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Control of seed and root development by WIPK-activated transcription factor, NtWIF in tobacco plants

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Abstract Mitogen-activated protein kinase (MAPK) is one of the critical mediators of signaling systems by constituting phosphorylation pathways together with upstream kinases. Despite the importance, few direct targets have so far been identified, making it difficult to understand the whole features of so called MAPK cascade. Notably, a novel transcription factor, which was recently identified from tobacco, is activated upon phosphorylation by wound-induced protein kinase (WIPK), a typical MAPK in tobacco plants, and designated as NtWIF (*Nicotiana tobacum* WIPK interacting factor). To identify genes that are controlled by NtWIF, a microarray screening was performed using NtWIF overexpressing transgenic tobacco, and a set of affected clones were isolated. Approximately 28% of them were related to defense, confirming the critical role of WIPK in stress response. In addition, 15% of the clones were related to differentiation, suggesting pleiotrophic effects of NtWIF. When NtWIF was overexpressed or silenced by RNAi in tobacco plants, abnormal seed development and root growth were observed. Seeds of RNAi lines exhibited immature endosperm, resulting in germination failure. Root growth was retarded in both transgenic seedlings due to abnormal cell numbers in meristem. These results implicated the participation of phytohormones, of which function might be controlled by NtWIF. Since NtWIF possesses a B3-DNA binding motif, which specifically recognizes auxin-responsive elements found in many auxin-responsive genes, the present finding suggested that WIPK/NtWIF functions not only in stress response but also in developmental process through hormonal control.

Key words: Auxin, microarray, mitogen-activated protein kinase, *Nicotiana tabacum*, root growth, seed development.

Among signal transduction pathways, mitogen-activated protein kinase (MAPK) cascade plays a central role as a controller of gene expression in response to extracellular signals. Recent genome projects on mammals and plants have identified more than 800 genes encoding MAPKs, among which 20 are from Arabidopsis (Jonak et al. 2002; Nakagami et al. 2005). In addition, genes for up-stream enzymes of MAPK were also identified; 10 MEKs (MAPK/ERK kinase) and 60 MEKKs (MEK kinase) in Arabidopsis. These kinases were considered to contribute not only to defense against biotic and abiotic stresses (Yang et al. 2001; Jonak et al. 1996; Seo et al. 1995; Zhang and Klessig 1998), but also to other physiological processes, such as regulation of cell growth and cell cycle (Soyano et al. 2002, 2006; Bogre et al. 1998; Hirt 2000; Ligterink 2000; Ligterink and Hirt 2001;Morris 2001). However, identification of direct targets which are phosphorylated by MAPKs has been limited. To date, several transcription factors related to stress response have generally been recognized as targets, which change their localization, protein level, DNA binding and transcriptional activity upon phosphorylation (Yang et al. 2003; Hazzalin and Mahadevan 2002). In plants, for example, phosphorylation of rice OsEREBP1 by BWMK1 was found to enhance its binding to a GCC box (AGCCGCC), resulting in expression of various pathogeneris-related (PR) genes and elevated resistance to pathogen attack (Cheong et al. 2003). Similarly phosphorylation of tobacco NtWRKY1 by SIPK enhanced its binding to a W-box (TGAC) of class I basic chitinase gene (CHN50), resulting in transcriptional activation (Menke et al. 2005).

Plant MAPKs are also considered to be involved in osmoregulation and hormonal signaling, including auxin-induced cell growth and differentiation (Mockaitis and Howell 2000; Kovtun et al. 1998, 2000; Mizoguchi et al. 1994). A recent survey has suggested that MAPK cascade actively participates in auxin signal transduction.

Abbreviations: MAPK, mitogen-activated protein kinase; PR, pathogenesis-related; WIF, WIPK-interacting factor; WIPK, wound-induced protein kinase.

List of microarray-identified genes can be found in the PDF file attached as supplementary data to this article on the JSPCMB web site (http://www.jspcmb.jp/)

This article can be found at http://www.jspcmb.jp/

For instance, a MAPK was activated by auxin in roots of *Arabidopsis seedlings* (Mockaitis and Howell 2000), and a MAPK, which was activated through oxidative stress, negatively regulated early auxin signal transduction (Kovtun et al. 2000). *Arabidopsis* MKK7 negatively regulated polar auxin transport, which in turn affected growth and development (Dai et al. 2006). These observation suggest MAPKs to pleiotrophically function in a broad range of physiological responses in plants.

