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https://doi.org/10.5109/12858

出版情報：九州大学大学院農学研究院紀要. 53 (2), pp.459-465, 2008-10-28. Faculty of Agriculture, Kyushu University
バージョン：
権利関係：

J. Fac. Agr., Kyushu Univ., **53** (2), 459–465 (2008)

# Identification of cDNAs Encoding the WD Repeat Protein and Auxin–regulated Protein in Nicotiana Glutinosa Leaves Induced in Response to Tobacco Mosaic Virus–infection at 25 °C

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(*Received June 9, 2008 and accepted July 16, 2008*)

*Nicotiana glutinosa* represents resistance to tobacco mosaic virus (TMV)– infection by causing a localized hypersensitive response (HR) at 25 °C. The fluorescent differential display (FDD) analysis followed by reverse transcription (RT)–PCR indicated that two transcripts encoding a WD–repeated protein and a putative auxin–regulated protein were significantly increased upon TMV–infection at 25 °C. The two full–length cDNAs (*Ng0881* and *Ng1851*), which encode the WD repeat protein and auxin–regulated protein, were cloned, and the complete nucleotide sequences were determined. The deduced amino acid sequence for *Ng0881* has seven repetitive WD repeats, sharing 81% identical residues with the homologue from *Oryza sativa*, while that for *Ng1851* shares 68% identical residues with the auxin–regulated protein from *Oryza sativa*. It could be speculated that translational products of the genes *ng0881* and *ng1851* may serve as signal mediators required for TMV–resistance.

## INTRODUCTION

Pathogen attacks are a continual threat to the survival of plants. Once pathogen invaded, plants induce various defense–related gene expressions in a time window between a few minutes to several hours, and they are therefore able to defend themselves against a wide variety of pathogens. The plant defense response is activated when a plant resistance gene product recognizes, directly or indirectly, a specific elicitor encoded by pathogen avirulence genes (Nimchuck *et al*., 2003). The specific recognition, referred to as gene–for–gene disease resistance (Flor, 1971), requires the presence of resistance (*R*) and avirulence (*Avr*) genes in the host and pathogen, respectively, and often leads to the activation of a defense response that is accompanied by a hypersensitive response (HR), a form of programmed cell death, at the site of infection (Hammond–Kosack *et al*., 2003; Kunkel *et al*., 2002).

The local HR proceeds by rapid calcium and ion fluxes, an extracellular oxidative burst, and accumulation of salicylic acid. These events activate a signal transduction pathway that leads to the induction of kinase cascades (Kunkel *et al*., 2002). This early response is followed by a later response that includes cell wall strengthening, production of antimicrobial compounds, such as phytoalexines and synthesis of pathogenesis–related proteins. In addition, jasmonic acid, ethylene, and nitric oxide have been implicated as signaling molecules that mediate plant defense responses (Nimchuck *et al*., 2003). The final result of these events is a halt in pathogen growth and an acquisition of immunity to subsequent attacks by a broad range of pathogens, known as systemic acquired resistance (Dempsey *et al*., 1999). Although a vast amount of sequence information of *R* genes as well as chemical signals for a signal transduction have accumulated for diverse plant species, a detailed network of *R* gene mediated resistance remained unclear.

*Nicotiana glutinosa* is a diploid tobacco plant and displays TMV–resistance mediated by *N*–gene (Culver *et al*., 1991). The *N*–gene is a member of *R* genes and confers resistance to the viral pathogen TMV (Whitham *et al*., 1994). That is, TMV–infection at 25 °C causes hypersensitive cell death in leaves. In contrast, when *N. glutinosa* infected with TMV was kept at 35 °C, no local lesions were formed on the leaves, but systemic infection occurred (Samuel, 1931). These observations led us to the expectation that comparison of the gene expression in response to TMV–infection at 25 °C with that at 35 °C would provide a clue to understand the *N*–gene mediated TMV–resistance of *N. glutinosa*. In the present study, we analyzed the gene expression in TMV–inoculated *N. glutinosa* leaves using fluorescent differential display (FDD) followed by reverse transcription (RT)–PCR. In this paper, we described identification and sequence analysis of the *N. glutinosa* genes induced upon TMV–infection at 25 °C in TMV–resistance.

## MATERIALS AND METHODS

### Plant material

The plants (*N. glutinosa*) were germinated and grown in a temperature–controlled green house at 25 °C under natural light. Eight–week–old plants were placed in growth cabinets at 25 °C or 35 °C under a 16/8 h light/dark cycle before infected with TMV. After 3 days, mature leaves were detached, inoculated with or without

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* **Abbreviations:** AMV, avian myeloblastosis virus; FDD, fluorescent differential display; HR, hypersensitive response; PCR, polymerase chain reaction; RT, reverse transcription; TMV, tobacco mosaic virus

TMV (10 μg/ml) in phosphate buffer (pH 7.0) using carborundum (600 mesh) as an abrasive, and incubated at 25 °C or 35 °C. After 6 h, leaves were frozen in liquid nitrogen immediately and stored at −80 °C until extraction of RNA.

