Overexpression of AtDREB1A Causes a Severe Dwarf Phenotype by Decreasing Endogenous Gibberellin Levels in Soybean [Glycine max (L.) Merr.]

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Abstract
Gibberellic acids (GAs) are plant hormones that play fundamental roles in plant growth and developmental processes. Previous studies have demonstrated that three key enzymes of GA20ox, GA3ox, and GA2ox are involved in GA biosynthesis. In this study, the Arabidopsis DREB1A gene driven by the CaMV 35S promoter was introduced into soybean plants by Agrobacterium-mediated transformation. The results showed that the transgenic soybean plants exhibited a typical phenotype of GA-deficient mutants, such as severe dwarfism, small and dark-green leaves, and late flowering compared to those of the non-transgenic plants. The dwarfism phenotype was rescued by the application of exogenous GA3 once a week for three weeks with the concentrations of 144 μM or three times in one week with the concentrations of 60 μM. Quantitative RT-PCR analysis revealed that the transcription levels of the GA synthesize genes were higher in the transgenic soybean plants than those in controls, whereas GA-deactivated genes except GmGA2ox4 showed lower levels of expression. The transcript level of GmGA2ox4 encoding the only deactivation enzyme using C20-GAs as the substrates in soybean was dramatically enhanced in transgenic plants compared to that of wide type. Furthermore, the contents of endogenous bioactive GAs were significantly decreased in transgenic plants than those of wide type. The results suggested that AtDREB1A could cause dwarfism mediated by GA biosynthesis pathway in soybean.

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Introduction
Gibberellic acids (GAs) are a class of essential hormones that play a key role in plant growth and developmental processes during the entire life cycle [1]. Three major oxidase gene families of GA20ox, GA3ox and GA2ox participate in GA synthesis by a series of conversions from geranylgeranyl diphosphate [2]. The levels of GAs are homeostatically modulated through the negative feedback regulation of the expression of GA20ox and GA3ox genes and positive feed-forward regulation of GA2ox genes [3,4].

To date, there are eight GA20ox genes from GmGA20ox1 to GmGA20ox8, six GA3ox genes from GmGA3ox3 to GmGA3ox6, and ten GA2ox genes from GmGA2ox1 to GmGA2ox10 that were identified in soybean, which were divided to four distinct subgroups (I, II, III, and C20 GA2ox) [5]. The GA20ox and GA3ox genes belong to subgroups I and II, respectively. The GmGA2ox genes except GmGA2ox4 belong to subgroup III, which also includes Arabidopsis GA2ox1 to GA2ox6 [5]. The function of the subgroup III members are to deactivate bioactive GAs and hydroxylate C19-GA substrates [6]. In Arabidopsis, overexpression of AtGA20ox1, -2, -3, -4, -5 and -6 resulted in dwarfism and reductions in bioactive GA levels [6]. In contrast, knockout mutants of five C19-GA 2-oxidases genes showed lower bioactive GAs content and growth retardation, indicating that the Arabidopsis C19-GA 2-oxidases mainly inactivate GA pathway [6]. In soybean, GmGA2ox4 may potentially receives only C20 (GA12 and GA53, precursors of bioactive GAs) as substrates and belongs to subgroup C20 GA2ox5 [5], which also includes AtGA2ox7 and AtGA2ox8, spinach GA2ox3, and OsaGA2ox4, -5, -6. Ectopic expression of AtGA2ox7 and AtGA2ox8 in transgenic tobacco (Nicotiana tabacum) also led to a dwarf phenotype [7]. This was also found with the activation of OsGA2ox6 in rice [8]. However, C20 GA2ox5 were found to cause less severe GA-defective phenotypes than C19 GA2ox5 in rice [9].

DREB (dehydration responsive element binding) transcription factors encode dehydration responsive element binding protein (DREB1 and DREB2) and contain a conserved AP2/EREBP
application of exogenous GA3. The transcript expression level of plants caused dwarf phenotype, which can be rescued by the [15,18,19].

dwarfism by overexpression of infected explants were briefly washed in washing medium, and

treatment reversed the dwarfism caused by overexpression of [14,16,17], but failed to rescue the dwarfism by overexpression of AtDREB1A in Arabidopsis and tobacco [15,18,19].

Here, we reported that overexpression of AtDREB1A in soybean plants caused dwarf phenotype, which can be rescued by the application of exogenous GA3. The transcript expression level of GmGA2ox4 was up-regulated in transgenic soybean plants, which decreased the levels of bioactive GAs as regarding on the dwarfism of soybean.