In previous studies, we identified a target protein of WIPK (wound-induced protein kinase), a typical MAPK in tobacco, and found it to be a novel transcription factor designated as NtWIF (*Nicotiana tabacum* WIPK interacting factor) (Yap et al. 2005). NtWIF is a protein with 648 amino acids, and consists of three major domains; a B3-type DNA binding domain at the N-terminus, a trans-activation domain at the central region, and a WIPK-interacting domain at the C-terminus (Yap et al. 2005). Upon phosphorylation at the N-terminus by WIPK, its trans-activation activity significantly increased as shown by the dual-luciferase assay (Chung and Sano, unpublished observation). *NtWIF* transcripts were rapidly induced by wounding and pathogen attack. Its over-expression in tobacco transgenic plants conferred an elevated level of salicylic acid (SA), resulting in a constitutive expression of *PR-1a* and *PR-2* under non-stressed conditions, and in rapid development of the hypersensitive response (HR) to exhibit resistance to pathogen attack (Waller et al. 2006). These results linked WIPK with a transcription factor, thereby fulfilling the last step of MAPK cascade. However, native target genes of NtWIF remain to be determined.

In the present work, we attempted to identify genes, which are under the control of NtWIF by differential microarray screening. Results brought about not only many defense-related genes, but also other genes apparently unrelated to the defense, among which those involved in cell differentiation were notable. These microarray and transgenic approaches suggested NtWIF to dual function in defense and in differentiation and/or development.

Materials and methods

Plant materials

Wild type and transgenic tobacco plants (*Nicotiana tabacum* cv Xanthi nc) were grown in a growth cabinet at 23°C under a 14/10-h light/dark cycle. Transgenic NtWIF-overexpressing plants were prepared as previously described (Waller et al. 2006), and RNAi plants were constructed using the pKANNIBAL (Wesley et al., 2001) vector designed for producing hairpin RNA with a loop. For sense orientation, the coding region of *NtWIF1* cDNA was amplified by PCR using forward (5′-TATCTAGATGTCATTTCCTCAGGGTCA-AC-3′) and reverse (5′-TAGGATCCTTCCAAAACAGGGTG-GAATCTC-3′) primers. For anti-sense orientation, the same region was amplified using forward (5′-TAGTCGACGAT-GTCATTTCCTCAGGGTCAAC-3′) and reverse (5′-TACA-ATTGCTTCCAAAACAGGGTGGAATCTC-3′) primers. All PCR products were introduced into the pGEM-T Easy vector as described above. Digested fragments were ligated to corresponding sites of the pKANNIBAL vector, which was introduced into *Agrobacterium tumefaciens* strain LBA4404 cells. Resulting plasmid was digested by *Not*I and ligated to the corresponding site of pART27, which was introduced into *A. tumefaciens* strain LBA4404 cells. Tobacco transformation was performed as described previously (Yap et al., 2002).

Microarray screening

Differential hybridization of tobacco microarrays (Katoh et al. 2003) was performed using a mixed probe of Cy3-labeled cDNA from wild type and Cy5-labeled cDNA from NtWIF overexpressing transgenic plants (S7), or Cy5-labeled cDNA from wild type and Cy3-labeled cDNA from S7 line. Total leaf RNA was extracted from each three independent plants which were grown under non-stressed conditions, and poly(A)$^+$ mRNA was prepared using GeneElute mRNA miniprep kit (Sigma, St. Lois, MO) according to the manufacture's instruction. Each mRNA sample was transcribed in the presence of Cy3-dCTP or Cy5-dCTP (Amersham Phamacia biotech, Dübendorf, Switzerland) by Label Star Array kit (Qiagen).Duplicated hybridization was performed three times for each mixture to confirm reproducibility.

RNA blot hybridization

Total RNA was isolated by the hot-phenol method and 20-μg aliquots were fractionated by formaldehyde/agarose gel electrophoresis and transferred to a nylon membrane (Hybond-N, Amersham). After crosslinking using a UV crosslinker (RPN 2501, Amersham), the membrane was subjected to hybridization with appropriate ^{32}P-labeled probes at 42°C for 16 h. After being washed several times, the membrane was used to expose either BAS (Fuji Film) or x-ray film (Kodak).

