Final Report 1998-2002

Grant # NAG2-1189

Molecular Genetics of Root Thigmoresponsiveness in *Arabidopsis thaliana*

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I. Introduction

My laboratory is investigating the molecular mechanisms that allow plant roots to use gravity and touch as growth guides. We are using a molecular genetic strategy in *Arabidopsis thaliana* to study these processes. When *Arabidopsis thaliana* seedlings grow on tilted hard-agar surfaces, their roots develop a wavy pattern of growth which appears to derive from a succession of left-handed and right-handed circumnutation-like processes triggered by gravity and touch stimulation (Okada and Shimura, 1990; Rutherford et al., 1998; Rutherford and Masson, 1996). Interestingly, mutations that affect root waving on tilted hard-agar surfaces can be identified and characterized. Some of these mutations affect root gravitropism, while others appear to be responsible for the production of abnormal waves (no waves, compressed or square waves, coils) without affecting gravitropism. The specific objectives of this project were to functionally characterize two genes (*WVD2* and *WVD6*) which are required for root waving on tilted agar surfaces, but not for root gravitropism. Specific objectives included a physiological and cytological analysis of the mutants, and molecular cloning and characterization of the corresponding genes. As summarized below, we have reached these objectives. We have also identified and partially characterized other mutations that affect root skewing on hard-agar surfaces (*sku5-1* and *ago1*), and have completed our work on the root-wave phenotype associated with mutations in genes of the tryptophan biosynthesis pathway (Lynn et al., 1999; Rutherford et al., 1998; Sedbrook et al., 2000, 2002).

Below, we briefly describe our progress on the cloning and characterization of *WVD6*, WVD2 and *SKU5*, and provide a list of papers (published, or in preparation) that derived from this grant. We also discuss the biological implications of our findings, with special emphasis on the analysis of *WVD2*. Future experimental prospects on our analysis of *WVD2* are detailed in a proposal for renewal that was favorably reviewed by the NASA Fundamental Biology Program (00-OBPR-01-058) last year. A grant was awarded to continue this work (grant # NAG2-1492). Most of this progress report was already included in this proposal, and in last year's progress report for the period 1998-2001.

II. Progress report

2.1. Characterization of *wvd6-1*. *wvd6*-1 was identified in a collection of promoter-trap T-DNA insertion lines for its inability to develop root waves or to skew on tilted hard-agar surfaces. It was also characterized by a slower rate of organ growth. The *wvd6-1* mutation co-segregated with a double-T-DNA insert that was genetically distinct from another insert originally identified at the *HSF4* locus in this line. The tandem T-DNA duplication was inserted within chromosome 2 sequences, adjacent to a translocation that resulted in the juxtaposition of *RHD3* sequences (chromosome 3) with chromosome-2 sequences. The translocation breakpoint was located in the first intron of *RHD3* on chromosome 3, 33 bps downstream of the translation start site, and a

few bps downstream of the T-DNA insertion site on chromosome 2. Hence, the *wvd6* translocation disrupted the *RHD3* locus, known to encode a GTP-binding protein of unknown function, which has been implicated in the regulation of root hair formation and cell enlargement (Wang et al., 1997).

To demonstrate that *rhd3* mutations confer a dampened root wave phenotype, we tested root waving on seedlings carrying two other *rhd3* alleles, obtained from John Schiefelbein (University of Michigan, Ann Harbor). Results confirmed that *rhd3* mutant roots do not wave or skew on tilted hard-agar surfaces. *RHD3* was originally characterized for its defect in root hair formation (*Root Hair Defective 3*), and in cell expansion. It should however be emphasized that the dampened root-wave phenotype is not a simple consequence of reduced growth rates, for many other mutants that are characterized by similar growth-rate alterations are still capable of skewing (see *wvd2-1*) or waving (for instance, see Rutherford et al., 1998).

To determine if the waving and skewing defects of *rhd3* resulted from the structural alterations in root hairs, we tested other root hair-deficient mutants for their ability to wave and skew on tilted hard agar surfaces. Results indicated no correlation between the two phenotypes. While *rhd1* and *rhd3* did not wave or skew on such surfaces, *rhd6* skewed more than wild type, and *rhd2* and *rhd4* waved and skewed like wild type. Hence, WVD6/RHD3 appears to be important for root waving and skewing, in addition to being essential for root hair development, and the former phenotype is not a consequence of the latter.

2.2. Characterization of *sku5*. *sku5* is a T-DNA-tagged mutation that confers increased root skewing from the vertical on tilted hard-agar surfaces, lower rates of root growth, and slower kinetics of graviresponse. The *SKU5* gene was cloned and shown to encode a protein that shares 25% sequence identity with ascorbate oxidases, laccases and putative pectine esterases. Computer programs predicted that the protein might be GPI (glycosyl-phosphatidylinositol) – anchored. In agreement with this model, transgenic plants expressing SKU5-GFP fusions, as well as preliminary immunolocalization experiments suggested that the protein is located in the cell wall (Sedbrook et al., 2000). John Sedbrook identified this mutation, initiated its physiological characterization, and cloned the *SKU5* gene at the end of his PhD program, and then as a postdoctoral fellow in my laboratory. He then moved to the Somerville lab (Carnegie Institution of Washington, Department of Plant Biology) where he continued his work on *sku5*. A manuscript describing the initial analysis of *SKU5* is in press (Sedbrook et al., 2002).

