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Isolation and Characterization of a Novel Mutation That Confers Gibberellin-Sensitive Dwarfism in *Arabidopsis thaliana*

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Gibberellins (GAs) regulate diverse aspects of plant growth and development. Despite extensive analysis of the GA-metabolic pathway, only a few genes have been identified as regulatory components of GA metabolism. In searching for those genes, we screened and isolated a novel dominant mutant, *GA-sensitive dwarf1-1D* (*gsd1-1D*), from *Arabidopsis thaliana*. This mutant exhibited the characteristic phenotypes of GA-deficient mutants, including semi-dwarfism, dark-green leaves, late-flowering, and reduced fertility. Exogenously applied GA rescued the *gsd1-1D* mutant phenotypes, implying that this phenomenon was likely due to a reduced level of GA. Likewise, transcripts of GA-responsive genes were affected by this *gsd1-1D* mutation, which genetic analysis showed to be semi-dominant and monogenic. Chromosomal mapping of the *GSD1* locus indicated that it resides on the middle of Chromosome 3, where no loci related to GA metabolism exist. These results suggest that the *GSD1* locus encodes a novel regulatory component controlling the bioactive GA level in *A. thaliana*.

Keywords: dominant, GA deficiency, gibberellin, semi-dwarfism

As sessile and multicellular organisms, plants must coordinate growth and differentiation between cells in response to both endogenous and external stimuli. Plant hormones, now well-characterized, play pivotal roles in this coordination (Davies, 2004). For example, gibberellins (GAs) control diverse aspects of development, including germination, stem elongation, flowering, and floral/fruit development (Richards et al., 2001). Molecular genetics studies with GA-deficient mutants, in which normal growth can be restored by application of exogenous GA, have identified most of the genes for GA-metabolic enzymes (Koornneef and Van der Veen, 1980; Hedden and Phillips, 2000). Biologically active GAs are synthesized from geranylgeranyl diphosphate that is converted to ent-kaurene, which in turn is oxidized to ent-kaurenoic acid. The ent-kaurenoic acid becomes further oxidized to GA_{12}, which is subject to oxidative modification at C-20 and to 3-β hydroxylation, thus producing biologically active GA_1 and GA_4. Hydroxylation of C-2 by GA 2-oxidases (GA2ox) inactivates those biologically active GAs (Hedden and Phillips, 2000).

Cellular GA concentrations are regulated by both endogenous and exogenous stimuli. Expression of *ent*-copalyl diphosphate synthase (CPS), catalyzing the first committed step of GA biosynthesis, is associated with particular developmental stages, in a cell type-specific manner (Silverstone et al., 1997). Expression of *GA 20-oxidases* (*GA20ox*) and *GA 3-oxidases* (*GA3ox*) genes, whose products catalyze the final steps of GA biosynthesis, are down-regulated by GA itself. In contrast, *GA2ox* genes are induced by GA (Chiang et al., 1995; Cowling et al., 1998; Xu et al., 1999; Yamaguchi and Kamiya, 2000; Olszewski et al., 2002). Furthermore, other plant growth regulators, including auxin, brassinolide, and polyamines, may affect GA biosynthesis by altering the expression of genes for GA metabolic enzymes, including *GA20ox*, *GA3ox*, and *GA2ox* (Ross et al., 2000; Bouquin et al., 2001; Alcázar et al., 2005). These genes are also controlled by external stimuli, e.g., photoperiod and red/far-red light (Wu et al., 1996; Yamaguchi et al., 1998). However, the molecular mechanisms by which such stimuli affect the expression of genes for GA-metabolic enzymes are largely unknown.

Several regulatory factors control GA biosynthesis. For example, a basic leucine zipper protein of tobacco, designated RSG (for REPRESSION OF SHOOT GROWTH), up-regulates ent-kaurene oxidase (KO), thereby increasing the cellular GA concentration (Fukazawa et al., 2000; Ishida et al., 2004). In addition, the KNOX homeodomain protein, NTH15 (*Nic-*

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Abbreviations: GA, gibberellin; gsd, gibberellin sensitive dwarf; SSLP, simple sequence length polymorphism.

otiana tabacum homeobox 15); a MADS domain protein, AGL15 (AGAMOUS-LIKE 15); and AP2-type transcription factors, DDF1 (DWARF AND DELAYED FLOWERING1)/DDF2 (DWARF AND DELAYED FLOWERING2) have been characterized to reduce levels of bioactive GA (Tanaka-Ueguchi et al., 1998; Sakamoto et al., 2001; Magome et al., 2004; Wang et al., 2004a). However, how plants regulate those levels remains poorly understood. Besides transcriptional regulation, it is conceivable that post-transcriptional regulation of the GA metabolic enzymes may account for dynamic changes in GA concentrations in response to endogenous and exogenous stimuli. Obviously, additional regulatory factors for GA metabolism must be identified.