Phenotypic observation

Seeds of transgenic or wild type plants were sterilized in 70% ethanol for 1 min and then in 1.6% hypochloride supplemented with 0.1% Tween-20 for 15 min under vigorous shaking. After being rinsed in sterile distilled water, approximately 100 seeds were placed on a plate containing germination medium (Murashige and Skoog MS basal medium supplemented with 3% sucrose and 8 g l^{-} agar, adjusted to pH 5.8) and allowed to grow at 23°C under a 16/8 h light/dark cycle. Germination rate was examined 10 days after germination, and the means values were calculated from triplicate samples. Phenotype was observed 3 weeks later to determine root growth parameters (length of primary root and numbers of lateral roots). For histological analysis, root tips (about 0.5 cm) were fixed in 1.6% (v/v) paraformaldehyde and 2.5% (v/v) glutaraldehyde in 50 mM phosphate buffer (pH 6.8) for 24 h at 4°C. After fixation, root tips were dehydrated by ethanol and embedded in Technovit 7100 (Kulzer, Wehrheinm, Germany) (Yeung 1999). Serial sections (10 μm) were prepared by cutting samples with a Histoknife H (Kulzer, Wehrheinm, Germany) on rotary microtome (Leica RM2145, Wetzlar, Germany)

stained with 0.05% toluidine blue, and photographed using a light microscope (Nikon E600, Japan).

Result and discussion

Transgenic tobacco lines

To identify genes that are controlled by NtWIF, transgenic tobacco lines were prepared, in which NtWIF was suppressed (RNAi lines) or over produced (sense-transgenic lines). Among 6 RNAi transformants, two lines (R4 and R20) were selected in this study, and among 5 over-producing ones, one line (S7) was used as previously described (Waller et al., 2006) (Figure 1A). Apparent phenotypic features of these transgenic lines did not differ from those of wild type plants during vegetative growth (Figure 1B, showing wild type, S7 and R20). Using these materials, we attempted to identify NtWIF controlled genes and their physiological functions.

Differential microarray screening

A microarray screening was performed using the array containing 3766 cDNA clones prepared from root and leaf tissues of *Nicotiana sylvestris*, and 48 cDNA clones prepared from wounded or TMV-treated *N. tabacum* (Katoh et al. 2004). Probes were prepared from non-stressed wild type or *NtWIF* overexpressing transgenic line 7 (S7) plants. Initial screening brought out 178 clones, which showed over 3-fold higher expression in S7 than in wild type plants. The average false positive rate was calculated to be less than 1% (Supplementary Table 1). When identified genes were classified according to their putative functions, 49 (27.5%) were found to be related to defense, suggesting NtWIF to play a critical role in general defense reaction (Figure 2). In addition, 19 (10.7%) were shown to be involved in metabolism, 16 (9%) were putative transcription factors and 26 (14.6%) were related to cell wall and cytoskeleton architectures (Figure 2). To verify the accuracy of microarray results, representative genes were selected and subjected to RNA blot hybridization (Figure 3). RNA samples were prepared not only from wild type and S7 plants, but also from *NtWIF*-RNAi line (R20), so that the accuracy of gene identification can be confirmed by the opposite feature of transcript accumulation to the overexpressing S7. Expression was examined by transcript accumulation profile upon wounding stress for wild type and R20 plants. Results showed that *NtWIF* was markedly suppressed in R20, indicating effective RNAi suppression. A defense marker gene *PR-1b* showed a similar accumulation profile as *NtWIF*, confirming NtWIF to function in defense response. In addition, genes encoding a cell wall-related endo-chitinase and auxin-induced mRNA (*pCNT115*) were also up regulated in the control but silenced in R20 line. These genes were