**FDD analysis**

Total RNA was extracted from the *N. glutinosa* leaves by the method of (Shirzadegan *et al*., 1991). A nonradioactive differential display method was carried out according to the manufacturer's instructions for Fluorescence Differential Display kit (TaKaRa BIO) with a minor modifications. Genomic DNA was removed by treatment with RNase–free DNase I (TaKaRa BIO). Total RNA isolated from healthy or TMV–infected leaves was reverse transcribed in a 20 μL reaction mixture with Avian myeloblastosis virus (AMV) reverse transcriptse (TaKaRa BIO) and a subset of specific oligo (dT) primers ($T_{13\sim16}$ MM, where M=A,C,G). Amplification of cDNA fragments was done in 20 μL reaction mixture consisting of 2 μL of the reverse transcribed cDNA, 0.5 μM arbitrary primer, 0.25 μM anchored oligo (dT)–primers, 0.1 mM of each dNTP, 1×LA PCR Buffer, 1 mM $MgCl_2$, 0.025 U LA Taq™ polymerase (TaKaRa BIO). The first cycle was done at 94 °C for 2 min, 40 °C for 5 min, and 72 °C for 5 min, the subsequent 34 cycles were at 94 °C for 30 sec, 38 °C for 2 min, and 72 °C for 1 min, and the final extension was at 72 °C for 5 min. The amplified cDNAs were separated on 4% polyacrylamide gel containing 7 M urea. Fluoroimages were scanned with a molecular imager FX (BIO–RAD). The differentially expressed cDNA fragments were excised from the gel and purified. DNA was re–amplified by PCR using the same set of primers for 40 cycles, as described above.

**Nucleotide sequencing**

The nucleotide sequences of cDNA fragments ligated into pGEM–T vector (Promega) were determined using a thermo sequenase fluorescent labeled primer cycle sequencing kit containing 7–deaza–dGTP (Amersham Pharmacia Biotech) and a DNA sequencer DSQ–1000 (Shimadzu). DDBJ, EMBL, GeneBank databases were searched using the BLAST algorithm (BLASTN, BLASTX, and PSI–BLAST)(Altschul *et al*., 1990; Altschul *et al*., 1997). Sequence alignments were made with the program CLUSTAL W (Thompson *et al*., 1994).

**RT–PCR analysis**

Total RNA was extracted using RNeasy Plant Mini kit (QIAGEN) from the TMV–infected *N. glutinosa* leaves at 25 °C or 35 °C. The first strand cDNA was synthesized using 1 μg of total RNA, oligo d(T) primer and AMV reverse transcriptase (TaKaRa BIO). Semi–quantitative RT–PCR was done, as described in Burton *et al*. (2000), and the amplified DNA fragments were analyzed on 1.5% agarose gels.

**Sequencing of full–length cDNA clones**

The amplification of the 5' ends of cDNA was done using a 5'–Full RACE Core Set (TaKaRa BIO) according to the manufacturer's instructions. In brief, total RNA (1 μg) was isolated from the *N. glutinosa* leaves 6 h after TMV–infection at 25 °C or 35 °C. The first strand cDNA was synthesized by RT using 5'–phosphorylated primers, 5'–TCCTAATCCTACA–3' and 5'–ATACACTTCACT–3', specific to *Ng1851* and *Ng0881*, respectively. The full length cDNAs of *Ng1851* and *Ng0881* were amplified by PCR using gene specific primers (*Ng1851* forward primer; 5'–AAGTGCTCCAATTGCATTCA–3', *Ng1851* reverse primer; 5'–GAGCTGAGGATTGTGAAGAG–3', *Ng0881* forward primer; 5'–GTAAATAAGGCTACATGTTT–3', *Ng0881* reverse primer; 5'–ACACCG CATTTGTTTCCAAG–3'). To enrich the 5'–RACE products, the first PCR products was re–amplified using nested primers (*Ng1851* forward primer; 5'–TCTGTGAAGGGTGTCAGATC–3', *Ng1851* reverse primer; 5'–GCAGGGACCAATTAAGGCGT–3', *Ng0881* forward primer; 5'–TCTCATTACTTGCTGGAGCC–3', *Ng0881* reverse primer; 5'–TCAGAGC ATTTTCTATTAAA–3'). The amplified products were purified using GENECLEAN Ⅱ (Funakoshi) and sequenced, as described above.

**Nomenclature**

The *N. glutinosa* cDNAs are denoted using the 5'–primer's numbers given first, followed by the 3'–oligo (dT) primer's numbers, and the number to discriminate the cDNA fragments amplified by the same combination of primer set (e.g., the single cDNA amplified by the 5'–primer No. 14 and the 3'–oligo (dT) primer No. 3 is referred to as *Ng1431*, and double cDNA fragments amplified by the 5'–primer No. 11 and the 3'–oligo (dT) primer No. 8 are referred to as *Ng1181* and *Ng1182*). The genes for cDNAs *Ng1431* and *Ng1181* are designated as *ng1431* and *ng1181*, and their translational products are referred to as Ng1431p and Ng1181p, respectively.

