggested that the *WD* gene present in the transgenic plants inhibited fungal growth in the root tissues, which are vital for absorption and transfer of water and nutrients to stems and leaves.

Discussion

Many early attempts to boost disease resistance through the constitutive expression of defense-related transgenes have been reported, but more often than not, those attempts have had setbacks. The transgenic plants might have reduced size (Chen and Chen 2002) or altered morphology (Li et al. 2004). Dalton et al. (2011) also reported trade-offs between the production of transgene product and biomass growth. Our study has shown that the use of tissue-specific promoters is a wise choice as they limit gene expression to the infection sites or the most vulnerable tissues of the plant. Chan et al. (2005) described that the tissue-specific expression of antimicrobial genes is desirable in terms of: 1) bio-safety issues evolving from the unexpected toxic and/or allergic response to transgene products and 2) enhancing the productivity of transgenic plants because the transgene expression is induced only in the necessary plant tissues.

Recently, we isolated two root-specific promoters, *LjNRT2* and *AtNRT2.1*, from *Lotus japonicus* and *Arabidopsis thaliana*, respectively. Transgenic plants harboring *LjNRT2-GUS* showed GUS staining only in the root tissues (Kong et al. 2013). In this study, those two root-specific promoters were respectively linked to *wasabi defensin* gene. Transgenic tobacco harboring the *LjNRT2::WD* gene and transgenic tomato harboring the *AtNRT2.1::WD* gene showed protein accumulation only in the roots (Figure 4). The strong root-specific expression of the *WD* gene driven by the *NRT2* promoters in transgenic plants would be highly beneficial for the genetic improvement of plants in terms of conferring high resistance to major fungal pathogens that specifically attack the roots.

Khan et al. (2011) reported that transgenic tomato plants expressing the *WD* gene showed broad-spectrum resistance to *B. cinerea, Alternaria solani, F. oxysporum*, and *Erysiphe lycopersici*. Ntui et al. (2011) reported that the co-expression of *chitinase* and *WD* genes conferred resistance to *F. oxysporum* f. sp. *nicotianae* in transgenic tobacco. Those authors used the CaMV 35S promoter to express the antifungal genes in the transgenic plants. Here, we were interested in whether *WD* gene expression in the roots of transgenic plants using a root-specific promoter enhances plant resistance to *F. oxysporum* infection. The results of antifungal activity measurement of root extracts, fungal root colonization assay, and in vitro whole plant bioassay, indicate high resistance to *F. oxysporum* comparable to the data reported by Ntui et al. (2011) in transgenic tobacco plants expressing the same *WD* gene using the CaMV 35S promoter.

In the antifungal activity measurement, significantly small numbers of fungal colonies were formed on media containing the root extracts of the transgenic lines at 48 and 72 hpi, compared to the leaf extracts of the same transgenic lines and both the root and leaf extracts of the non-transformed plants. For instance, CFU values

at 72 hpi of the root extracts from transgenic tobacco line 4 and tomato line 3 were 2.9 and 3.4, respectively, whereas those of the leaf extracts from the same plants were 8.4 and 15.3, respectively (Figure 5). Although the CFU values of the leaf extracts were higher than those of the root extracts in the transgenic plants, those values were lower than the CFU values of both the root and leaf extracts from the control plants. This result suggested that the WD protein produced in the roots of the transgenic plants strongly inhibited fungal growth and indirectly induced systemic acquired resistance in the leaves of the transgenic plants (Ryals et al. 1996).

The assay for fungal root colonization showed that in transgenic tobacco line 4 and tomato line 3 plants, 14.8% and 13.3% of the roots were colonized by the fungus, respectively (Table 2). Non-transformed tobacco and tomato plants showed much higher percentages of colonized roots, namely, 79.2% and 82.3%, respectively. This result indicates that the root-specific expression of antifungal genes prevented root colonization by the pathogen. We thus suggest that the reduced symptoms observed in our transgenic plants are associated with the reduction in fungal root colonization (Gao et al. 1995; Ntui et al. 2011; Shawa et al. 2010). Consequently, an in vitro whole plant assay was carried out to test disease resistance of the transgenic plants to *F. oxysporum*. Approximately 3–4 days after inoculation, fungal mycelia readily grew, causing browning at the stem base of the control plant, which eventually wilted and died within 10 dpi. However, transgenic plants expressing the *WD* gene remained green and continued to survive beyond 21 dpi with mild symptoms observed only at the stem base.

All together, the results of the three disease assays clearly demonstrate that the expression of *wasabi defensin* (*WD*) gene under the control of the root-specific promoters, *LjNRT2* and *AtNRT2.1*, provides significant of protection to *F. oxysporum* in transgenic tobacco and tomato plants. The results suggest that transgenic lines show high of resistance to *F. oxysporum* and other soil-borne pathogens under field conditions. The root-specific accumulation of transgene products is also expected to contribute to the public acceptance of GM crops, such as tomato.

Acknowledgements

We gratefully acknowledge Dr. Masahiro Nishihara, Iwate Biotechnology Research Center, for providing antibody against WD protein, and Prof. Yoshimiki Amemiya, Graduate School of Horticulture, Chiba University, for his suggestions on disease resistance assay for *Fusarium oxysporum*.

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