ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2014) **12**(S1); S70–S73
doi:10.1017/S1479262114000306

Genome-wide expression analysis in a dwarf soybean mutant

Feng Zhang[1,2†], Yanting Shen[2,3†], Shi Sun[4†], Jianqiu Guo[4†], Congcong Li[2,3], Cunxiang Wu[4], Qing Li[2,3], Hai Nian[5], Xianzhong Huang[1]*, Zhixi Tian[2]* and Tianfu Han[4]*

[1]*Key Laboratory of Agrobiotechnology, College of Life Sciences, Shihezi University, Shihezi 832003, People's Republic of China,* [2]*State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, Beijing 100101, People's Republic of China,* [3]*University of Chinese Academy of Sciences, Beijing 100039, People's Republic of China,* [4]*MOA Key Laboratory of Soybean Biology (Beijing), Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, People's Republic of China, and* [5]*College of Agriculture, South China Agricultural University, Guangzhou 510642, People's Republic of China*

Abstract

Plant height is important for crop yield improvement. In this study, a dwarf mutant, *Gmdwarf1*, was screened from a γ-ray-treated soybean population. Compared with the wild type, the mutant exhibited later germination, smaller and darker green leaves, and less-elongated shoots. Genome-wide transcriptome detection through RNA-seq analysis revealed that not only gibberellin-related genes but many other genes involved in hormone biosynthetic pathways were also significantly influenced in the mutant. We presumed that *Gmdwarf1* might play essential roles in the plant hormone pathways. Future functional analysis of this dwarf mutant would help us to understand the underlying mechanisms and be beneficial for improving soybean yield.

Keywords: Soybean; *GmDwarf1*; Gene expression

Introduction

Dwarf genes play an important role in the improvement of crop yield. In the 1960 s, plant breeders developed cereal varieties with shorter stems, which improved lodging resistance and, in turn, increased yield (Khush 2001). The new varieties prevent many people across the world from starving, which was well known as the 'Green Revolution'. Currently, there are a large number of modern wheat varieties that contain semi-dwarfing alleles (Borner *et al.* 1996).

Molecular identification has revealed that the genes responsible for the Green Revolution in wheat (Peng *et al.* 1999) and rice are involved in the gibberellin (GA) biosynthetic/signalling pathway (Hedden 2003, Monna *et al.* 2002). It has been found that GA plays important roles in the control of dwarf and plant development, including seed germination, leaf expansion, stem elongation (Plackett *et al.* 2011, Sun and Gubler 2004) and stress (Achard *et al.* 2006).

It has been predicated that the current crop production must be doubled by 2050 to meet the food demand of the increasing world population. Soybean [*Glycine max* (L.) Merr.], one of the most important crops, is a main source of protein and oil for both humans and animals. Dwarf mutant analysis is essential for soybean yield improvement. In this study, a severe soybean dwarf mutant screened from a γ-ray-treated population was characterized. RNA-seq analysis revealed that many genes related to hormone metabolism exhibited significant differences in the wild type and dwarf mutant. Treatment with

* Corresponding authors. E-mail: zxtian@genetics.ac.cn, hantianfu@caas.cn, or xianzhongh@shzu.edu.cn
†These authors contributed equally to this study.

GA_3 (Gibberellin 3) could partially restore the mutant phenotype. Our results indicate that the responding gene may play essential roles in many hormone pathways.

Materials and methods

Screening of Gmdwarf1

Gmdwarf1 was screened from a γ-ray-treated (with 250 Gy) population of the soybean variety Huaxia 3 in 2006. After treatment under short-day conditions and with 10 mg/l GA_3, M2 (indicate second-generation) mature seeds were obtained at the Campus Farm, Chinese Academy of Agricultural Sciences in Beijing.

Plant growth conditions and material collection

The soybean plants were grown in the normal season of 2012 and 2013 at the Experimental Station of the Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, in Beijing. Four seeds were planted in each pot. Nine pots were used for each experiment.

For RNA-seq analysis, 10-day-old roots and cotyledons were collected from the wild-type and mutant plants after germination and immediately frozen in liquid nitrogen. Each sample was collected from at least five independent plants and pooled together.

RNA-seq library construction and sequencing

Total RNA was isolated using the TRIzol reagent (Invitrogen, http://www.lifetechnologies.com/). RNA-seq libraries were constructed following the method described previously (Severin *et al.* 2010). RNA sequencing was carried out on a Hi-Sequation 2000 analyser (illumina, http://www.illumina.com/systems.ilmn).