2.3. Isolation and characterization of *wvd2-1*

2.3.1. Isolation of *wvd2-1*

We used a *Ds*-based, activation tagging strategy (Wilson et al., 1996) to isolate genes that are important for the regulation or occurrence of the circumnutation-like process that accompanies root waving and skewing on hard-agar surfaces. This strategy relies on a *Ds* transposable element that carries a strong CaMV 35S promoter driving transcription outward through the element's end. Hence, it allows the identification of gain-of-function mutations associated with increased or ectopic expression of targeted genes, including some involved in the regulation of cell expansion (Wilson et al., 1996). We generated a collection of plants that carry independently transposed copies of this *Ds* element, and screened it for mutants with defects in root waving and/or skewing on hard-agar surfaces (Hilson, Carroll and Masson, unpublished data). One of the mutants identified in this screen (*wvd2-1*) displayed dampened waves on tilted agar surfaces, opposite skewing compared to wild type, and thick roots (Fig 1).

The original *Ds* line (*tn116*) used to generate this collection was constructed in the L*er* background (Wilson et al., 1996). On the other hand, the *Ac* element used to mobilize *Ds* was in the No-O background (D. Smith, personal communication). To avoid any confounding effects due to ecotype-background effects (modifiers), we backcrossed the mutation at least 4 times with either No-O or L*er*. Results described in this report were obtained with lines that derived from at least 3 successive backcrosses, except when stated otherwise (Yuen, Pearlman and Masson, unpublished data).

2.3.2 Phenotypic analysis of *wvd2-1*

wvd2-1 roots did not wave on tilted hard-agar surfaces. They skewed from the vertical in the opposite direction compared to wild type (Fig. 1A). Interestingly, the degree of *wvd2-1* root skewing appeared to depend more upon plate tilting or agar content (strength of the medium) than that of wild type roots (Fig. 1B). This result suggests that the mutation increased the roots' ability to sense the obstacle generated by the agar surface, or to respond to it. It also shows that the thick root phenotype associated with *wvd2-1* (see below) does not interfere with the root's ability to develop the circumnutation-like process that accompanies root skewing.

Mutant roots appeared shorter than wild-type (Fig. 1A), and the slower rate of root growth correlated with radial expansion of the roots (Fig. 2). This fat-root phenotype derived from increased radial cell expansion of all the tissues (Fig. 2, table 1). In addition, *wvd2-1* roots carried more root hairs. Mutant hypocotyl epidermal cells were shorter and radially expanded, and cell files appeared twisted on etiolated seedlings (Fig. 3). Inflorescence stems of mature plants were thicker and shorter than wild type. Internodes were shorter, siliques were often shorter and thicker, and leaves were smaller. Seed size, on the other hand, was only slightly affected by the mutation (Table 2).

2.3.3. Physiological analysis of *wvd2-1*

We characterized the ability of mutant seedlings to respond to exogenous hormones, as well as to agents that stabilize or destabilize the cortical microtubules. Results indicated that *wvd2-1* did slightly affect the ability of mutant roots to respond to exogenous auxin (IAA, figure 4B or NAA) and ACC (Fig. 4C). These effects were however much smaller than those observed on typical auxin- or ethylene-response mutants (Chen et al., 1998), suggesting that they might be consequences of the morphological defects associated with *wvd2-1*.

The mutation was found to have no effects on root-growth sensitivity to cytokinin (BA. Fig. 4D) or gibberellin GA7 (data not shown). However, it enhanced root-growth sensitivity to oryzalin (Fig. 4E) and trifluralin, two microtubule destabilizers.

2.3.4. The cortical microtubule arrays are disorganized in mutant lateral root cap and epidermal cells

Whole-mount *in situ* immunolocalization assays were used to image the cortical microtubules in the root tip (Goodbody and Lloyd, 1994). Confocal microscopy revealed that cortical microtubules in lateral root cap (Fig. 5) and epidermal cells (not shown) were mis-aligned in mutant CEZ cells compared to wild type.

2.3.5. Genetic analysis of *wvd2-1*

In F2 segregating populations derived from a cross between *wvd2-1* (deprived of *Ac*) and wild type, the phenotypes segregated as a semi-dominant mutation (Chi-square = 0.013; n=472). Similar analyses revealed that the Wvd2 phenotype co-segregated with hygromycin resistance, a trait carried by the *Ds* element, in 114 segregating F2 families (Pearlman, 1999).