## RESULTS AND DISCUSSION

**FDD of cDNAs induced upon TMV–infection at 25°C**

It is known that TMV–resistance exhibited by N. glutinosa is temperature sensitive, occurring only at temperatures below 28 °C. At temperatures above 28 °C, HR is suppresses and TMV spreads systemically (Samuel *et al*., 1931). On the expectation that the genes induced in response to TMV–infection at 25 °C but not at 35 °C would be implicated in TMV–resistance, we first carried out FDD of mRNAs between the leaves treated at 25 °C and those at 35 °C using twenty–four 5'–arbitrary primers and nine 3'–oligo (dT) primers. Since the TMV–infection onto the *N. glutinosa* leaves was preceded by wounded treatment, the FDD analysis was additionally done for mRNAs derived from wounded leaves at 25°C and 35°C as a control. Each of the 5'–primers was paired with one of the nine 3'–oligo (dT) primers, that is, 216 combinations of PCR primer sets were used to amplify cDNAs. In this analysis, about a half of the combinations produced cDNA fragments. Among the cDNA fragments, we obtained 17 cDNA fragments, which were dif-

ferentially displayed between the leaves TMV–infected at 25 °C and 35 °C. Fig. 1 shows a representative result of the differential display analysis, where the cDNA fragment was amplified using the 5'–primer No. 8 and the 3'–oligo (dT) primer No. 8.

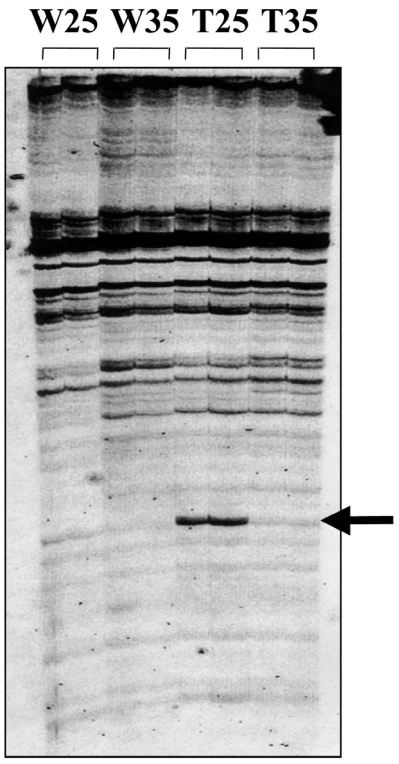


**Fig. 1.** Differential Display of cDNA Fragments from the TMV–infected *N. glutinosa* Leaves.
Total RNA was extracted from the *N. glutinosa* leaves 6 h after TMV–infection (T) or mock–infection (W) at 25 °C (25) and 35 °C (35). The PCR reactions were done on two different dilutions of each cDNA sample to minimize artifacts. An arrow indicates the position of the cDNA fragments that were recovered from the gel and further analyzed.

Seventeen cDNA bands of differentially expressed mRNA were excised from the gels and re–amplified by PCR using a corresponding set of primers. The resulting cDNA fragments were cloned into a pGEM–T vector and their nucleotides were sequenced. A homology search revealed that the nucleotide sequences of 6 cDNA fragments had similarity to those of known genes in plants, such as *N. tabacum* and *A. thaliana*. They include the genes encoding elicitor inducible protein, elicitor responsive protein, cell wall–plasma membrane linker protein, GTP cyclohydrolase Ⅱ, NADH dehydrogenase and metallothionein–like protein type 2, as given in Table 1. In addition, the nucleotide sequence of eight cDNAs showed similarity to those of genes encoding putative or hypothetical proteins, as presented in Table 1. In contrast, three cDNA sequences had no similarity to other known genes and known domain structures in databases, as given in Table 1. The result suggested that 17 possible translational products may be candidate proteins involved in formation of HR, and thereby function as defensive proteins toward TMV–infection.

**RT–PCR analysis**

To corroborate the result obtained by the FDD analysis, RT–PCR was done for 17 genes using individual specific primers. For this purpose, total RNA was isolated from the *N. glutinosa* leaves 6 h after TMV–infection or mock–infection at 25 °C and 35 °C, and RT–PCR was done as described under Material and Methods (Fig. 2). Transcripts of the genes *ng0881* and *ng1851* encoding a WD repeat protein and an auxin–inducible protein were significantly induced only upon TMV–infection at 25 °C but not upon TMV–infection at 35 °C and mock–infections. In addition, a slight inducible expression in response to TMV–infection at 25 °C was observed for genes *ng1191*, *ng1181*, *ng0752*, and *ng1171*. These results demonstrate the inducible expression of 6 genes in response to TMV–infection at 25 °C (Table 1). In contrast, little induction upon TMV–infection at 25 °C was

**Table 1.** cDNA Induced in Response to TMV Infection at 25 °C

| cDNA | Homologue | Accession No. | Identity (%) | Specie | Length (bp) |
|---|---|---|---|---|---|
| *Ng0681* | No hits found | | | | 262 |
| *Ng0751* | Putative steroid membrane binding protein | AC078840 | 66 | *Oryza sativa* | 705 |
| *Ng0752* | Elicitor inducible protein | AB040410 | 96 | *Nicotiana tabacum* | 550 |
| *Ng0753* | Putative serine–threonine kinase receptor–associated protein | AP005008 | 64 | *Oryza sativa* | 423 |
| *Ng0754* | Elicitor resposible protein | AB040409 | 92 | *Nicotiana tabacum* | 417 |
| *Ng0881* | Putative WD–40 repeat protein | AP003623 | 81 | *Oryza sativa* | 296 |
| *Ng1171* | No hits found | | | | 413 |
| *Ng1181* | Unknown protein (At4g35840) | AK118593 | 31 | *Arabidopsis thaliana* | 444 |
| *Ng1182* | Similarity to cell wall–plasma membrane linker protein | AP001306 | 27 | *Arabidopsis thaliana* | 333 |
| *Ng1191* | GTP cyclohydrolase Ⅱ | AF403706 | 58 | *Malus domestica (Apple)* | 491 |
| *Ng1192* | NADH dehydrogenase (Ubiquinone) | M63034 | 96 | *Oenothera bertiana* | 463 |
| *Ng1431* | No hits found | | | | 299 |
| *Ng1851* | Putative auxin–regulated protein | AC137608 | 68 | *Oryza sativa* | 315 |
| *Ng1852* | Putative zinc finger protein | AE005172 | 84 | *Arabidopsis thaliana* | 304 |
| *Ng1771* | Putative ubiquitin–conjugating enzyme | AC092697 | 87 | *Oryza sativa* | 493 |
| *Ng1772* | Metallothionein–like protein type 2 | U46543 | 94 | *Nicotiana glutinosa* | 487 |
| *Ng1891* | Hypothetical protein At2g35880 | AC007017 | 44 | *Arabidopsis thaliana* | 548 |