In searching for additional regulatory factors for GA metabolism, we have screened semi-dwarf mutants that can be rescued by exogenous GA. Here, we report a novel dominant GA-sensitive dwarf mutant, *GA-sensitive dwarf1-1*(*gsd1-1D*), in *Arabidopsis*. Its phenotypes are similar to those of GA-deficient mutants. Our objective was to conduct genetic analyses and determine possible roles for GSD1 in GA metabolism.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis thaliana ecotype Col-0 was used as the wild type. The *gsd1-1D* mutant was identified from transposon-tagging pools, N41981 (The Nottingham Arabidopsis Stock Centre, NASC, USA). CS3432 (tetraploid *gi-2 co-1* Col-0 *Arabidopsis*) was obtained from the Arabidopsis Biological Resource Center (ABRC). General growth conditions of plants were as described (Soh et al., 1999; Yang et al., 2003). Plants were grown in soil at 22-23°C under either long days (LD; 16-h photoperiod) or short days (SD; 8-h photoperiod). For the gibberellin application, plants were grown in soil and sprayed twice a week with 100 μM GA_3 solutions starting at Day 7 after sowing. Plant height, silique length, fertility rate, and flowering time were recorded as described by Tyler et al. (2004).

RT-PCR

Total RNA from 2-week-old plants was extracted with an RNeasy Miniprep kit (Qiagen, Germany). Reverse transcription (RT)-PCR analysis was performed by treating 2 μg of total RNA with RNase-free DNase, then reverse-transcribing it using the Superscript II RT-PCR kit (Invitrogen, USA), according to the manufacturer's instructions. For semi-quantitative RT-PCR expression studies, the following primers were used: *AtGA3ox1*, 5′-GCGACTAACCAAATCGGT-3′ and 5′-GGACAAACCGGGTAGTGA-3′; *AtGA20ox1*, 5′-ATCTCTGAGGCCTGTAAG-3′ and 5′-GAAGGATGGTAAGAGATG-3′; *LTP-like* (*At2g45180*), 5′-CTCCTTCACATTCATCCCAAA-3′ and 5′-TGAGGAACTTTCTTGCCACA-3′; and *Ubiquitin*, 5′-GATCTTTGCCGGAAAACAATTGGAGGATGGT-3′ and 5′-CGACTTGTCATTAGAAAGAAAGAGA- TAACAGG-3′.

Genetic Mapping

The chromosomal location of the *gsd1-1D* mutation was determined by genetic mapping with SSLP (Simple Sequence Length Polymorphism) markers, as described by Lukowitz et al. (2000). F_2 seeds were obtained from the cross between the *gsd1-1D* mutant (Col background) and Ler plants, and then scored for dwarf phenotypes. DNA was prepared from individual F_2 wild type-like plants and used for SSLP mapping. For fine mapping of *gsd1-1D*, we generated SSLP markers that can detect polymorphism between Ler and Col, based on data for Insertion/Deletion polymorphism (InDel; The Arabidopsis Information Resource, TAIR). Primers for the MOD1-1 SSLP marker included 5′-GATTCCCACTTATCAACCATCTCC-3′ and 5′-CGAGTATTACAAACCAAGCTTCAG-3′. For the MIF6-1 marker, the primers were 5′-ACATATCGCGGTCATTGTCA-3′ and 5′-CTCGGGAATTTCTCATCCAA-3′.

RESULTS

Isolation of *gsd1-1D* Mutant

We isolated a mutant by genetic screening for semi-dwarfism from transposon-tagged mutant pools in *A. thaliana*. This mutant, designated *GA-sensitive dwarf1-1* (*gsd1-1D*), showed smaller and dark-green leaves, compared with the wild type (Fig. 1A). It also exhibited delayed flowering and semi-dwarf phenotypes (Fig. 1B, 1C). Late-flowering occurred regardless of photoperiodic conditions, but was enhanced under SD. Floral morphology also was altered, with anther development being impaired. As a result, fertility was lower than for the wild type (Fig. 2). The pleiotropic phenotypes of the *gsd1-1D* mutant were reminiscent of GA-deficient and GA-insensitive mutants, suggesting this *gsd1-1D* mutation impairs GA metabolism or the GA-signaling pathways.