To demonstrate that *wvd2-1* is caused by *Ds* insertion, we re-introduced the *Ac* element in the homozygous *wvd2-1* background, and sought revertants in the corresponding progeny. Of 24 individuals analyzed, 2 revertants were identified which developed wild-type root waving, skewing and anisotropic growth phenotypes (Fig. 6). Hence, all aspects of the Wvd2 phenotype appeared to derive from the insertion of *Ds* in the *WVD2* locus (Pearlman, 1999).

To demonstrate that these revertants were truly derived from *Ds* excision, we used inverse-PCR to clone genomic sequences flanking the 3' end of *Ds* in *wvd2-1* plants. This genomic DNA was then used as a probe to clone a larger and overlapping piece of genomic DNA, which was sequenced. A primer pair was designed and used to PCR-amplify a genomic fragment that overlaps with the original *Ds* insertion site in the two revertants. Results showed that these revertants derived from *Ds* excision from the locus, leaving in place a 7-bp insertion footprint (Fig. 6)(Pearlman, 1999)

2.3.6. Molecular analysis of *wvd2-1*

We used the same cloned genomic DNA probe to clone a full-length cDNA (Newman et al., 1994) and the corresponding gene. Both were sequenced. Primer extension was then used to map the 5'end of the *WVD2* transcript (Yamada et al., 1998). Northern analysis revealed that *wvd2-1* tissues contain higher levels of *WVD2*

transcript than wild-type, *eto1 - 3* or *erh1* - 3 tissues (Fig. 7). A cDNA corresponding to the mutant transcript was cloned and sequenced. Results showed that the *Ds* element was inserted in the 5' untranslated region of *WVD2*. Orientation of *Ds* in *wvd2-1* was such that its divergent 35S promoter could drive expression of full-length *WVD2* -transcript.

We used RT-PCR to determine the pattern of *WVD2* expression in wild-type plants. Results indicated that all tissues analyzed (root tips, hypocotyls, siliques, flowers) contained a low level of WVD2 transcripts (usually below levels of detection by Northern blot) (Pearlman, 1999).

2.3.7. *WVD2* encodes a novel protein

Sequence analysis and comparison with databases revealed that *WVD2* potentially encodes a novel 23-kD protein with no homologies to proteins of known function in the databases. The WVD2 protein contains an 80-amino acid region that shares homology with a highly conserved domain found in a number of predicted plant proteins of unknown function, as well as with 7 other predicted proteins in *Arabidopsis* (Fig. 8B). WVD2 was predicted to be cytoplasmic.

2.3.8. WVD2 controls root growth behavior on agar surfaces

Most of our functional analysis of *WVD2* had so far involved an analysis of plants over-expressing the protein, implying the possibility that WVD2 might not necessarily function in controlling root growth behavior in wild type backgrounds. To eliminate this possibility, we developed gene knockout strategies aimed at eliminating WVD2 function in plants. We first expressed in *wvd2-1* a silencing construct that carried an inverted duplication of *WVD2*, separated by the *GUS* open reading frame (Smith et al, 2000). Multiple transgenic lines were recovered and shown to express decreased levels of transcripts derived from *WVD2* and its closest paralog (*WDL1*, also named F7018.11 in figure 8B), compared to untransformed *wvd2-1* plants, although *WVD2* transcripts were still more abundant in these lines than in wild type seedlings. Interestingly, the silenced seedlings displayed a largely wild-type phenotype, except that their roots now skewed more to the right when grown on agar-based media than wild type. Indeed, the angle of skewing was 20.8 +/- 1.0 degrees for NoO wild type seedlings, and 25.0 +/- 0.6 for silenced seedlings in one experiment involving growth on vertical 1.5% agar-based GM medium (t test p value of 0.001). This root-growth behavior phenotype was opposite to that of *wvd2-1* (In similar experiments, *wvd2-1* mutant roots would always skew to the left; the skewing angle was –21.8 +/- 1.6 in one such experiment), or that of *WVD2* or *WDL1* over-expressors (data not shown).

A similar root skewing phenotype was also found to be associated with a newly isolated intragenic suppressor mutation in *wvd2-1* (*wvd2-2*), that introduced a premature stop codon within the highly conserved KLEEK domain of the protein (R152Stop; data not shown). Hence, this knockout analysis of *WVD2* function fully supports a role for *WVD2* and *WDL1* in controlling root skewing on tilted hard agar surfaces.