Computational analysis of sequencing data and determination of differentially expressed genes (DEGs)

After quality control, the raw sequencing data were mapped to the soybean reference genome (http://www.phytozome.net/soybean) using TopHat2 (Trapnell *et al.* 2009) with a default parameter. DEGs were determined using edgeR (Robinson *et al.* 2010). Gene Ontology (GO) analysis was carried out using agriGO (Du *et al.* 2010) and WEGO (Ye *et al.* 2006).

Results and discussion

Phenotypic characterization of the Gmdwarf1 *mutant in soybean*

A dwarf mutant, *Gmdwarf1*, was screened from a γ-ray-treated population of the soybean variety Huaxia 3.

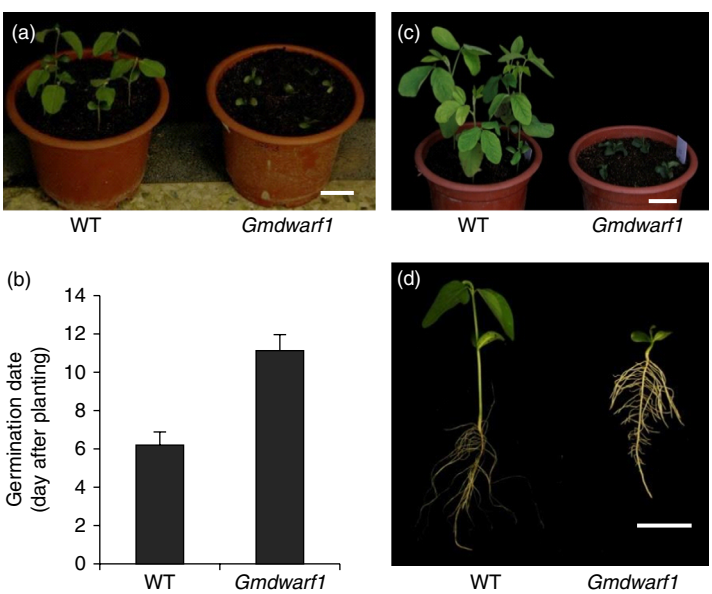


Fig. 1. Phenotypes of soybean *Gmdwarf1* mutant and wild-type (WT) plants. (a) Phenotypes of 3-week-old *Gmdwarf1* mutant and WT plants (scale bar 5 cm). (b) Difference in the germination date between *Gmdwarf1* mutant and WT plants. (c) Four-week-old *Gmdwarf1* mutant and WT plants (scale bar 5 cm). (d) Difference in the root phenotype of 10-day-old *Gmdwarf1* mutant and WT plants (scale bar 5 cm).

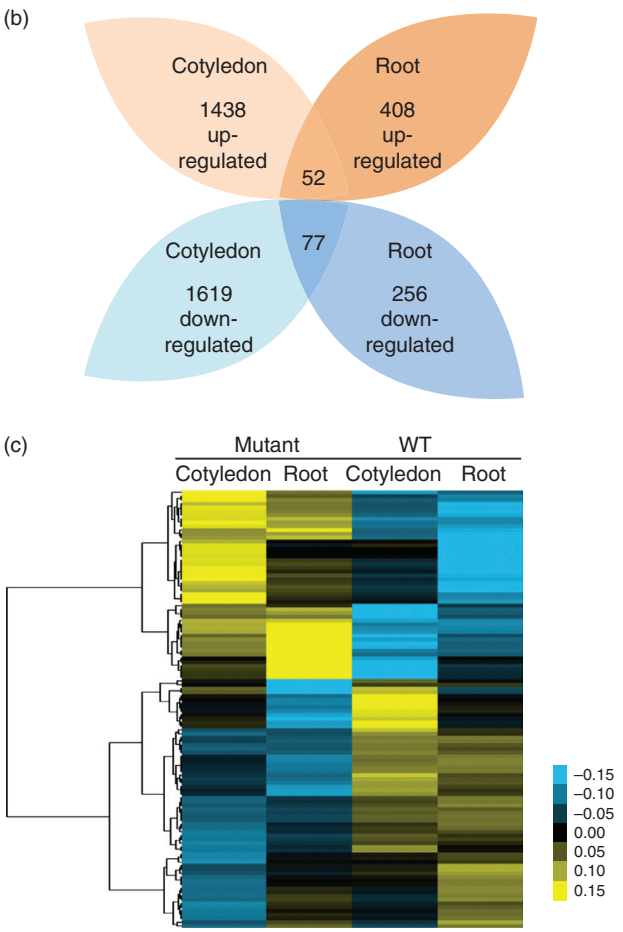
https://doi.org/10.1017/S1479262114000306 Published online by Cambridge University Press