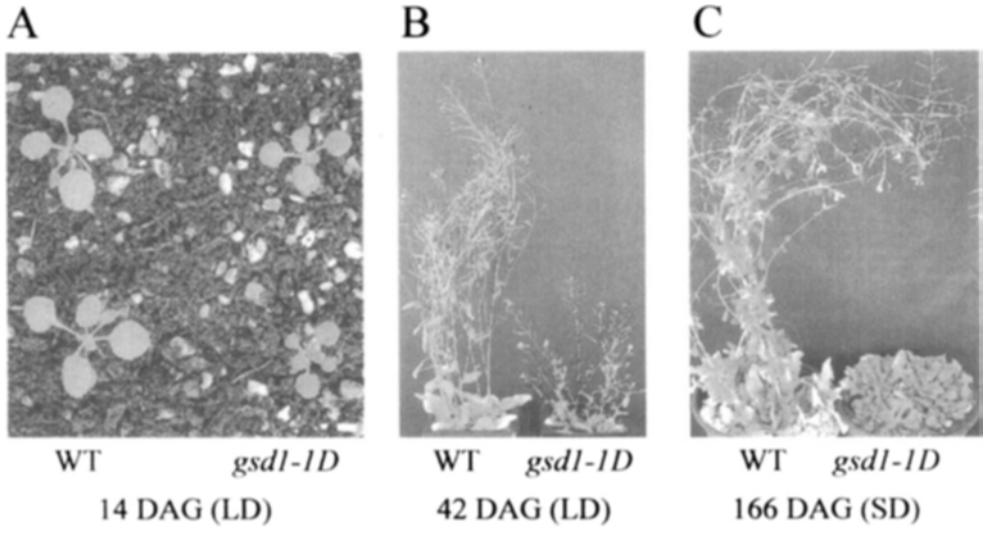

Figure 1. Phenotypes of *gsd1-1D* mutant and wild-type plants. **A.** Seedlings were grown in soil for 14 d under long-day (LD) conditions. Scale bar indicates 5 mm. **B.** Plants were grown in soil for 42 d under LD conditions. **C.** Plants were grown for 166 d under short-day (SD) conditions. DAG, days after germination.

Application of Exogenous GA Rescues Phenotypes of *gsd1-1D* Mutant

To test if our *gsd1-1D* mutant phenotypes were due to reduced levels of bioactive GA or defects in GA-signaling, we examined whether exogenously applied GA could rescue the *gsd1-1D* mutant phenotypes. Plants were sprayed with GA_3 twice per week. The semi-dwarfism and delayed-flowering phenotypes were restored to normal phenotypes by this treatment (Fig. 2). In addition, the impaired floral development and partial sterility were attenuated by exogenous GA_3. These results suggest that the *gsd1-1D* mutant phenotypes were not caused by defective GA-signaling, but likely by a reduction in bioactive-GA levels.

Expression of GA-Responsive Genes and GA-Biosynthetic Genes

Developmental responses to GA involve changes in gene expression, including feedback control of GA-biosynthetic genes (Ogawa et al., 2003). To test whether GA-dependent gene expression was altered in the *gsd1-1D* mutant, we measured transcript levels of three GA-responsive genes (Ogawa et al., 2003) -- *lipid transfer protein* (*LTP*)*-like*, *GA3ox1*, and *GA20ox1* -- in wild-type and *gsd1-1D* mutant plants. Transcripts for *GA20ox1* and *GA3ox1* were increased, whereas that of *LTP-like* was reduced in the mutant, compared with levels in the wild type (Fig. 3). This result is consistent with our hypothesis that the *gsd1-1D* mutant has less bioactive GA.

Genetic Analysis of gsd1-1D Mutant

F_1 seedlings derived from crosses between the wild type (Col) and the *gsd1-1D* mutant had shortened petioles, dark-green leaves, and semi-dwarfism (Fig. 4A, 4B). F_2 plants derived from the wild-type and mutant plants segregated at 3:1 (data not shown). These genetic analyses indicated that the *gsd1-1D* mutant is dominant and monogenic. Although this mutation was identified from a transposon-tagged pool, it did not show co-segregation with the transposon.