2.4. List of Publications Related to This Grant

2.4.1. Published papers

Rutherford, R., Gallois, P., and Masson, PH. (1998). Mutations in *Arabidopsis thaliana* Genes Involved in The Tryptophan Biosynthesis Pathway Affect Root Waving on Tilted Agar Surfaces. *Plant J. 16:* 145-154

Sedbrook, J., Boonsirichai, K., Chen, R., Hilson, P., Pearlman, R., Rosen, R., Rutherford, R., Batiza, A., Carroll, K., Schulz, T., and Masson, P.H. (1998). Molecular Genetics of Root Gravitropism and Waving in *Arabidopsis thaliana. ASGSB Bull. 11*: 71-78

Lynn, K, Fernandez, A., Aida, M., Sedbrook, J., Tasaka, M., Masson, P.H., Barton, M.K. (1999). The *PINHEAD/ZWILLE* Gene Acts Pleiotropically in *Arabidopsis* Development and Has Overlapping Functions with the *ARGONAUTE1* Gene. *Development* 126: 469-481

Chen, R., Rosen, E., and Masson, P.H. (1999). Gravitropism in Higher Plants. *Plant Physiol. 120*: 343-350

Rosen, E., Chen, R., and Masson, P.H. (1999). Root Gravitopism: A Complex Response to a Simple Stimulus? *Trends in Plant Sciences 4*: 407-412

Chen, R., Guan, C., Boonsirichai, K., and Masson, P.H. (2002). Complex Physiological and Molecular Processes Underlying Root Gravitropism. *Plant Mol. Biol. 49*: 305-317

Masson, P.H., Tasaka, M., Morita, M.T., Guan, C., Chen, R., and Boonsirichai, K. (2002). *Arabidopsis thaliana*: A Model for the Study of Root and Shoot Gravitropism. In "*The Arabidopsis Book*" (Meyerowitz and Somerville, eds.). The American Society of Plant Biologists, Rockville, MD (DOI/10.1199/tab.0043, http://www.aspb.org/publications/arabidopsis/).

Boonsirichai, K., Guan, C., Chen, R., and Masson, P.H. (2002). Root Gravitropism: An Experimental Tool to Investigate Basic Cellular and Molecular Processes Underlying Mechanosensing and Signal Transmission in Plants. *Ann. Rev. Plant Biol. 53*: 421-447 (DOI: 10.1146/annurev.arplant.53.100301.135158)

Sedbrook, J., Carroll, K.L., Hung, K.F., Masson, P.H., Somerville, C.R. (2001). "The *Arabidopsis SKU5* Gene Encodes an Extracellular GPI-Anchored Glycoprotein Involved in Directional Root Growth. Plant Cell, in press.

2.4.2. Thesis

Pearlman, R. (1999). Analysis of WVD2, a Locus Involved in Radial Expansion and Root Waving in *Arabidopsis thaliana*. PhD Thesis, University of Wisconsin – Madison

2.4.3. Manuscripts in preparation

Yuen, C, Pearlman, R., Silo-Suh, L, Carroll, KL, Hilson, P, Masson, PH (2001). Increased Expression of a Novel *Arabidopsis thaliana* Gene (*WVD2*) Promotes Radial Cell Expansion, Decreased Elongation and Altered Root-Growth Behavior. In preparation

Yuen, C, Sedbrook, J, Masson, PH (2001). Mutations in *RHD1* and *RHD3* affect root waving and skewing on tilted hard-agar surfaces. In preparation

III. Conclusions

The *wvd2-1* mutation affects root-growth behavior and cellular expansion without affecting the root's ability to respond to gravistimulation, nor the kinetics of gravitropism. Some aspects of the mutant phenotype appear to be modulated by plate tilting and agar content, suggesting an involvement of WVD2 in the transduction of, or response to environmental cues. Hence, this mutation reveals a new pathway/process that may, in the long term, become an excellent target for engineering plants that better respond to environmental parameters encountered during space flight, but still retain their ability to respond to gravitational accelerations when grown on Earth or other planets.

Regulation of cell growth is an essential component of morphogenesis in plants. The orientation of maximal anisotropic diffuse growth rate appears to be associated with the main pattern of cortical microtubule and cellulose microfibril orientation in plant cells. Interestingly, the Wvd2 phenotype is associated with defects in cellular elongation, radial expansion and cortical microtubule orientation within elongation zone cells, in addition to altered root growth behavior. This phenotype is due to over- or ectopic expression of a wild-type protein. Hence, molecular analysis of the WVD2 protein, identification of proteins it interacts with and of processes it affects, and identification of phenotypes associated with loss-of-function mutations in the same locus will provide clues on specific processes that regulate anisotropic diffuse growth and morphogenesis in plants.

The combination of phenotypes displayed by *wvd2-1* mutant plants (shorter and stockier plants, normal seed set) is particularly attractive to space-exploration programs, considering the growth-space limitations in spacecrafts. These traits are also praised by Plant-Breeding programs attempting to improve crop cultivars for production on Earth, because they confer increased resistance to lodging by wind and rain. The molecular basis of this mutation (ectopic expression of a wild-type gene) strongly suggests that it will be possible to engineer such phenotypes in other species. Hence, a better understanding of *WVD2* function should provide an excellent

basis for designing efficient engineering and breeding strategies allowing the development of plants that are *better* adapted to the specific environments found both during space-flights, on Earth and on other planets. As such, this proposal is of direct relevance to the Fundamental Space Biology Program, which emphasizes: "*Research that applies (fundamental) knowledge (in the biological sciences) to NASA's other goals of enabling human exploration of space and improving the quality of life of Earth is also encouraged*" (NRA 00-OBPR-01).