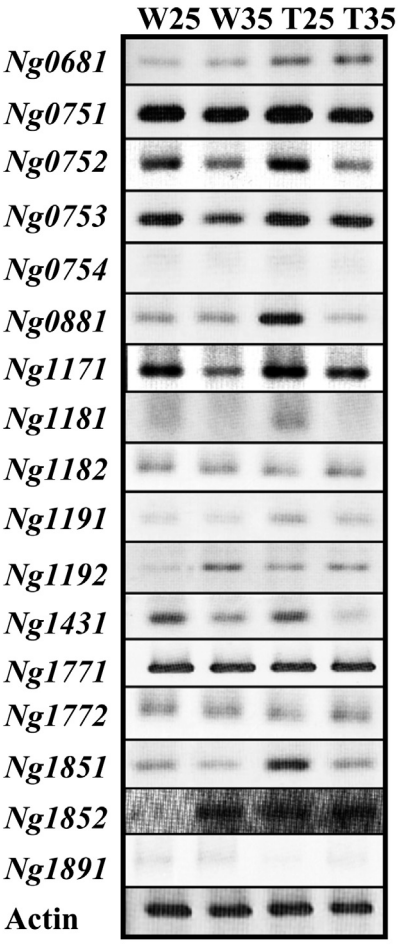


**Fig. 2.** RT–PCR of RNAs Isolated from the TMV–infected *N. glutinosa* Leaves.
Total RNA was extracted from the *N. glutinosa* leaves 6 h after TMV–infection (T) or mock–infection (W) at 25 °C (25) and 35 °C (35), as described under Materials and Methods. Then, RT–PCR was done using specific primers for individual cDNA fragments. The amplification of cDNA encoding actin shown at the bottom was done to serve as a control.

observed for other remaining genes by the RT–PCR analysis: the genes *ng1431* and *ng0753* were induced in response to both wounding and TMV–infection at 25 °C, *ng0681* and *ng1191* were induced upon TMV–infection both at 25 °C and 35 °C, and the other 7 genes seemed to be constitutively induced in response to wounding and TMV–infection at 25 °C and 35 °C (Table 1). The discrepancy of the results obtained by FDD and RT–PCR may be attributed to a technical difference. A specific primer set was used in RT–PCR, whereas arbitrary primers were used in the FDD analysis. It is thus assumed that amplification by RT–PCR seems to produce more specific cDNA fragments than that by FDD. It is likely that the expression of 6 genes seems to be induced upon TMV–infection at 25 °C, and in particular, two genes *ng0881* and *ng1851* are significantly induced in response to TMV–infection at 25 °C. It is thus suggested that the possible translational products, WD repeat protein and auxin–inducible protein, of the two genes are involved in TMV–resistance.

**Full–length cDNA**

The cDNA fragments obtained by the FDD analysis were cDNA fragments located in the 3'–terminal regions of the respective cDNAs. Hence, to gain more information of the two genes, we amplified the 5'–terminal regions of the two cDNAs by the 5' RACE method using a 5'–Full RACE Core Set, as described under Materials and Methods. Sequence analysis of the amplified cDNA fragments gave the complete nucleotide sequences of the cDNAs, *Ng0881* and *Ng1851*. The nucleotide sequence data for *Ng0881* and *Ng1851* appear in the DDBJ, EMBL, and GenBank nucleotide sequence data bases under accession Nos. AB233412 and AB233413, respectively.

The *Ng0881* and *Ng1851* cDNAs have 1364 and 1956 nucleotides with open reading frames of 1008 and 1731 nucleotides, respectively. Their putative translational products, Ng0881p and Ng1851p, are composed of 336 and 577 amino acid residues with Mr of 36,700 and 64,593, respectively. Gly (8.9%), Ser (8.6%), and Val (8.6%) are the most abundant amino acids in Ng0881p, while Leu (9.5%) and Val (8.3%) are in Ng1851p. In addition, Ng0881p contains relatively a large amount of Trp (2.4%), while Ng1851p has of Tyr (3.5%). A hydropathy profile generated from the deduced amino acid sequence reveal a general hydrophilic structure of