To test whether the dominance by *gsd1-1D* was due to a haplo-insufficient or a gain-of-function mutation, we performed gene-dosage experiments (Timpte et al., 1994). Triploid plants with two copies of the *GSD1* wild-type allele and one copy of the *gsd1-1D* mutant allele were generated by crossing the homozygous *gsd1-1D* mutant to a tetraploid line. If the *gsd1-1D* mutant phenotype was due to such haplo-insufficiency, the triploid plants would have exhibited a wild-type phenotype. However, our F_1 triploid plants (*GSD1/GSD1/gsd1-1D*) displayed a semi-dwarf phenotype (Fig. 4B) similar to that of a *GSD1/gsd1-1D* heterozygous mutant, thereby suggesting that *gsd1-1D* is likely a gain-of-function mutation.

Finally, to determine the chromosomal location of the *gsd1-1D* mutation, we selected 125 F_2 plants with a wild-type phenotype, as derived from a cross between the wild type (Ler) and the *gsd1-1D* mutant. These were used for genetic mapping with SSLP markers. The *GSD1* locus resides on the middle of

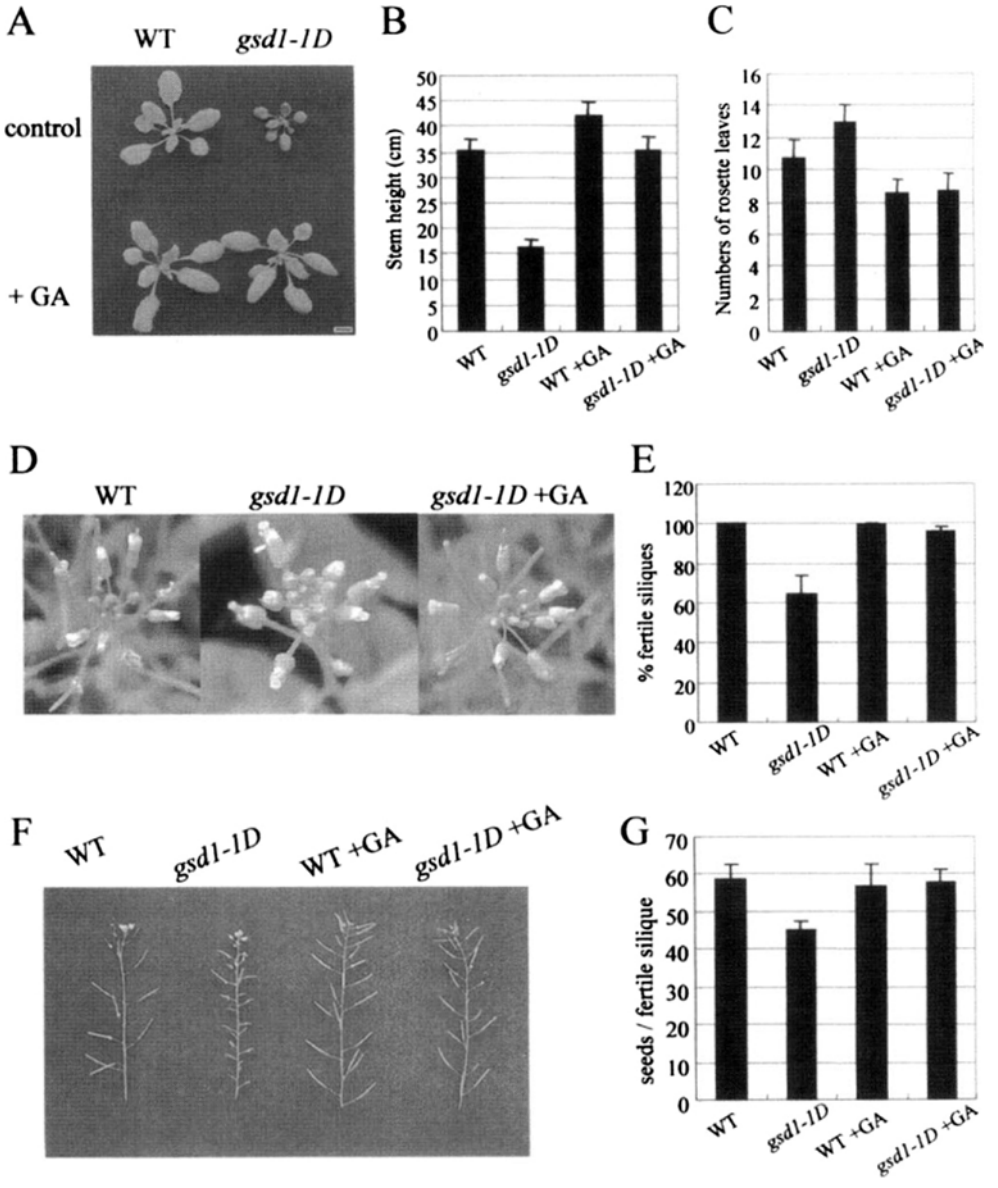

Figure 2. Effects of exogenous GA on phenotypes of *gsd1-1D* mutant. Plants were grown in soil under LD (16-h photoperiod), either with (+GA) or without spraying with 100 μM of GA_3. **A.** Plant morphology of 18-d-old mutant and wild-type (WT) plants. Scale bar shows 5 mm. **B.** Heights of 60-d-old plants. Values are averages for at least 15 plants. **C.** Numbers of rosette leaves at flowering stage. Values are averages for at least 15 plants. **D.** Primary inflorescence stems from 44-d-old mutant and WT plants, grown under LD conditions. **E.** Percent of fertile siliques on primary inflorescence. Values are averages for at least 15 plants. **F.** Primary inflorescence stems from mutant and WT plants. **G.** Number of seeds per fertile silique on primary inflorescence, as counted from at least 10 fertile siliques per plant. Values are averages for at least 10 plants. Error bars indicate standard deviations.