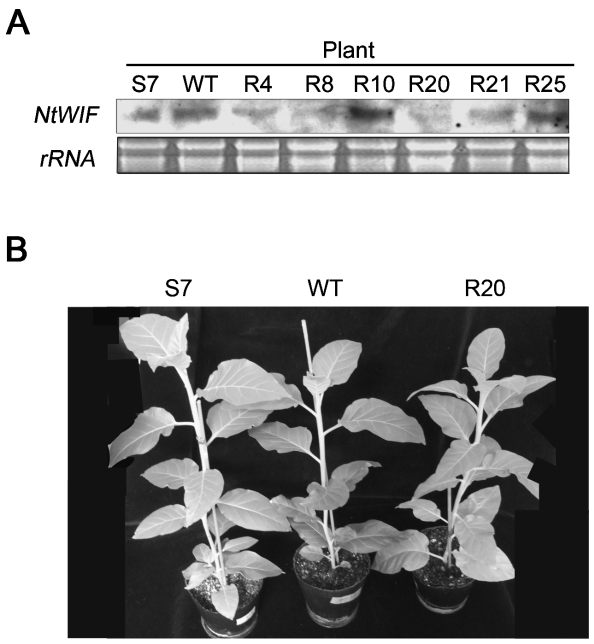

Figure 1. Transgenic plants. (A) RNA blot hybridization. Total RNA was isolated from healthy leaves of wild type (WT) and NtWIF-RNAi lines (R4, R8, R10, R20, R21 and R25) were wounded by punching, and floated in water for 30 min. Samples from the NtWIF-overexpressing line (S7) were not treated. Total RNA was isolated from leaves, and a 20-μg aliquot per lane was fractionated by gel electrophoresis and transferred onto a nylon membrane, which was subjected to hybridization using ^{32}P-labeled *NtWIF* cDNA as a probe (upper panel). Equal loading of RNA was monitored with rRNA (lower panel). (B) Phenotypes of transgenic NtWIF-overexpressing line (S7), NtWIF-RNAi line (R20) and wild type plants. Photographs were taken 6 weeks after vegetative propagation.

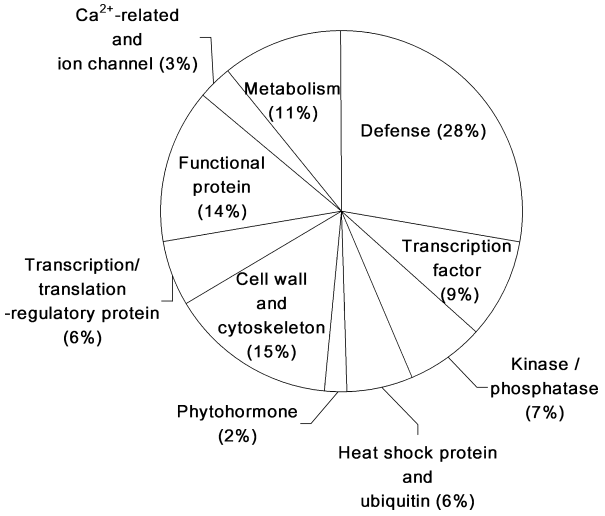

Figure 2. Functional classification of microarray-identified genes. Circle chart shows classification of genes which showed over 3-fold higher expression in S7 than in wild type plants. Among totally 178 identified genes, 49 (27.5%) were for defense, 26 (14.6%) for cell wall and cytoskeleton, 19 (10.7%) for metabolism, 16 (9%) for putative transcription factors, 13 (7%) for kinase/phosphatase, 10 (5.6%) for heat shock protein and ubiquitin, 3 (2%) for non-stress hormone, 11 (6.1%) for transcriptional/translational regulatory protein, 25 (14%) for functional protein, 6 (3%) for Ca^{2+}-related and ion channel.

constitutively expressed in the S7 line. The results confirmed that the microarray results were reliable for identification of differentially regulated genes, and also suggested that NtWIF was involved not only in defense reaction but also in the process of cell wall and cytoskeleton formation.

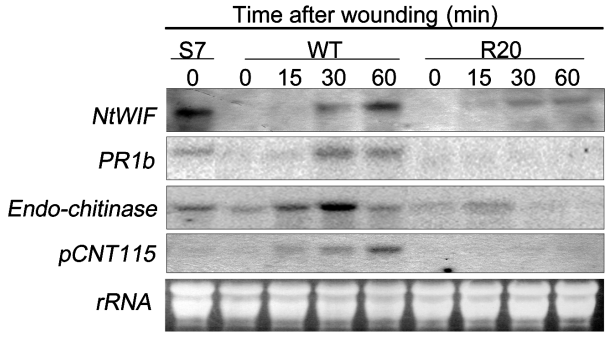

Figure 3. Verification of differential expression profiles. Among genes which showed over 3-fold higher expression in *NtWIF*-overexpressing transgenic line (S7) in comparison with the wild type (WT) control, those for PR-1b (accession no. D90197), endo-chitinase (X16938) and auxin induced mRNA (pCNT115, X56267) were subjected to RNA blot hybridization analysis to confirm their differential expression patterns among untreated S7, wound-treated wild type and R20 lines. Healthy wild type and R20 leaves were wounded by punching, and floated in water for indicated time periods. Leaves from the S7 line were not treated. Total RNA was isolated and a 20-μg aliquot per lane was fractionated by agarose gel electrophoresis and subjected to RNA blot hybridization with indicated ^{32}P-labeled cDNA probes. rRNA was employed as the loading control.