Materials and Methods

Plasmid Construction

The plasmids pUC18 (TaKaRa) deleted the sites between BamHI and Pml and the paragraph of pZY102 with 35S-GUS-NOS sequence were digested with restriction endonuclease HindIII, and then the two linearized parts were linked together (thereafter named as pUC18-pZY102). A 663 bp opening reading frame (ORF) of AtDREB1A was amplified from the cDNA of Arabidopsis ecotype Columbia using reverse transcriptase PCR and ligated into pGEM -T Easy vector at the multiple cloning site (Promega). The primers were designed as 5’GGATCCCTTTACAGAAACATACCA3’ and 5’GGTACC-CACTCGTTTCTGGTGTTTA3’ with the BamHI and Kpfl sites, respectively. The ORF paragraph of AtDREB1A digested with BamHI/Kpfl was cloned into the site of GUS position of the intermediate vector of pUC18-pZY102. After sequencing confirmation, the paragraph of 35S-AtDREB1A-NOS from pUC18-pZY102 was inserted into pZY101 vector at HindIII site, which was named pZY101-AtDREB1A. The resulting binary vector was introduced into Agrobacterium tumefaciens strain EHA101 by the freeze-thaw method [21], which was then used for further genetic soybean transformation.

Soybean Transformation

Mature soybean seeds of cultivar Huachun 5 bred in Guangdong Subcenter of National Center for Soybean Improvement were surface sterilized for 13.5 h using chlorine gas produced by mixing 4.2 ml of 12 N HCl with 100 ml sodium hypochlorite in tightly sealed desiccators [22]. The cotyledonary-node method described herein was modified from that described previously [23] and the brief methodology is given below.

Each of explants was prepared by removing the root and the majority of the hypocotyl approximately 3–5 mm below the cotyledonary-node after four days germination in germination medium(B3 salt/B3 vitamins, 30 g/L sucrose, 3 g/L phytagel, pH 5.8). The cotyledonary-nodes were wounded by making 10 slices with the blade perpendicular to the hypocotyls and inoculated in the 30 ml co-cultivation suspension for 30 min, and then transferred on co-cultivation medium (B5 salt (0.1x)/B5 vitamins, 30 g/L sucrose, 3 g/L phytagel, 3.9 g/L MES, 0.25 mg/L GA3, 0.15 g/L Na-thiofate, 0.4 g/L L-cysteine, 0.15 g/L DL-dithiothreitol, 0.04 g/L Acetosyringone, pH 5.4) as abaxial side down under dark condition. Three days later, the infected explants were briefly washed in washing medium, and transferred to shoot inducing medium(B5 salt/B5 vitamins, 30 g/L sucrose, 3 g/L phytagel, 0.39 g/L MES,1.67 mg/L 6-BA, 100 mg/L Timentin, 200 mg/L Cefotaxime, 5 mg/L Glufosinate, pH 5.7) and shoot elongation medium (MS salt/MS vitamins, 30 g/L sucrose, 3 g/L phytagel, 0.59 g/L MES, 5 mg/L Aspartagine, 5 mg/L Glutamine, 0.4 mg/L IAA, 0.5 mg/L GA3, 1 mg/L Trans-Zeatin Riboside, 100 g/L Timentin, 200 mg/L Cefotaxime, 5 mg/L Glufosinate, pH 5.7), cultured for four weeks respectively. Elongated shoots were placed into rooting medium containing 0.5 mg/L IBA. Primary positive plants were screened with 135 mg/L Liberty (AgrEvo) [24], and identified by DNA and RNA analysis.

Exogenous GA3 Treatment

Three-week-old transgenic soybean seedlings of T3 generation were sprayed with a GA3 solution of 0, 60, 144, or 288 μM (in 10% ethanol) once a week for three consecutive weeks or with a GA3 solution of 60 μM (in 10% ethanol) three times in one week. The plants of wide type were treated with 10% ethanol as control. The plant height, leaf area and chlorophyll content were measured two weeks after the treatment.

Chlorophyll Content Measurement

The chlorophyll content of the first and second expanding trifoliate leaves in transgenic and wide-type plants were measured by Portable chlorophyll content meter (SPAD-502, Spectrum Technologies, Inc), and each measurement repeated three times.