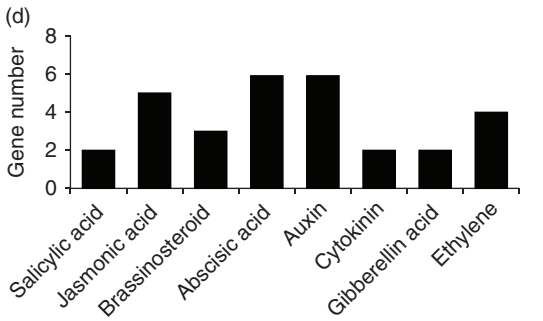
The *Gmdwarf1* mutant germinated later than the wild type (Fig. 1(a)). On average, germination was delayed by 5 day in the mutant (Fig. 1(b)). After germination, the *Gmdwarf1* mutant exhibited an extremely dwarf phenotype and no obvious internode. The leaves of the mutant were much smaller and darker green than those of the wild type (Fig. 1(c)). In addition, elongated roots were observed in the mutant (Fig. 1(d)).

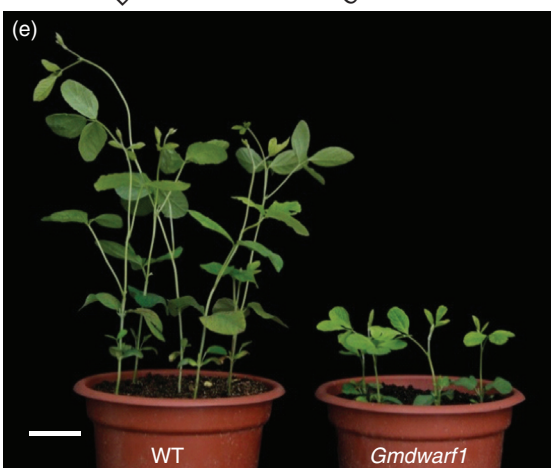
The phenotype of *Gmdwarf1* indicated that it might be caused by a mutation related to the GA biosynthetic or response pathway (Peng and Harberd 2002, Thomas and Sun 2004). To determine the genes that would be affected by the mutation, the RNA of 10-day-old roots and cotyledons of the wild type and dwarf mutant were extracted and used for RNA sequencing.

(a)

Samples	Start reads	Unique mapped reads
WT cotyledon	73,686,200	47,990,311 (65.13%)
WT root	64,918,962	34,353,987 (52.92%)
Mutant cotyledon	68,822,326	50,688,865 (73.65%)
Mutant root	56,095,226	29,838,964 (53.19%)







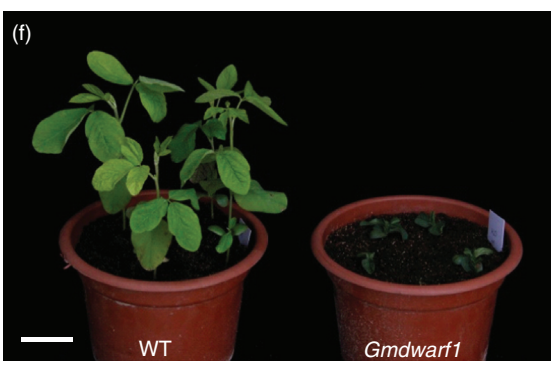


Fig. 2. Differentially expressed genes (DEGs) in *Gmdwarf1* mutant and wild-type (WT) plants detected through RNA-seq analysis. (a) Statistics of the mapped read ratios from RNA-sequation (b) Venn diagram of DEGs in the samples. (c) Heat map of DEG expression in *Gmdwarf1* mutant and WT plants. (d) DEGs related to endogenous stimulus response. (e) Phenotype of *Gmdwarf1* mutant and WT plants after treatment with 140 μM GA_3 (scale bar 5 cm). (f) Phenotype of *Gmdwarf1* mutant and WT plants after treatment with water (scale bar 5 cm).

https://doi.org/10.1017/S1479262114000306 Published online by Cambridge University Press

Gmdwarf1 led to different expression of hormone pathway-related genes

In total, more than 263 M reads (~26.3 Gb) were obtained from RNA-seq. The average reads for each sample were 65.8 M (~6.58 Gb; Fig. 2(a)). After trimming of adaptor sequences and filtering of low-quality reads, 52–73% of high-quality unique reads (excluding multiple mapped reads) were aligned to the soybean genome. Among the four samples, the mapped read ratios in the cotyledons were higher than those in the roots (Fig. 2(a)).