Functional diversity

Functional classification of array-identified genes indicated NtWIF to be involved in a broad range of cellular activity (Supplement Table 1). First, defense-related genes appeared to be the main target, as a high level of up-regulation was evident in both biotic- and abiotic-stress responsive genes, including *PR-1b* and *PR-Q* for the former, and *TAS14* and *osmotin* for the latter. Genes involved in ROS production, in stress hormone biosynthesis such as ethylene, and in secondary metabolism such as putresine methyltransferase and cytochrom p450 were also up-regulated by NtWIF. These results are consistent with our previous findings showing an enhanced resistance to tobacco mosaic virus-infection in NtWIF over-expressing plants (Waller et al. 2006).

Second, genes involved in cell wall and cytoskeleton formation were greatly affected. Encoded proteins included cell wall degradation enzymes such as β-galactosidase, β-D-xylosidase, β-1,3-glucanase and chitinase, cell wall expansion-related enzyme, and cell wall synthesis enzyme such as cellulose synthase and putative pectinacetylesterase. They regulate plant growth, development and morphology in coordination, as exemplified by expansin LeEXP1 for fruit softening (Chen et al. 2001) and cell-wall invertase for phloem unloading during seed development (Miller and Chourey 1992). In addition, they are also involved in protection against environmental stress and in releasing signal

Table 1. Differentially expressed genes related to cell wall and cytoskeleton.

Description	S7/WT (fold)	p-value	Origin
putative pectinacetylesterase	12.17	0.00167	*A. thaliana*
GDP-D-mannose pyrophosphorylase	5.24	0.00680	*N. tabacum*
fructose-1,6-bisphosphate aldolase	4.72	0.00076	*Pisum sativum*
similar to dTDP-D-glucose 4,6-dehydratase	4.66	0.00001	*A. thaliana*
putative actin-binding protein	4.65	0.00095	*Malus domestica*
putative microfibril-associated protein	4.00	0.00110	*A. thaliana*
actin-depolymerizing factor 2	4.00	0.00095	*Petunia hybrida*
glucose-6-phosphate isomerase	3.96	0.00167	*Spinacia oleracea*
kinesin-like protein	3.82	0.00054	*N. tabacum*
pectinesterase-like protein	3.77	0.00066	*A. thaliana*
Ntbfruc1	3.74	0.00880	*N. tabacum*
beta-galactosidase	3.67	0.00207	*Prunus armeniaca*
putative pyrophosphate—fructose-6-phosphate 1-phosphotransferase	3.54	0.00280	*A. thaliana*
putative syntaxin protein	3.50	0.00353	*Oryza sativa (japonica cultivar-group)*
endo-chitinase	3.50	0.00416	*N. tabacum*
putative expansin	3.41	0.00184	*Capsicum annuum*
alpha-tubulin	3.36	0.00320	*N. tabacum*
xyloglucan endotransglycosylase	3.22	0.00599	*Malus domestica*
cellulose synthase catalytic subunit	3.05	0.01331	*N. alata*
alpha-galactosidase-like protein	3.29	0.00103	*A.s thaliana*
fructose-biphosphate aldolase	3.25	0.00281	*Mesembryanthemum crystallinum*
fructose-bisphosphate aldolase	3.23	0.00504	*Persea americana*
putative 6-phosphogluconolactonase	3.22	0.00071	*Elaeis guineensis*
beta-galactosidase like protein	3.14	0.00219	*A. thaliana*
beta-D-xylosidase	3.06	0.00194	*Prunus persica*
putative nucleotide sugar epimerase	3.02	0.00038	*A. thaliana*

Among 26 cell wall and cytoskeleton-related genes initially identified, those showing more than 3-fold higher expression in transgenic S7 plants than in wild type control are listed.

molecules, as seen from β-1,3-glucanase and chitinase functioning not only in defense responses against fungal infection (Mauch et al. 1988) but also in physiological and developmental processes including embryogenesis, microsporogenesis and flowering (Neale et al. 1990; Grosset et al. 1990; Balandin and Castresana 1997).