Chromosome 3, between MOD1-F and NIT1.2 (Fig. 4C). In its vicinity, no loci exist that are related to GA metabolism or signaling, which suggests that *GSD1* defines a new genetic locus controlling GA metabolism in *Arabidopsis*.

DISCUSSION

Coordinated regulation of the bioactive phytohormone level is critical to the developmental plasticity of higher plants coping with environmental fluctua-

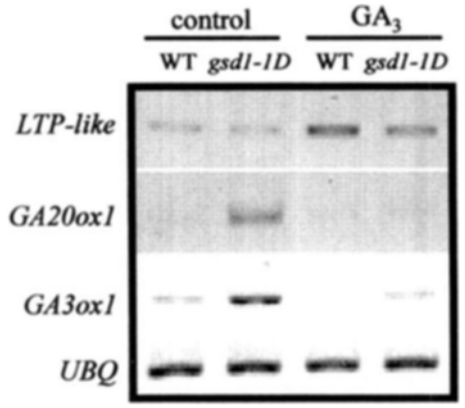

Figure 3. Expression of GA-responsive genes. Ten-day-old seedlings grown on MS-suc media were incubated for 24 h with MS solution (-) or MS solution containing 50 μM of GA_3 (+). Afterward, total RNAs were extracted for semi-quantitative RT-PCR analysis. *UBQ10* served as control. Similar results were obtained twice, from two independent experiments.

tions (Haberer and Kieber, 2002; Ljung et al., 2002; Wang et al., 2004b; Kwon and Choe, 2005). In the case of gibberellins, only a few molecular components have been characterized to control genes encoding GA-metabolic enzymes. These include a KNOX protein, NTH15 (Sakamoto et al., 2001); the bZIP protein RSG (Fukazawa et al., 2000); AP2 transcription factors, DDF1/DDF2 (Magome et al., 2004); and a MADS-domain protein, AGL15 (Wang et al., 2004a). However, the molecular components and regulatory mechanisms by which plants control their GA metabolism remain poorly characterized. Here, we have described the isolation and characterization of a novel dominant mutant, *gsd1-1D*, in *Arabidopsis*.