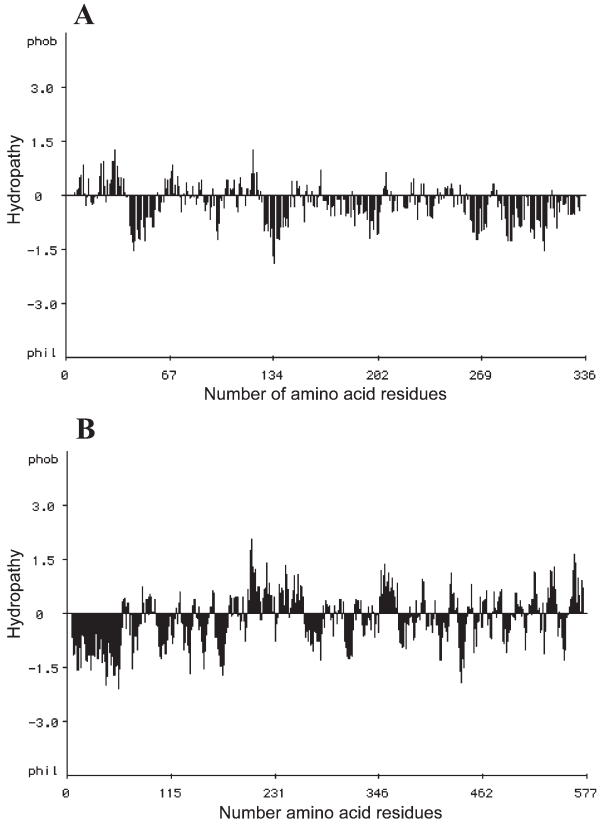


**Fig. 3.** Hydropathy Profiles Based on the Deduced Amino Acid Sequences of Ng0881p (A) and Ng1851p (B).
Hydropathy profiles were drawn using the algorithm of the Kyte and Doolittle (Kyte *et al.*, 1982). The hydropathic index is the mean value of 11 successive residues. Hydrophobic domains are indicated by a positive index and hydrophilic ones by a negative index.

Ng0881p, with the exception of the N–terminal region where three hydrophobic segments are predicted, suggesting that the N–terminal sequence may function as a leader peptide signal (Fig. 3). In contrast, Ng1851p is predicted to be folded into two domains, N– and C–domains; the N–terminal one–third has a hydrophilic structure, while the C–terminal two–thirds is predicted to be hydrophobic structure, as shown in Fig. 3.

The deduced amino acid sequences of the putative translational products Ng0881p and Ng1851p were compared with those of other known proteins included in databases, and the amino acid sequences are aligned with those of their individual homologous proteins for maximum similarity with the CLUSTAL W program (Thompson *et al*., 1994), as shown in Fig. 4. This comparison showed that Ng0881p is closely related to a WD