Third, many genes encoding transcription factors were also up-regulated. They included WRKY-type proteins such as NtWRKY3 and WRKY2, MYB family proteins such as LBM1, LBM3 and LBM4, EREBP/AP2-type transcription factors such as Tsi 1, and basic leucine zipper transcription factors such as TGA2.2 and TGA1b. They are reported to play important roles in regulation of many genes involved in diverse physiological function including defense and development MYB proteins for stress and defense response (Daniel et al. 1999; Sugimoto et al. 2000) and for regulation of meristem formation and seed development (Schmitz et al. 2002; Penfield et al. 2001); TGA family proteins for defense response by interacting with NPR1 to activate SA-inducible genes (Zhang et al. 2003), and for flower organ formation (Corinna et al. 2005); and WRKY-type transcription factors as downstream factors of stress-related MAPK (Kim and Zhang 2004). The array data thus suggested that NtWIF may pleiotrophically contribute to diverse physiological functions, among which defense response and developmental control appeared to be unique. Whether its effect is direct or indirect remain to be determined.

Seed development

Phenotypic features of transgenic lines (S7 and R20)

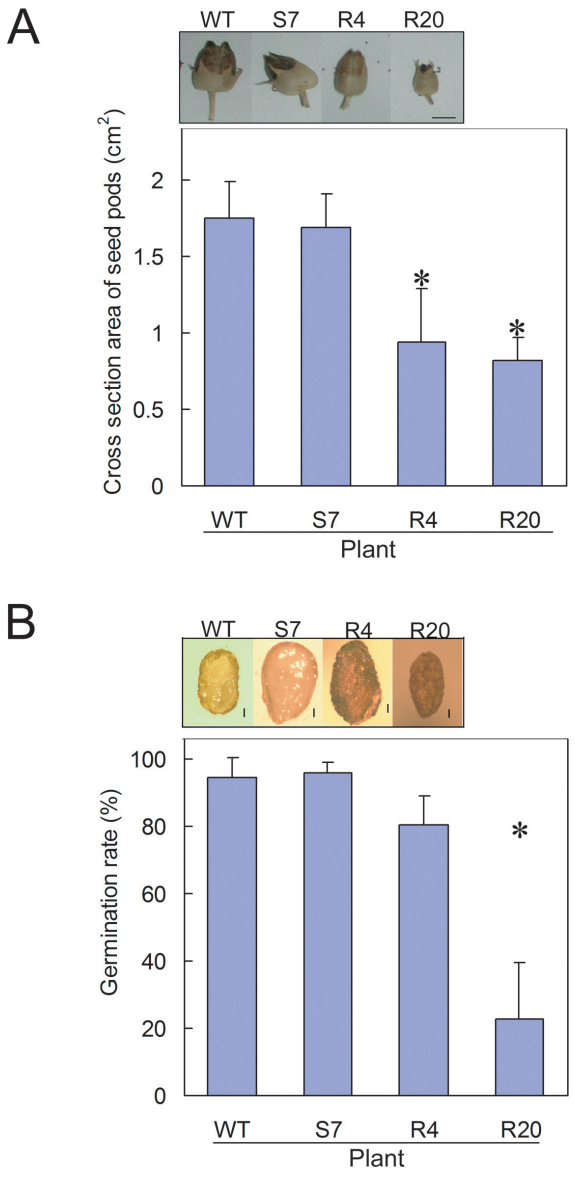

Figure 4. Size comparison of seed pods and germination rate. (A) Size of seed pod. Pod size is represented as a mean of values estimated from width and height lengths from transgenic (S7, R4 and R20) and wild type (WT) plants. Photographs (upper panel) depict corresponding pods to each sample. Bar indicates 0.5 cm. (B) Germination rate. Seeds were placed on germination medium, and germination rate was calculated 10 days after germination as a mean of triplicate samples with standard deviation. Asterisks indicate a significant difference from wild type (t-test; *$P<0.05$). Photographs (upper panel) depict corresponding seed to each sample taken after cutting in half. Bar indicates 0.1 mm.