IV. Tables and figures

Table 1. Relative surface area and length of root cells in different tissues of the mature zone. **A.** Surface area. Transverse sections were made through the mature zone of roots of 7-day old Ler, No-O and *wvd2-1* seedlings (15<n<23), and analyzed under a microscope. Digitized images were taken and quantified, using the NIH Image Analysis program. All cells in focus in one section per root were measured. The data were normalized to Ler. **B.** Relative cell length. Root tips of 3-day old seedlings were chemically cleared and were analyzed under Nomarski optics (8<n<20). Standard deviations are indicated in parentheses.

Relative cell surface	Epidermis	Cortex	Endodermis	Central Cylinder
Ler	1 (0.1)	1 (0.1)	1 (0.12)	1 (0.08)
No-O	0.8 (0.12)	0.7 (0.06)	0.7 (0.15)	0.7 (0.12)
Wvd2-1	2 (0.35)	2.5 (0.5)	2 (0.26)	1.5 (0.25)
Relative cell length	Epidermis	Cortex	Endodermis	Central cylinder
Ler	1 (0.3)	1 (0.4)		
No-O	1.15 (0.2)	1 (0.1)		
Wvd2-1	0.8 (0.25)	0.7 (0.1)		

Note 1. Cell numbers for each tissue were also counted, and found not to differ significantly between genotypes (t-test p >0.2).
Note 2. These data were obtained on *wvd2-1* seedlings that had not yet been introgressed.

Table 2. Seed length and width for wild type and *wvd2-1* mutant plants (n = 160).

	Length (mm)	Width (mm)
No-O	0.75 (0.06)	0.44 (0.04)
Wvd2-1	0.73 (0.05)	0.48 (0.03)

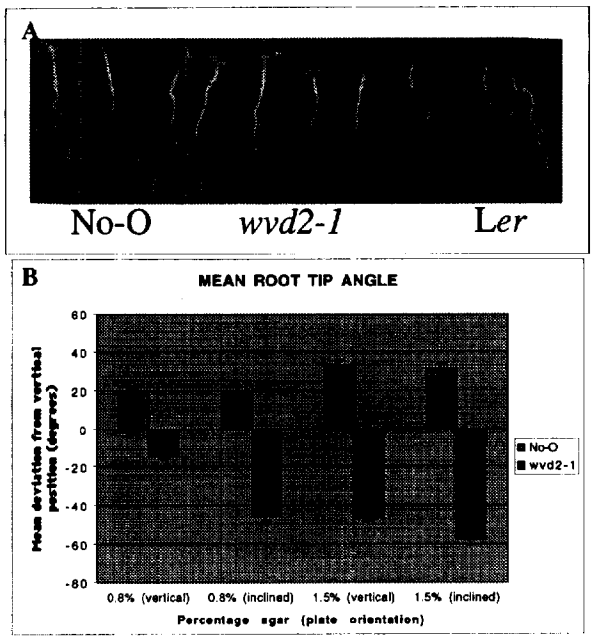

Figure 1. A. Wavy root-growth phenotype of 7-day old No-O, *wvd2-1* and L*er* seedlings on tilted hard-agar surfaces (Reproduced from [62]). **B.** Quantification of root tip angles from the vertical for 7-day old seedlings grown on the surface of vertical or tilted media containing the amounts of agar defined under the graph (in %)(n=38-52). Experimental conditions were as described in [5]. Vertical bars represent standard errors.

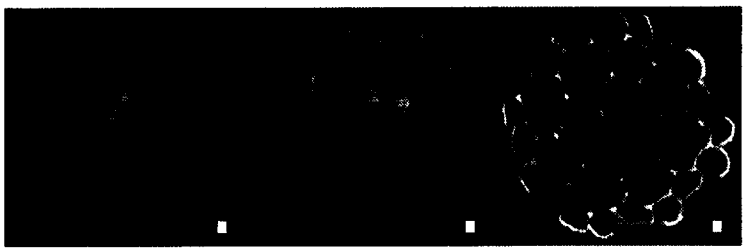

Figure 2. No-O, L*er* and *wvd2-1* roots have the same number of cells in each tissue layer, but *wvd2-1* roots are thicker due to radial cell expansion of all cell types. Fresh transverse sections in the mature zone of roots from 5 day-old seedlings, at the same magnification. Cross bars in each panel represent 1 μm (Reproduced from [62])

Figure 3. Etiolated *wvd2-1* hypocotyls exhibit right-handed cell file rotation. Seedlings were grown in the dark for 5 days on media containing 0.8% agar. Epidermal cells were visualized with Nomarski optics. All hypocotyls are shown at the same magnification (reproduced from [62]).