DEG detection revealed that there were 793 DEGs in the roots of the wild type and mutant. In the mutant, 460 DEGs were up-regulated and 333 were down-regulated (Fig. S1(A), available online). In total, 3186 DEGs were found in the cotyledons of the mutant, including 1490 that were up-regulated and 1696 that were down-regulated (Fig. S1(B), available online). Of the total DEGs, 52 that were up-regulated and 77 that were down-regulated were shared by both the cotyledons and roots (Fig. 2(b)). Their expression patterns are shown in Fig. 2(c). GO annotation demonstrated that the DEGs were involved in different biological processes (Fig. S1(C), available online). Further analysis revealed that these DEGs were enriched in the process of endogenous stimulus response (GO:0009717; Fig. S1(D), available online), in which GA-related genes only make up a small portion. However, many genes involved in other hormone pathways were detected (Fig. 2(d)). This may indicate that *Gmdwarf1* may not play essential roles in only a single hormone biosynthetic pathway.

Treatment with 140 μM GA_3 could partially restore the mutant phenotype (Fig. 2(e)), whereas that with water could not (Fig. 2(f)). Genetic analysis through crossing with the wild type revealed that the segregating ratio of the wild type to the mutant in the F2 population was almost equal to 3:1 (65:31; $\chi^2_{3:1} = 2.72 < \chi^2_{0.05} = 3.84$), indicating the *Gmdwarf1* phenotype was controlled by a single recessive gene. Future cloning and functional analysis of *Gmdwarf1* would help us to understand the underlying mechanisms in the plant hormone pathways.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000306

Acknowledgements

This study was supported by the open project of the State Key Laboratory of Plant Cell and Chromosome Engineering, Institute of Genetics and Developmental Biology, Chinese Academy of Sciences, National Natural Science Foundation of China and China Agriculture Research System (grant no. 31222042, grant no. 91131005 and CARS-04).

References

Achard P, Cheng H, De Grauwe L, Decat J, Schoutteten H, Moritz T, Van der Straeten D, Peng JR and Harberd NP (2006) Integration of plant responses to environmentally activated phytohormonal signals. *Science* 311: 91–94.

Borner A, Plaschke J, Korzun V and Worland AJ (1996) The relationships between the dwarfing genes of wheat and rye. *Euphytica* 89: 69–75.

Du Z, Zhou X, Ling Y, Zhang Z and Su Z (2010) AgriGO: a GO analysis toolkit for the agricultural community. *Nucleic Acids Research* 38: W64–W70.

Hedden P (2003) The genes of the green revolution. *Trends in Genetics* 19: 5–9.

Khush GS (2001) Green revolution: the way forward. *Nature Reviews Genetics* 2: 815–822.

Monna L, Kitazawa N, Yoshino R, Suzuki J, Masuda H, Maehara Y, Tanji M, Sato M, Nasu S and Minobe Y (2002) Positional cloning of rice semidwarfing gene, *sd-1*: rice "green revolution gene" encodes a mutant enzyme involved in gibberellin synthesis. *DNA Research* 9: 11–17.

Peng JR and Harberd NP (2002) The role of GA-mediated signalling in the control of seed germination. *Current Opinion in Plant Biology* 5: 376–381.

Peng J, Richards DE, Hartley NM, Murphy GP, Devos KM, Flintham JE, Beales J, Fish LJ, Worland AJ, Pelica F, Sudhakar D, Christou P, Snape JW, Gale MD and Harberd NP (1999) 'Green revolution' genes encode mutant gibberellin response modulators. *Nature* 400: 256–261.

Plackett ARG, Thomas SG, Wilson ZA and Hedden P (2011) Gibberellin control of stamen development: a fertile field. *Trends in Plant Science* 16: 568–578.

Robinson MD, McCarthy DJ and Smyth GK (2010) edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26: 139–140.

Severin AJ, Woody JL, Bolon YT, Joseph B, Diers BW, Farmer AD, Muehlbauer GJ, Nelson RT, Grant D, Specht JE, Graham MA, Cannon SB, May GD, Vance CP and Shoemaker RC (2010) RNA-seq atlas of *Glycine max*: a guide to the soybean transcriptome. *BMC Plant Biology* 10: 160.

Sun TP and Gubler F (2004) Molecular mechanism of gibberellin signaling in plants. *Annual Review of Plant Biology* 55: 197–223.

Thomas SG and Sun TP (2004) Update on gibberellin signaling. A tale of the tall and the short. *Plant Physiology* 135: 668–676.

Trapnell C, Pachter L and Salzberg SL (2009) Tophat: discovering splice junctions with RNA-seq. *Bioinformatics* 25: 1105–1111.

Ye J, Fang L, Zheng H, Zhang Y, Chen J, Zhang Z, Wang J, Li S, Li R and Bolund L (2006) WEGO: a web tool for plotting GO annotations. *Nucleic Acids Research* 34: W293–W297.