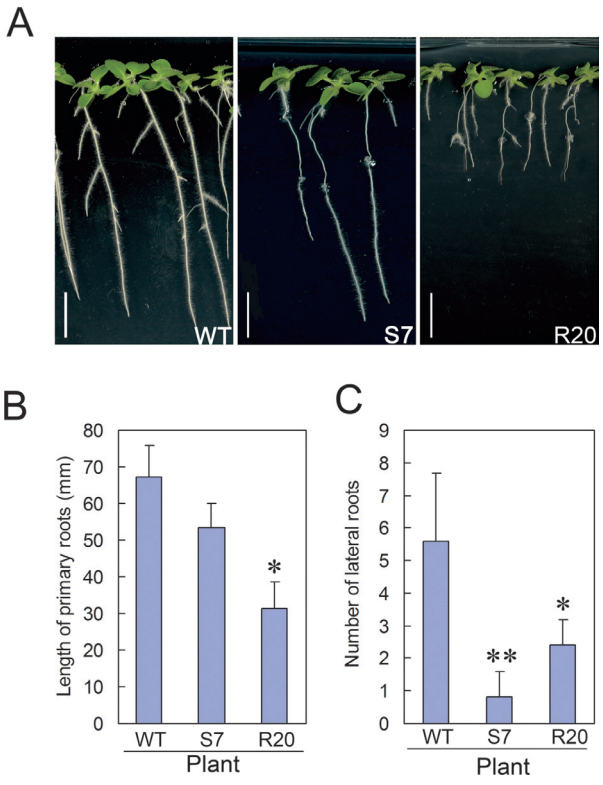

Figure 5. Root growth and development. (A) Seedlings. Seeds of wild type (WT), S7 or R20 were plated on vertical agar plates, incubated for 3 weeks and photographed. Vertical bar stands for 1 cm. (B) Length of primary roots. Root length of indicated plants was measured from more than 15 samples and mean values presented with standard deviation from triplicate experiments. (C) Number of lateral roots. Lateral root number of indicated plants was measured from more than 15 samples and mean values presented with standard deviation from triplicate experiments. Asterisks indicate a significant difference from wild type (t-test; *$P<0.05$, **$P<0.01$).

apparently did not differ from those of wild type plants during vegetative growth (Figure 1B). However, a distinct difference was observed in pod development and seed germination. Pod development appeared to be retarded in RNAi lines showing an average size at maturation to be significantly smaller than that of S7 and wild type plants (Figure 4A). This was especially distinct in R20 line, showing less than half size to the control. Pod size of S7 line was similar to that of wild type (Figure 4A). When allowed to germinate on MS agar plates, wild type and S7 seeds normally germinated, whereas R4 and R20 germinated at a reduced frequency with 80% and 22% that of the control, respectively (Figure 4B). This inability of germination was due to defective and/or incomplete development of embryo and endosperm as seen from vacant seed coats (Figure 4B). These results indicated that NtWIF plays a critical role in seed development, perhaps independently of defense response.

Root growth

One of distinct phenotypic differences between wild type and transgenic plants was root development, as a clear retardation of growth was observed 3 weeks after germination (Figure 5A–C). Average length of primary roots in wild type was 67.2 mm, while it was 53.5 mm in S7 and 31.4 mm in R20, showing respective reduction of 20.4% and 53.3% (Figure 5A and B). Average number of lateral roots was also reduced; 5.6 in wild type, while 2.4 in R20 and less than 0.8 in S7 transgenic plants (Figure 5C). When root meristem was visually observed with microscope, the cell number was apparently increased in S7 but decreased in R20 lines in comparison with wild type samples (Figure 6A–B). This was evident by quantification of cell numbers per 250 μm, showing 22 in wild type, 43 in S7 and 11 in R20, each being double and half of the control (Figure 6B). These features indicated that retarded root growth might be due to abnormal cell numbers, either excess (S7) or deficiency (R20). Since auxin plays a critical role in root formation (Casimiro et al. 2001; Guo et al. 2005), the results suggested that auxin function was disturbed in transgenic lines.