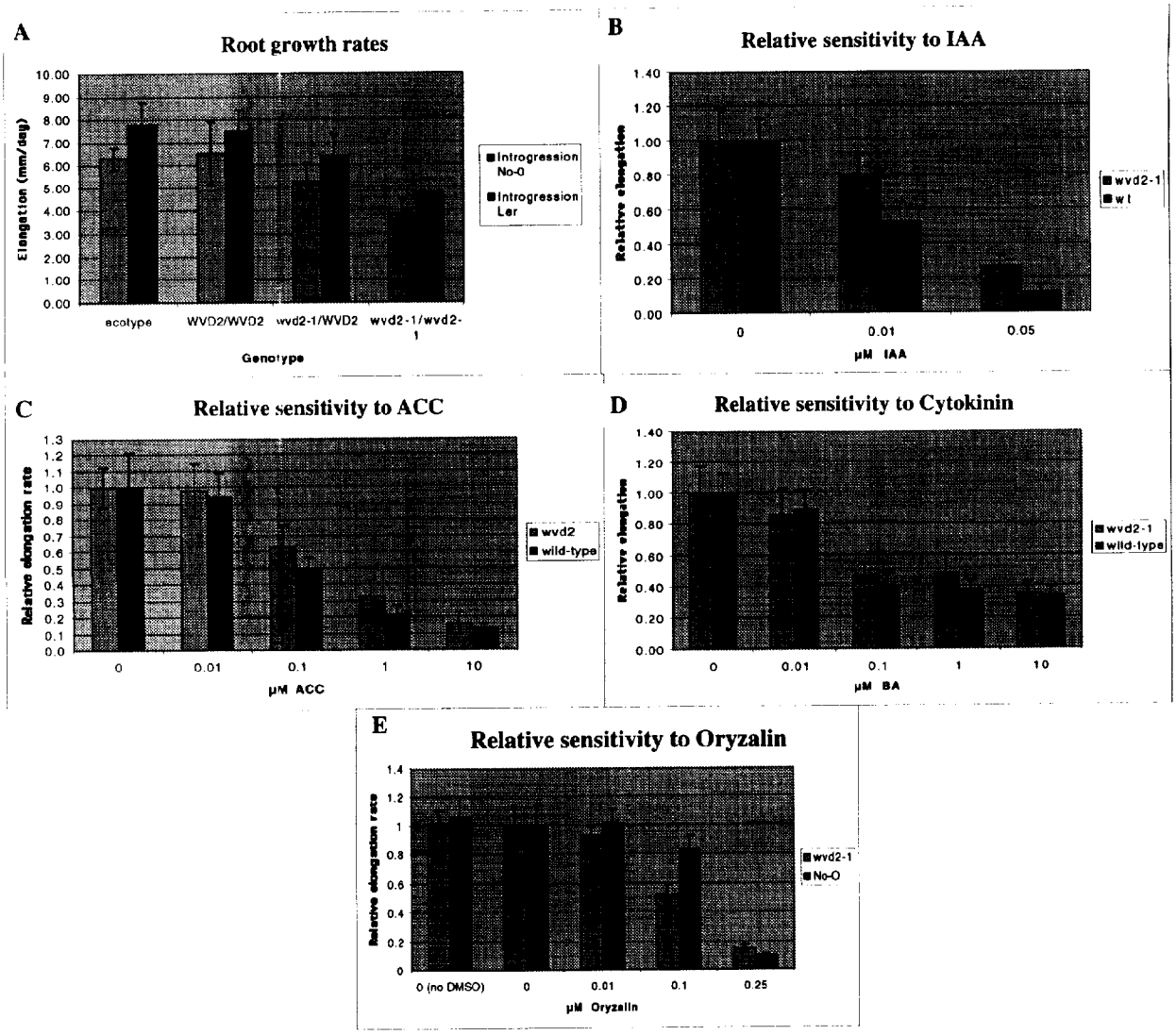

Figure 4. Growth rate (mm/24hours) of wild type (No-O, blue, or L*er*, red), heterozygous and homozygous *wvd2-1* roots (introgressed into No-O, blue, or L*er*, red) over a period of 48 to 96 hours following seedling transplantation onto fresh medium **(A)**. Relative growth rates of wild-type (No-O) and *wvd2-1* mutant roots on vertical agar surfaces with the indicated concentrations of IAA **(B)**, ACC **(C)**, BA **(D)**, oryzalin **(E)**, or without added compounds **(A)**. For each assay, absolute growth was measured during a period of 24 hours following transfer of 5-day old seedlings onto the medium containing the compound under study (B, C and D), or between 24 and 72 hours after transfer (E). All measurements were then converted into relative values by dividing each absolute value by the corresponding growth obtained on a medium without drug. Absolute growth values (in mm) on control media were (for *wvd2-1* and No-O, respectively): 2.70+/-0.49 and 7.14+/-0.93 (B); 2.70+/-0.34 and 6.13+/-1.31 (C); 3.19+/-0.56 and 7.09+/-0.95 (D), and 6.33+/-0.63 and 11.58+/-1.68 (E). All media used in each experiment were corrected to contain the same amount of solvent (0.1% ethanol for B, 0.05% DMSO for D and 0.1% for E). In the oryzalin assay, an additional control was included, with no DMSO. (n>=35). Vertical bars represent stardard deviations.