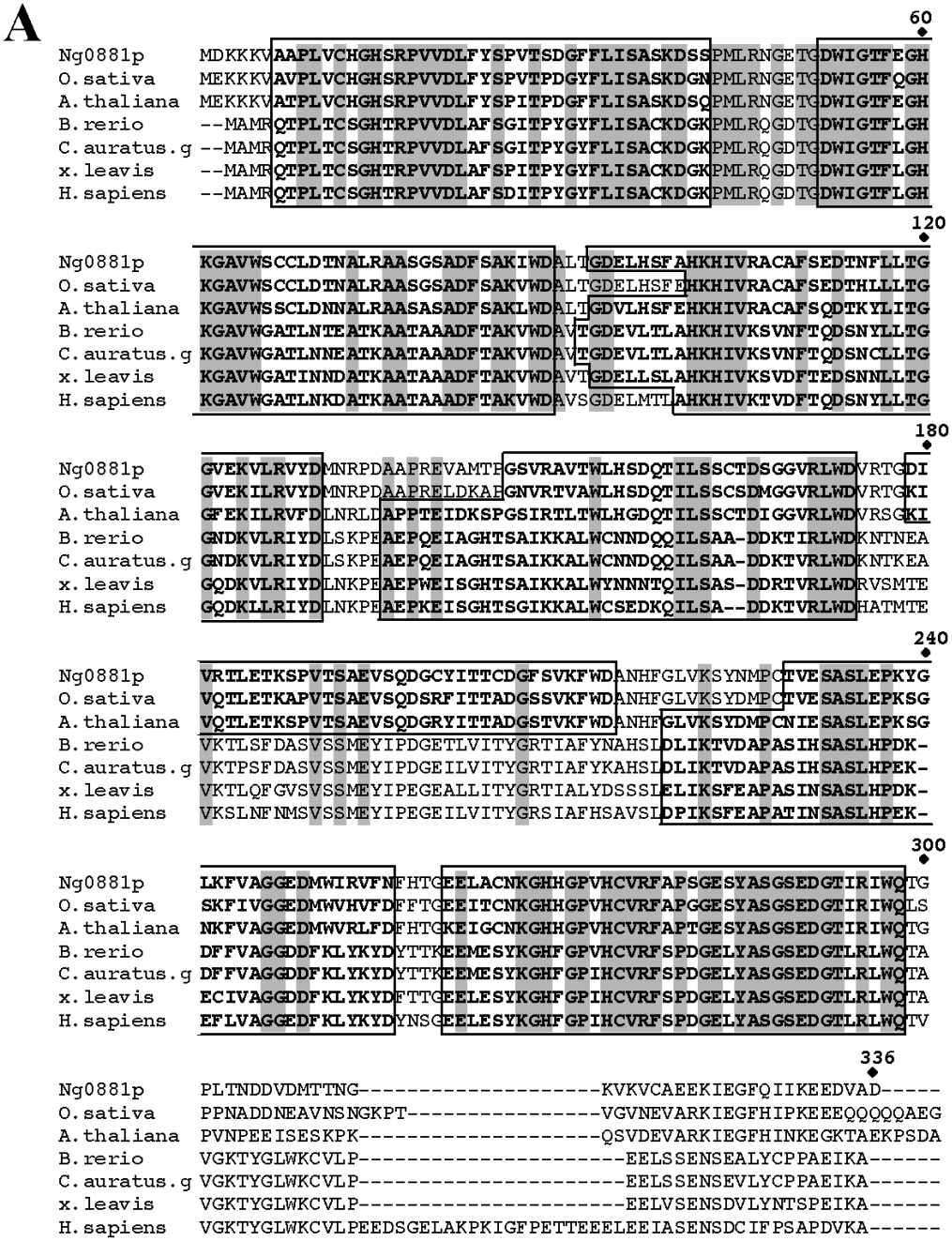


**Fig. 4.** Sequence Comparison of Homologous Proteins.
A, The deduced amino acid sequences of Ng0881p is aligned with those of homologous proteins from *O. sativa* (japonica cultivar–group) (Q69X61), *A. thaliana* (Q9LW17), *Brachydanio rerio* (Q7ZW92), *Carassius auratus gibelio* (Q98U02), *Xenopus laevis* (Q642N8), and human (AB024327). B, Sequence alignment of the deduced amino acid sequence of Ng1851p with those of homologues from *O. sativa* (Q6I581) and *A. thaliana* (Q949V9). The completely conserved residues are highlighted in grey, and WD–repeats are boxed in the Ng0881p homologous proteins. Amino acid residues are numbered according to those of Ng0881p and Ng1851p.