Concluding remarks

Recent studies have revealed that MAPK cascade mediates a variety of signal transduction pathways, which are not always simply liner but complicatedly connected with each other (Jonak et al. 2002; Zwerger and Hirt 2001). For example, a single MAPK-mediated cascade was suggested to function in diverse pathways depending on physiological conditions (Nakagami et al. 2005). NPK1-MEK1-NTF6 pathway showed dual functions, which were involved not only in R gene-mediated resistance but also in regulation of cytokinesis (Jin et al. 2002). In previous studies, we identified NtWIF as a down-stream

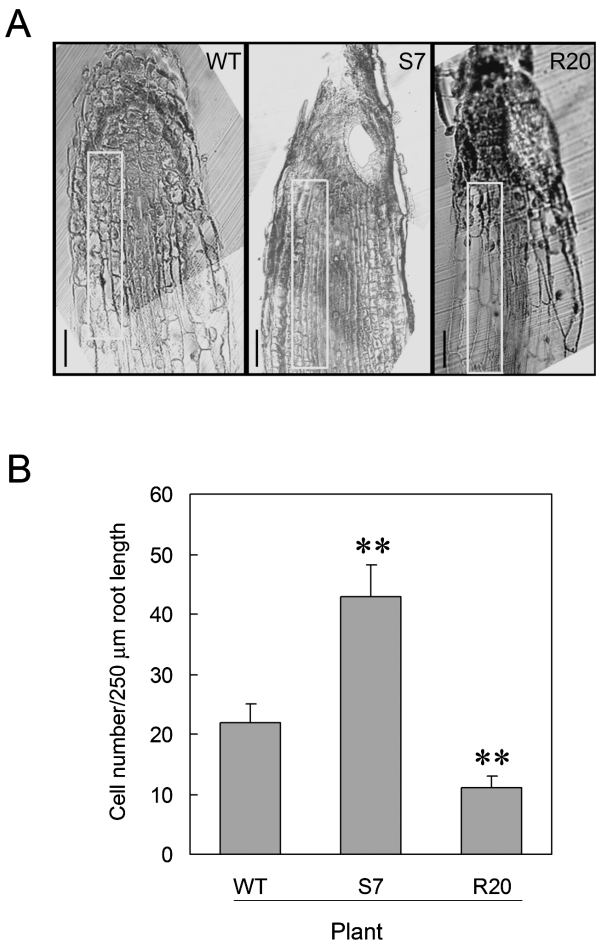

Figure 6. Phenotype of root meristem. (A) Microscopic observation. Root tip from indicated plant was sectioned and observed under a microscope. Bar stands for 50 μm. (B) Comparison of cell numbers. Cell number of primary roots at meristem zones of 250 mm (indicated by boxes in A) was counted and plotted by mean values with standard deviation from triplicate experiments. Asterisks indicate a significant difference from wild type (t-test; $^{**}P<0.01$).

factor of WIPK. Functionally it was clearly involved in wound and pathogen resistance responses (Waller et al. 2006). However, structurally it exhibited a unique feature, having a region with a high homology to the B3-type DNA binding domain at the N-terminus. The B3 domain was identified in maize VP1 (McCarty et al. 1991), Arabidopsis ABI3 (Giraudat et al. 1992) and auxin response factors (Ulmasov et al. 1999), and suggested to be involved in regulation of auxin- and abscisic acid-responsive genes (Suzuki et al. 2001). Such structural properties allowed us to speculate that NtWIF might possess other physiological functions such as auxin and ABA responses in addition to defense responses. Subsequent microarray screening and functional classification of identified genes strongly suggested that NtWIF is indeed involved not only in defense response but also in a broad range of cellular activity including cytokinesis and organogenesis. In this context, NtWIF may be assigned as one of the

fundamental proteins that pleiotrophically contribute to diverse physiological functions, among which defense response and developmental control appears to be representative.

This idea was substantiated by transgenic approach, which showed that disturbance of NtWIF and its downstream genes clearly induced phenotypic abnormalities in seed and root development. The absence of NtWIF caused a decrease of seed pod size, incomplete development of embryo and endosperm, and defective development of primary and lateral roots of seedlings. These morphological abnormalities have been known to be the results of imbalance of hormonal and/or signaling molecules (Feldman 1984; McCarty 1995; Osmont et al. 2007) For instance, initiation and emergency of lateral roots were reported to be closely associated with not only auxin but also cell cycle phase, nutrients such as sucrose and nitrogen, and ABA-mediated signaling (Casimiro et al. 2003). Seed development was also shown to be affected by cross talking between different signaling molecules such as sugars, light and phytohormones including ABA and GA (Smeekens 2000). These observation together with our present findings suggest that a set of diverse genes could be simultaneously controlled by NtWIF during development, and that WIPK-NtWIF cascade possesses at least dual functions, defense response under stressed condition and developmental control during ordinary growth.

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