We propose that GSD1 functions as a regulator of GA metabolism, based on the following observations. First, pleiotropic phenotypes of our *gsd1-1D* mutant are similar to those of GA-deficient or defective GA-

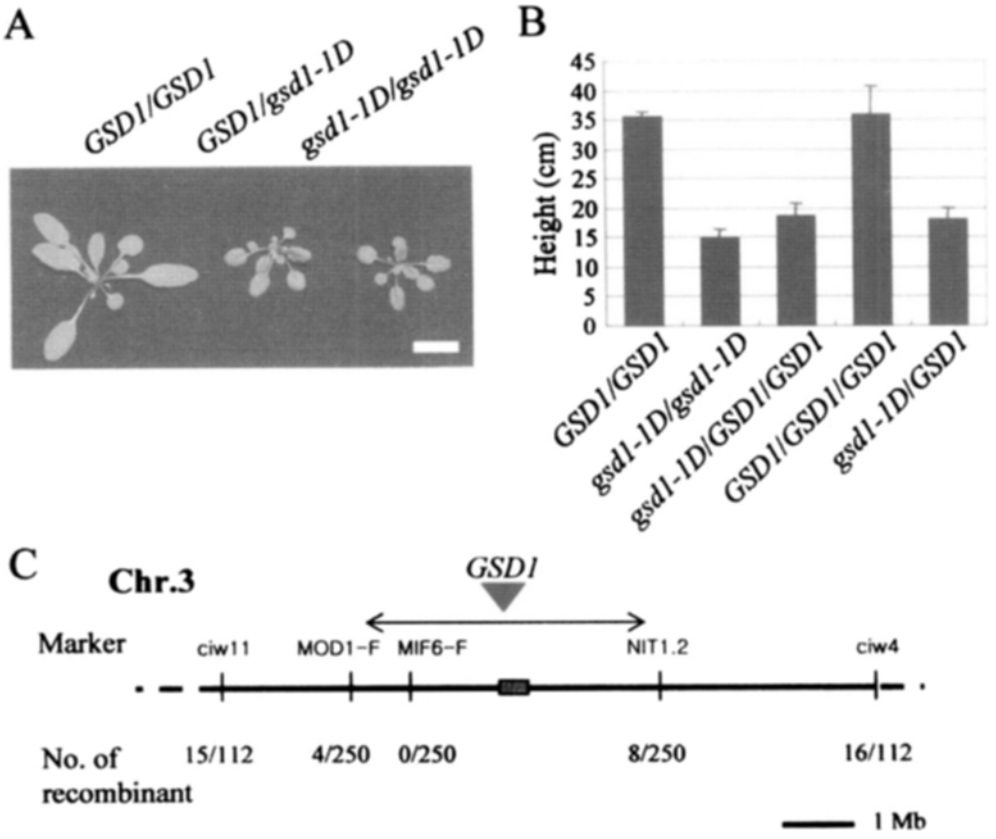

Figure 4. Genetic analyses of *gsd1-1D* mutant. **A.** Morphologies of wild-type (*GSD1/GSD1*), *gsd1-1D* heterozygote (*GSD1/gsd1-1D*), and *gsd1-1D* homozygote (*gsd1-1D/gsd1-1D*) plants grown for 3 weeks under LD conditions. Scale bar represents 5 mm. **B.** Height phenotypes for adult plants. Lengths of primary inflorescences were averaged from at least seven plants grown for 8 weeks. Error bars indicate standard deviations. **C.** Genetic mapping of *GSD1* locus, with position shown along with actual number of recombinants. Hatched box indicates relative position of centromere for Chromosome 3.

signaling mutants, i.e., shortened petioles, small dark-green leaves, delayed flowering, altered GA-responsive gene expression, and reduced fertility. In particular, the late-flowering phenotype of the *gsd1-1D* mutant is more pronounced under short-day conditions, a phenomenon consistent with previous findings that GA plays a central role in the flowering of SD-grown plants (Wilson et al., 1992; Moon et al., 2003). Second, application of exogenous GA rescues the mutant phenotypes of *gsd1-1D* plants. This result indicates that the GA-signaling pathway is not altered by that mutant but, instead, the *gsd1-1D* mutation affects GA metabolism, lowering the level of bioactive GA. It is notable that seeds of the *gsd1-1D* mutant germinated normally (data not shown) and retained at least partial fertility (Fig. 3). In this respect, the *gsd1-1D* mutant resembles two GA-biosynthetic mutants -- *ga4* and *ga5* – that have fewer bioactive GAs than wild-type plants (Talon et al., 1990). Therefore, these findings suggest that GA biosynthesis is partially inhibited in the *gsd1-1D* mutant.