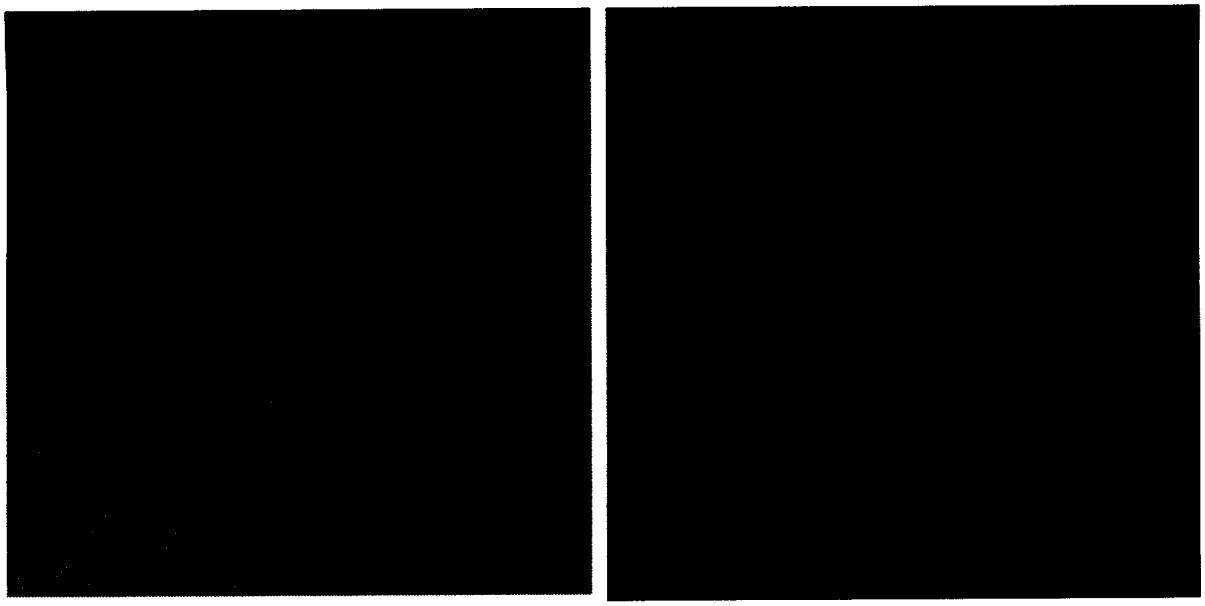

Figure 5. Whole-Mount *in situ* immunolocalization of cortical microtubules in the root tips of 5-day old No-O (A) and *wvd2-1* (B) seedlings. Experimental procedures were as described in [61], except that the following antibodies were used: MAS 078 monoclonal rat anti-tubulin antibodies as primary (clone YOL 1/34, Isotype Rat IgG2a), and FITC-conjugated affinity-purified goat anti-rat IgG as secondary antibody]. Fluorescence was observed using a confocal microscope.