Gene Expression Analysis

The six-week-old soybean seedlings of transgenic and wide type were extracted using Trizol reagent (Invitrogen). After RNa-free DNase (TaKaRa) treatment, approximately 1 μg total RNA was used for reverse transcription using the oligo (dT) primer and MLV (Invitrogen). qRT-PCR was performed using CFX96 (Bio-Rad, USA) and SYBR Green I (Bio-Rad, USA). Each of the cDNA samples was subjected to a real-time PCR analysis in triplicate. The data were normalized using the reference gene β-tubulin. The relative expressions of specific genes were quantified using the 2ΔΔCt calculation. The primer pairs used for q-RT-PCR are listed in Table S1.

Quantification of Endogenous GAs

The transgenic and wide type soybean plants were grown in 1/2 Hoagland solution for four weeks in growth chamber under 28°C, 16-h light and 24°C, 8-h dark condition. Samples were taken from the top part of young plants including apex, young stem and young leaves. The GAs contents were determined by the method of capillary electrophoresis-time of flight-mass spectrometry described previously [25].

Results

Overexpression of the Arabidopsis DREB1A Gene in Soybean Caused Severe Dwarf Phenotype

The Arabidopsis DREB1A gene driven by the CaMV 35S promoter was transferred into the soybean plants using Agrobacterium-mediated transformation of the cotyledon node. Unexpectedly, during the transformation process, some elongated shoots showed abnormal phenotype with no obvious stems (Fig. S1). Consequently, these shoots were later identified as positive plants. A total of 12 T0 lines were successfully regenerated. All the 35S::AtDREB1A transgenic plants exhibited severe dwarf phenotype (Fig. S2). Homozygous T3 plants of two independent
transgenic lines of AtDREB1A-L1 and AtDREB1A-L2 were selected for further analysis. The transgenic lines were more tolerance to the herbicide treatment than that of wide type (Fig. 1A). Moreover, qRT-PCR analysis showed the AtDREB1A was transcriptionally expressed in transgenic lines. However, under the GA3 treatment condition, its expression was decreased (Fig. 1B).

The 35S::AtDREB1A transgenic plants exhibited a severe dwarf phenotype with no observable internodes (Fig. 2A). The average length of internodes was only 19.58% and 22.08% of those in wide type, respectively. The height of transgenic plants was decreased by 79.91% and 80.05% of those in wide type, respectively (Table 1). The leaf area and color from the 1st trifoliate to 4th trifoliate were smaller and darker than those of wide-type (Fig. 2B, 2D, 2E). The chlorophyll contents of the 1st and 2nd trifoliate were 1.1-fold and 1.5-fold higher in transgenic plants than those of control plants (Fig. 2E). In addition, the transgenic plants showed the phenotypes of late flowering and podding. The flowering and podding stage were longer more than 20 days and 40 days than those of wide type, respectively (Table 1, Fig. S3). Furthermore, the transgenic seeds were much smaller in size with the grain weight only about 51.5% and 55.2% of those of wild type (Fig. 2C, 2D, 2E). The chlorophyll contents of the 1st and 2nd trifoliate were smaller and darker than those of wide-type (Fig. 2B, 2D, 2E). The chlorophyll contents of the 1st and 2nd trifoliate were 1.1-fold and 1.5-fold higher in transgenic plants than those of control plants (Fig. 2E). In addition, the transgenic plants showed the phenotypes of late flowering and podding. The flowering and podding stage were longer more than 20 days and 40 days than those of wide type, respectively (Table 1, Fig. S3). Furthermore, the transgenic seeds were much smaller in size with the grain weight only about 51.5% and 55.2% of those of wild type (Fig. 2C, 2D, 2E).

The Dwarf Phenotype of Transgenic Soybean were Rescued by the Application of Exogenous GA3

Overexpression of AtDREB1A caused dwarfism, dark-green leaves and late flowering, which resembles the previously identified typical phenotypes of GA-deficiency mutants [13]. This suggested that the phenotypic changes of AtDREB1A-overexpression plants were caused by GA3 deficiency. Application of GA3 at concentration of 144 µM once a week for three consecutive weeks rescued the dwarf phenotype in transgenic plants (Fig. 3). In addition, when application of GA3 at concentration of 60 µM and increased frequency to three times in one week, the plant height of transgenic plants also be rescued and even much taller than that of plants under 144 µM GA3 treatment and wide type (Fig. S4). The leaf area of was partly rescued and chlorophyll contents was fully rescued after GA3 treatment in transgenic plants (data not shown). However, the flowering time was didn’t rescued (data not shown).