It remains to be characterized how the *gsd1-1D* mutation affects GA metabolism. Our data from the gene-dosage experiments indicate that this mutation likely is not a loss-of-function allele but, rather, a gain-of-function allele. This gain-of-function nature implicates several possible functions for GSD1: 1) the *gsd1-1D* mutation might be a hypermorph; the wild-type protein may function as a negative regulator for the bioactive GA level, whereas the mutant protein may either repress GA biosynthesis or activate GA catabolism. 2) if the *gsd1-1D* mutation is a neomorphic allele, then the wild-type GSD1 may not be involved in GA metabolism. This issue could be resolved by further research on the positional cloning of the *GSD1* gene and the loss-of-function mutation of *gsd1-1D*. Moreover, the affected step of GA biosynthesis by the *gsd1-1D* mutation should be demonstrated in future studies.

One of the most desirable traits in crop breeding is dwarfism. Genetic engineering of the bioactive GA level has resulted in dwarf crop varieties that are resistant to wind and have increased yields (Evans, 1993; Spielmeyer et al., 2002; Sakamoto et al., 2003). Identification of the novel regulatory locus, *GSD1*, would not only shed light on the regulatory mechanisms of GA metabolism, by which plants have adapted their plastic development in response to endogenous/exogenous stimuli, but would also provide a novel opportunity for the generation of dwarf crop varieties.

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LITERATURE CITED

Alcázar R, Garcia-Martinez JL, Cuevas JC, Tiburcio AF, Altabella T (2005) Overexpression of *ADC2* in *Arabidopsis* induces dwarfism and late-flowering through GA deficiency. Plant J 43: 425-436

Bouquin T, Meier C, Foster R, Nielsen ME, Mundy J (2001) Control of specific gene expression by gibberellin and brassinosteroid. Plant Physiol 127: 450-458

Chiang HH, Hwang I, Goodman HM (1995) Isolation of the *Arabidopsis GA4* locus. Plant Cell 7: 195-201

Cowling RJ, Kamiya Y, Seto H, Harberd NP (1998) Gibberellin dose-response regulation of *GA4* gene transcript levels in *Arabidopsis*. Plant Physiol 117: 1195-1203

Davies PJ (2004) Plant Hormones: Biosynthesis, Signal Transduction, Action. Kluwer Academic Publishers, Dordrecht

Evans LT (1993) Crop Evolution, Adaptation, and Yield. Cambridge University Press, Cambridge

Fukazawa J, Sakai T, Ishida S, Yamaguchi I, Kamiya Y, Takahashi Y (2000) REPRESSION OF SHOOT GROWTH, a bZIP transcriptional activator, regulates cell elongation by controlling the level of gibberellins. Plant Cell 12: 901-915

Haberer G, Kieber JJ (2002) Cytokinins: New insights into a classic phytohormone. Plant Physiol 128: 354-362

Hedden P, Phillips AL (2000) Gibberellin metabolism: New insights revealed by the genes. Trends Plant Sci 5: 523-530

Ishida S, Fukazawa J, Yuasa T, Takahashi Y (2004) Involvement of 14-3-3 signaling protein binding in the functional regulation of the transcriptional activator REPRESSION OF SHOOT GROWTH by gibberellins. Plant Cell 16: 2641-2651

Koornneef M, van der Veen JH (1980) Induction and analysis of gibberellin-sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet 58: 257-263

Kwon M, Choe S (2005) Brassinosteroid biosynthesis and *dwarf* mutants. J Plant Biol 48: 1-15

Ljung K, Hull A, Kowalczyk M, Marchant A, Celenza J, Cohen JD, Sandberg G (2002) Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic

acid in *Arabidopsis thaliana*. Plant Mol Biol 50: 309-332

Lukowitz W, Gillmor CS, Scheible WR (2000) Positional cloning in *Arabidopsis*: Why it feels good to have a genome initiative working for you. Plant Physiol 123: 795-805

Magome H, Yamaguchi S, Hanada A, Kamiya Y, Oda K (2004) *dwarf and delayed flowering 1*, a novel *Arabidopsis* mutant deficient in gibberellin biosynthesis because of overexpression of a putative AP2 transcription factor. Plant J 37: 720-729

Moon J, Suh SS, Lee H, Choi KR, Hong JB, Paek NC, Kim SG, Lee I (2003) The SOC1 MADS-box gene integrates vernalization and gibberellin signals for flowering in *Arabidopsis*. Plant J 35: 613-623

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S (2003) Gibberellin biosynthesis and response during *Arabidopsis* seed germination. Plant Cell 15: 1591-1604

Olszewski N, Sun TP, Gubler F (2002) Gibberellin signaling: Biosynthesis, catabolism, and response pathways. Plant Cell 14: S61-S80