**B**

```
                                                                      58
Ng1851p     MKMVVEKKFDQEQVIEEFEDLTKDAGKIQEETLKKILEQNGGTEYMQQWGLNG--RSDPQ
O.sativa    -----MTICSCEETINEFEMLTRDAARVQKDTLKKILEINASAEYLQNFGLGG--RTDAE
A.thaliana  -MLEKVETFDMNRVIDEFDEMTRNAHQVQKQTLKEILLKNQSAIYLQNCGLNGNATDPEE

                                                                     118
Ng1851p     TFKNCVPIVTHNDLEPYIQRIADGDLSPILTGKPIETISLSSGTTQGKPKFVPFNDELME
O.sativa    SYKSCIPLCVHNDIEPYIQRIVDGDTSPVVTGEPITNLSLSSGTTHGKPKFIPFNDELLE
A.thaliana  AFKSMVPLVTDVELEPYIKRMVDGDTSPILTGHPVPAISLSSGTSQGRPKFIPFTDELME

                                                                     177
Ng1851p     STMKIFKTSFAFRNREFPIG-NGKALQFIYSSKQFKTKGGLAAGTATTNVYRNAQFKKTM
O.sativa    TTLQIYRTSYAFRNREYPIG-QGKALQFVYGSKQVITKGGILATTATTNLYRRQRYKEGM
A.thaliana  NTLQLFRTAFAFRNRDFPIDDNGKALQFIFSSKQYISTGGVPVGTATTNVYRNPNFKAGM

                                                                     237
Ng1851p     KAMCTPCCSPDEVIFGPDFHQSLYCHLLCGLIFRDEVQVVSSTFAHSIVHAFRTFEQVWE
O.sativa    KDIQSQCCSPDEVIFGPDFHQSLYCHLLCGLIYSEEVHSVFSTFAHSLVHAFQTFEEVWE
A.thaliana  KSITSPSCSPDEVIFSPDVHQALYCHLLSGILFRDQVQYVFAVFAHGLVHAFRTFEQVWE

                                                                     297
Ng1851p     ALVVDIREGVLSSRVTVPSIRLAMSKLLKPDPELADTIYNKCSRLSNWYGLIPELFPNTR
O.sativa    DLCTDIRDGVLSKKVTAPSIREAVSKILKPNPELADSIYKKCIGLSNWYGVIPALWPNAK
A.thaliana  EIVTDIKDGVLSNRITVPSVRTAMSKLLTPNPELAETIRTKCMSLSNWYGLIPALFPNAK

                                                                     357
Ng1851p     YIYGIMTGSMEPYLKKLRHYAGELPLLSADYGSSEGWVGVNVNPKLPPELVTYAVLPNIG
O.sativa    YVYGIMTGSMEPYLKKLRHYAGNLPLISADYGASEGWVGSNIDPTVPPEQVTYAVLPQVG
A.thaliana  YVYGIMTGSMEPYVPKLRHYAGDLPLVSHDYGSSEGWIAANVTPRLSPEEATFAVIPNLG

                                                                     409
Ng1851p     YFEFIPLGGNLNGVEQADS--------PVDLTEVKVGEEYEIVFTNFAGLYRYRLGDVVK
O.sativa    YFEFIPLEKPIGEETENSASIHYIESDPVGLTEVEVGKIYEVVITNFAGLYRYRLGDVVK
A.thaliana  YFEFLPVSETGEGEEKPVG-----------LTQVKIGEEYEVVITNYAGLYRYRLGDVVK

                                                                     469
Ng1851p     VKGFHNGTPELQFVCRRNLLLSINIDKNTEKDLQLAVEAASKRLVDEKLEVVDFTSQVNV
O.sativa    IARFHNSTPELQFICRRSLVLSINIDKNTEKDLQLAVEEASKFLEGEKLEVMDFTSFVER
A.thaliana  VIGFYNNTPQLKFICRRNLILSINIDKNTERDLQLSVESAAKRLSEEKIEVIDFSSYIDV

                                                                     529
Ng1851p     SADPGHYVIFWELSGEATDEMLQDCCNCLDKAFIDTGYVSSRKVNAIGALELRIVKRGTF
O.sativa    SSDPGRYVIFWELSGDASDEVLSSCANALDLAFIDAGYTGSRKIKTIGPLELRILRKGTF
A.thaliana  STDPGHNAIFWEISGETNEDVLQDCCNCLDRAFIDAGYVSSRKCKTIGALELRVVAKGTF

                                                                  577
Ng1851p     HKILDHFVGLGGAVSQFKTPRCVGPKNSSLLQILCSNVVENYVSTAFC-
O.sativa    KEILDHFLSLGGAVSQFKTPRFVNPSNSKVLQILSRNVTQSYFSTAYGF
A.thaliana  RKIQEHFLGLGSSAGQFKMPRCVKPSNAKVLQILCENVVSSYFSTAF--
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domain protein from *Oryza sativa*, sharing 81% amino acid residues. The WD motif (also known as the Trp–Asp or WD40 motif), originally identified in $\beta$–transduction (Matsumoto *et al*., 1993), is found in a multitude of eukaryotic proteins involved in a variety of cellular processes, such as signal transduction, protein trafficking, nuclear export, and RNA processing (Smith *et al*., 1999). The common feature of these proteins is the WD repeat, about 40 amino acid stretch typically ending in Trp–Asp, but exhibiting only limited amino acid sequence conservation, when present in a protein, the WD repeat is typically found as several (4–10) tandemly repeated units (Neer *et al*., 1994; van der Voorn *et al*., 1992). To date, several crystal structures of WD repeat proteins have been solved including the G protein $\beta$–subunit (Wall *et al*., 1995; Gaudet *et al*., 1996; Lambright *et al*., 1996; Sondek *et al*., 1996) and the Ski complex component Ski8 (Madrona *et al*., 2004; Cheng *et al*., 2004). A common feature of these structures is the arrangement of the WD repeats in a bladed $\beta$ propeller, thereby presenting possible protein interaction surfaces on the top, bottom, and side of the propeller structure. Ng0881p includes seven WD repeats conserved in all seven homologous proteins (Fig. 4). It is known that proteins containing WD repeats are often physically associated with other proteins and function as a scaffolding or protein–binding protein (Matsumoto *et al*., 1993; Smith *et al*., 1999; Neer *et al*., 1994; van der Voorn *et al*., 1992). Thus, WD repeat containing proteins either regulate the function of other proteins by modulating binding, or act to colocalize two or more other proteins.

Ng1851p is most closely related to the putative auxin–regulated protein from *O. sativa* (68%). The auxin–regulated protein was first identified from soybean as an early auxin–responsive gene *GH3* (Hagen *et al*., 1984). Subsequently, the tobacco auxin–responsive cDNA *Nt–gh3* was isolated as an early auxin–responsive cDNA by differential display from *N. tabacum* (Roux *et al*., 1997); *Nt–gh3* mRNA accumulates within a short time of about 10–15 min after auxin treatment. The plant hormone auxin is involved in various aspects of plant growth and development like cell elongation, cell division, cell differentiation, root formation and some tropisms (Davies, 1995). It is further reported that an auxin regulated pro-

tein FIN219 in *A. thaliana* which belongs to the GH3 family serves as a signaling component that has a role in the phytochrom A–mediated light inactivation of COP1 (Hsieh *et al.*, 2000): a key repressor of photomorphogenic development in the dark (McNellis *et al.*, 1994; Osterlund *et al.*, 1999).

