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

Abbreviations: GA, gibberellin; gsd, gibberellin sensitive dwarf; SSLP, simple sequence length polymorphism.
A Dominant GA-Sensitive Dwarf Mutant

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, CA-sensitive dwarfl-1 (gsdl-1 D), 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-tagged 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 Super-Script 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'-GGCGCTAATCCACCATCGG-3' and 5'-CGCACAAACCCGGTAGTA-3'; AtGA20ox1, 5'-ATCTCTGACGCTGTAAG-3' and 5'-GAAGGATGTAAGAGATT-3'; LTP-like (At2g45180), 5'-CTCTTTCACATCCCAA-3' and 5'-TGAGGAACTTTCAAC-3'; and Ubiquitin, 5'-GACACTGTGCGGAAAACAAATGGAGGATGGT-3' and 5'-CGACACTGTGCTTACAGAAGAGAGA-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). F2 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 F2 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 MODI-1 SSLP marker included 5'-GATCCCACCTCAACATATTGCA-3' and 5'-TGAGGAACTTTCAAC-3'. For the MIF6-1 marker, the primers were 5'-ACATATCCCTCTATCCTTAC-3' and 5'-CTCGGGAATTTCTATCCAA-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.