Richards DE, King KE, Ait-ali T, Harberd NP (2001) How gibberellin regulates plant growth and development: A molecular genetic analysis of gibberellin signaling. Annu Rev Plant Physiol Plant Mol Biol 52: 67-68

Ross JJ, O'Neill DP, Smith JJ, Kerckhoffs LHJ, Elliot RC (2000) Evidence that auxin promotes gibberellin A1 biosynthesis in pea. Plant J 21: 547-552

Sakamoto T, Kobayashi M, Itoh H, Tagiri A, Kayano T, Tanaka H, Iwahori S, Matsuoka M (2001) Expression of a gibberellin 2-oxidase gene around the shoot apex is related to phase transition in rice. Plant Physiol 125: 1508-1516

Sakamoto T, Morinaka Y, Ishiyama K, Kobayashi M, Itoh H, Kayano T, Iwahori S, Matsuoka M, Tanaka H (2003) Genetic manipulation of gibberellin metabolism in transgenic rice. Nat Biotechnol 21: 909-913

Silverstone AL, Chang CW, Krol E, Sun TP (1997) Developmental regulation of the gibberellin biosynthetic gene *GA1* in *Arabidopsis thaliana*. Plant J 12: 9-12

Soh MS, Hong SH, Kim BC, Vizir I, Park DH, Choi G, Hong MY, Chung YY, Furuya M, Nam HG (1999) Regulation of both light- and auxin-mediated development by the *Arabidopsis IAA3/SHY2* gene. J Plant Biol 42: 239-246

Spielmeyer W, Ellis MH, Chandler PM (2002) Semidwarf (*sd-1*), "green revolution" rice, contains a defective gibberellin *20-oxidase* gene. Proc Natl Acad Sci USA 99: 9043-9048

Talon M, Koornneef M, Zeevaart JAD (1990) Endogenous gibberellins in *Arabidopsis thaliana* and possible steps blocked in the biosynthetic pathways of the semidwarf *ga4* and *ga5* mutants. Proc Natl Acad Sci USA 87: 7983-7987

Tanaka-Ueguchi M, Itoh H, Oyama N, Koshioka M, Matsuika M (1998) Over-expression of a tobacco homeobox gene, NTH15, decreases the expression of a gibberellin biosynthetic gene encoding GA 20-oxidase. Plant J 15: 391-400

Timpte C, Wilson AK, Estelle M (1994) The *axr2-1* mutation of *Arabidopsis thaliana* is a gain-of-function mutation that disrupts an early step in auxin response. Genetics 138: 1239-1249

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun TP (2004) DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*. Plant Physiol 135: 1008-1019

Wang H, Caruso L, Downie AB, Perry SE (2004a) The embryonic MADS domain protein AGAMOUS-like 15 directly regulates expression of a gene encoding an enzyme involved in gibberellin metabolism. Plant Cell 16: 1206-1219

Wang KLC, Yoshida H, Lurin C, Ecker JR (2004b) Regulation of ethylene gas biosynthesis by the *Arabidopsis* ETO1 protein. Nature 428: 945-950

Wilson RN, Heckman JW, Somerville CR (1992) Gibberellin is required for flowering in *Arabidopsis thaliana* under short days. Plant Physiol 100: 403-408

Wu K, Li L, Gage DA, Zeevaart JAD (1996) Molecular cloning and photoperiod-regulated expression of gibberellin 20-oxidase from long-day plant spinach. Plant Physiol 110: 547-554

Xu YL, Li L, Gage DA, Zeevaart, JAD (1999) Feedback regulation of *GA5* expression and metabolic engineering of gibberellin levels in *Arabidopsis*. Plant Cell 11: 927-936

Yamaguchi S, Kamiya Y (2000) Gibberellin biosynthesis: Its regulation by endogenous and environmental signals. Plant Cell Physiol 41: 251-257

Yamaguchi S, Smith MW, Brown RSG, Kamiya Y, Sun TP (1998) Phytochrome regulation and differential expression of gibberellin 3β-hydroxylase genes in germinating *Arabidopsis* seed. Plant Cell 10: 2115-2126

Yang KY, Kim YM, Lee SH, Song PS, Soh MS (2003) Overexpression of a mutant basic helix-loop-helix protein HFR1, HFR1-DN105, activates a branch pathway of light signaling in *Arabidopsis*. Plant Physiol 133: 1630-1642