In conclusion, the present study identified two genes whose expressions are induced upon TMV–infection at 25 °C but not at 35 °C. It is known that *N, gultinosa* exhits TMV–resistance mediated N–gene at 25 °C but not at 35 °C. It is so assumed that the putative translational products Ng1851p and Ng0881p, may serve as a signaling component involved in TMV–resistance. Further study on the *N. glutinosa* cDNAs *Ng0881* and *Ng1851* would gain more insight into a signal transduction implicated in the N–gene mediated TMV–resistance of *N. glutinosa*.

## ACKNOWLEDGMENT

We should like to thank Dr. T. Kusakabe (Department of Applied Genetics and Pest Management, Faculty of Agriculture, Kyushu University) for free access to the molecular imager FX.

## REFERENCES

Altschul, S. F., Gish, W., Miller, W., Myers, E. W and D. J. Lipman 1990 Basic local alignment search tool. *J Mol Biol.*, **215**: 403–410

Altschul, S. F., Madden, T. L., Schaffer, A. A., Zhang, J., Zhang, Z., Miller, W and D. J. Lipman 1997 Gapped BLAST and PSI–BLAST: a new generation of protein database search programs. *Nucleic Acid Res.*, **25**: 3389–3402

Burton, R. A., Gibeaut, D. M., Bacic, A., Findlay, K., Roberts, K., Hamilton, A., Baulcombe, D. C and G. B. Fincher 2000 Virus–induced silencing of a plant cellulose synthase gene. *Plant Cell*, **12**: 691–705

Cheng, Z., Liu, Y., Wang, C., Parker, R and H. Song 2004 Crystal structure of Ski8p, a WD–repeat protein with dual roles in mRNA metabolism and meiotic recombination. *Protein Sci.*, **13**: 2673–2684

Culver, J. N., Lindebeck, A. G. C and W. O. Dawson 1991 Virus–host interactions: induction of chlorotic and necrotic responses in plants by tobamoviruses. *Annu Rev Phytopath.*, **29**: 193–217

Davies, P. 1995 in: Plant Hormones: *Physiology Biochemistry and Molecular Biology* Kluwer Academic Dordrecht

Dempsey, T., Shah, J and D. Klessig 1999 Salicylic acid and disease resistance in plant. *Crit Rev Plant Sci.*, **18**: 547–575

Flor, H. H. 1971 Current status of the gene–for–gene concept. *Annu Rev Phytopathol.*, **9**: 275–296

Gaudet, R., Bohm, A and P. B. Sigler 1996 Crystal structure at 2.4 angstroms resolution of the complex of transducin $\beta\gamma$ and its regulator, phosducin. *Cell* , **87**: 577–588

Hagen, G., Kleinschmidt, A and T Guilfoyle 1984 Auxin–regulated gene expression in intact soybean hypocotyls and excised hypocotyls sections. *Plant*, **162**: 147–153

Hammond–Kosack, K. E and J. E. Parker 2003 Deciphering plant–pathogen communication: fresh perspective for molecular resistance breeding. *Curr Opin Biotechnol.*, **14**: 177–193

Hsieh, H–L., Okamoto, H., Wang, M., Ang, L–H., Matsui, M., Goodman, H and X–W. Deng 2000 FIN219, an auxin–regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of Arabidopsis development. *Genes & Dev.*, **14**: 1958–1970

Kunkel, B. N and D. M. Brooks 2002 Cross talk between signaling pathways in pathogen defense. *Curr Opin Plant Biol.*, **5**: 325–331

Kyte, J and R. F. Doolittle 1982 A simple method for displaying the hydropathic character of a protein. *J Mol Biol.*, **157**: 105–132

Lambright, D. G., Sondek, J., Bohm, A., Skiba, N. P., Hamm, H. E and P. B. Sigler 1996 The 2.0 Å crystal structure of a heterotrimeric G protein. *Nature*, **379**: 311–319

Madrona, A. Y and D. K. Wilson 2004 The structure of Ski8, a protein regulating mRNA degradation: Implications for WD protein structure. *Protein Sci.*, **13**: 1557–1565

Matsumoto, Y., Sarkar, G., Sommer, S. S and R. B. Wickner 1993 A yeast antiviral protein, SKI8, shares a repeated amino acid sequence pattern with b–subunits of G proteins and several other proteins. *Yeast*, **9**: 43–51

McNellis, T. W., von, Arnim. A. G and X–W. Deng 1994 Overexpression of Arabidopsis COP1 results in partial suppression of light–mediated development: Evidence for a light–inactivable repressor of photomorphogenesis. *Plant Cell*, **6**: 1391–1400

Neer, E. J., Schmidt, C. J., Nambudripad, R and T. F. Smith 1994 The ancient regulatory protein family of WD–repeat proteins. *Nature*, **371**: 297–300

Nimchuck, Z., Euglem, T., Holt, Ⅲ, B. F and J. L. Dangl 2003 Recognition and response in the plant immune system. *Annu Rev Genet.*, **37**: 579–609

Osterlund, M. T., Ang, L–H and X–W. Deng 1999 The role of COP1 in repression of Arabidopsis photomorphogenic development. *Trends Cell Biol.*, **9**: 113–118

Roux, C and C. Perrot–Rechenmann 1997 Isolation by differential display and characterization of a tobacco auxin–responsive cDNA Nt–gh3, related to GH3. *FEBS Lett.*, **419**: 131–136

Samuel, G. 1931 Some experiments on inoculating methods with plant viruses, and on local lesions. *Ann Appl Biol.*, **18**: 494–507

Shirzadegan, M., Chistie, P and J. R. Seemann 1991 An efficient method for isolation for RNA from tissue cultured plant cells. *Nucleic Acids Res.*, **11**: 6055

Smith, T. F., Gaitatzes, C., Saxena, K and E. J. Neer 1999 The WD repeat: a common architecture for diverse functions. *Trends Biochem Sci.*, **24**: 181–185

Sondek, J., Bohm, A., Lambright, D. G., Hamm, H. E and P. B. Sigler 1996 Crystal structure of a G–protein $\beta\gamma$ dimmer at 2.1 Å resolution. *Nature*, **379**: 369–374

Thompson, J., Higgins, D and T. Gibso 1994 CLUSTALW: improving the sensitivity of progressive multiple sequence alignment through sequence weighting position specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, **22**: 4673–4690

van der Voorn, L and H. L, Ploegh 1992 The WD–40 repeat. *FEBS Lett*, **307**: 131–134

Wall, M. A., Coleman, D. E., Lee, E., Iniguez–Lluhi, J. A., Posner, B.A., Gilman, A. G and S. R. Sprang 1995 The structure of the G protein heterotrimer Gi $\alpha 1\beta 1\gamma 2$. *Cell*, **83**: 1047–1058

Whitham, S., Dinesh–Kumar, S. P., Choi, D., Hehl, R., Corr, C and B. Baker 1994 The product of the tobacco mosaic virus resistance gene N: similarity to toll and the interleukin–1 receptor. *Cell.*,**78**: 1101–1115