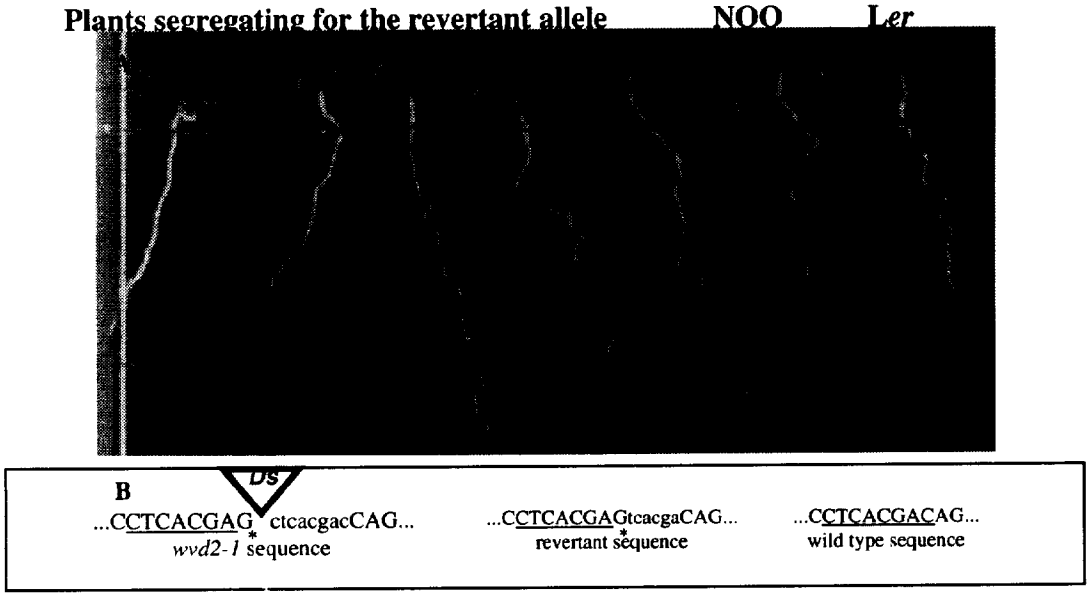

Figure 6. Wavy root growth phenotype of 7-day old wild type (No-O and L*er*, right), *wvd2-1* and *WVD2*^{R} revertant* seedlings (left) on tilted hard-agar surfaces ([5]) (A). Sequences of the wild-type (right), *wvd2-1* (left) and revertant (middle) alleles of *WVD2* around the original site of *Ds* insertion (triangle). The sequence that was duplicated during *Ds* transposition in the locus is underlined. New nucleotides derived from that duplication are represented in lower case. Reproduced from [62]

Figure 7. Northern blot analysis of RNAs extracted from 7-day old Col (1), L*er* (2), No-O (3), *wvd2-1* (4), *eto1* (5), *eto2* (6), *eto3* (7), *erh1* (8), *erh2* (9) and *erh3* (10) seedlings, after successive hybridizations with *eIF4A* (top band) and *WVD2* probes (Bottom band in 4, indicated by an arrow).

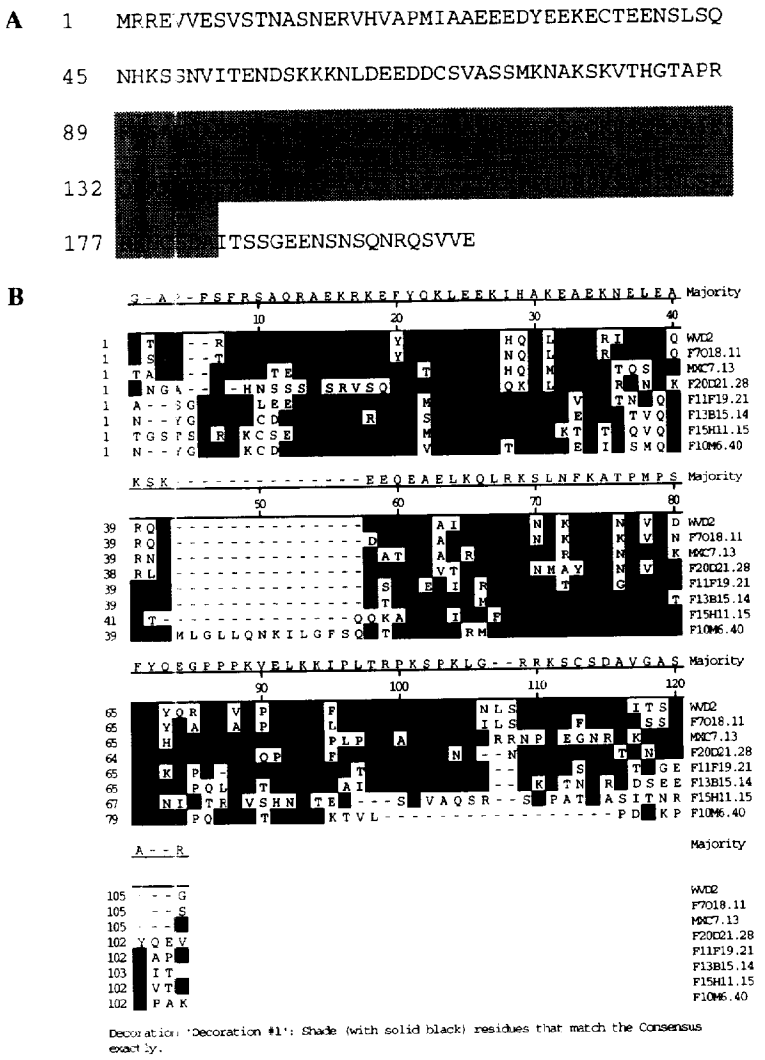

Figure 8. (**A**) Amino acid sequence of the WVD2 protein, showing the KLEEK domain (shadowed). (**B**) Sequence alignment of the KLEEK domains found in predicted *Arabidopsis thaliana* proteins (MegAlign program from DnaStar, Gap Penalty = 10, Gap Length Penalty = 10, PAM250 Matrix). Residues that match the consensus are boxed in black (B). Sequence gaps introduced by the alignment algorithm are represented by dashes, and consensus sequence is indicated on top of the alignments. Amino acid positions are indicated on the left of the sequence, and sequence codes on the right.

V. Bibliography

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*([62] in figure legends)

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