Table 1. Characterization of transgenic and WT soybean plants.

| Traits                      | WT   | L1   | L2   |
|-----------------------------|------|------|------|
| Plant height (cm)           | 35.00| 7.03±0.58 | 6.98±0.34B |
| Average length of internodes (cm) | 2.40±0.20A | 0.47±0.03B | 0.53±0.01B |
| Initial flower stage (day)  | 29.00±1.84A | 52.00±2.06B | 53.00±1.72B |
| Initial pod stage (day)     | 44.00±1.12A | 90.00±1.68B | 91.00±1.02B |
| Grain weight(g/100 grains)  | 30.43±0.26A | 8.63±0.20B | 3.35±0.26B |

Values are the mean of ten biological replicates ± SE, the same letter indicates no significant difference and different letters are significantly different by the analysis of variance (ANOVA), p<0.01. WT: wide type; L1: AtDREB1A transgenic line 1; L2: AtDREB1A transgenic line 2.

The Expression of GA Biosyntheses Genes Were Changed in the Overexpression of AtDREB1A Soybean Plants

The GA-20 oxidase, GA-3-β-hydroxylase and GA-2 oxidase are critical enzymes in GA biosyntheses. Quantitative RT-PCR analysis was performed to investigate these genes expression level in transgenic plants. Due to the tissue specific expression pattern among members, only GmGA20ox3, GmGA3ox6 and six GA2-oxidase genes were detected. The result showed that the relative mRNA expression of GmGA20ox3 and GmGA3ox6 were dramatically increased in two transgenic lines compared with those of wild-type plants (Fig. 4). While the mRNA level of GA2-oxidase genes of GmGA2ox1, GmGA2ox2, GmGA2ox6, GmGA2ox7 and GmGA2ox8 were down regulated in transgenic lines (Fig. 4). However, the transcriptional expression of another GA2-oxidase gene, GmGA2ox4, was significantly up-regulated in transgenic plants compared to that of wide type (Fig. 4).

Overexpression of AtDREB1A Reduced the Bioactive GAs

Regarding the data above, we propose that the GA biosynthesis was interfered by overexpression of AtDREB1A through regulating the GA synthase genes, especially for activating the expression of GmGA2ox4, which could decrease the bioactive level in transgenic soybean plants. Thus, the endogenous GAs content were examined using collected samples of apex, young leaves and stem.
Figure 2. Phenotypes of AtDREB1A-overexpressing soybean plants. A: Phenotypes of 3-week-old T3 transgenic and WT soybean plants. B: Leaf phenotype of 3-week-old T3 transgenic and WT soybean plants. C: Seeds of transgenic and WT soybean plants. D: The first to fourth leaf acreage of 3-week-old transgenic and WT soybean plants. E: The chlorophyll content of first and second leaf in transgenic and WT soybean plants. WT: wide type; L1: AtDREB1A transgenic line 1; L2: AtDREB1A transgenic line 2. Values are the mean of six biological replicates ± SE, the same letter on each column set indicates no significant difference and different letters are significantly different by the analysis of variance (ANOVA), p<0.05. doi:10.1371/journal.pone.0045568.g002

Figure 3. Effects of GA3 on phenotypic restoration. A: Phenotypes of transgenic plants under 0 μM, 60 μM, 144 μM or 288 μM GA3 treatments for 7 days and 14 days. B: The plant height of eight weeks of WT and transgenic plants after treated with or without 144 μM GA3 for two weeks. WT: wide type; L1: AtDREB1A transgenic line 1; L2: AtDREB1A transgenic line 2; L1+L2: AtDREB1A transgenic plants line 1 and 2. Values are the mean of six biological replicates ± SE, the same letter on each column set indicates no significant difference and different letters are significantly different by the analysis of variance (ANOVA), p<0.05. doi:10.1371/journal.pone.0045568.g003
of transgenic and wide type soybean seedlings after hydroponic cultivation for four weeks. Table 2 showed that the concentration of the bioactive GA_{1} in transgenic plants was reduced by 74.41\% compared with that of wide type, and the concentration of bioactive GA_{4} was even not detected. It demonstrated that \textit{AtDREB1A} transgenic plants are similar to GA deficient mutants. The contents of intermediates (GA_{19}) and producers (GA_{9} and GA_{24}) of GA20ox were decreased to 13.94\%, 28.01\% and 24.58\% of wide type in transgenic plants, respectively. Based on this observation, we speculated that GA20-oxidation might be partly impaired in transgenic plants, which was consistent with previous report [14]. Furthermore, some C20-GAs (GA_{12}, GA_{35} and GA_{24}) in transgenic plants accumulated at lower levels below the limit of detection compared with those of wide type. Taken together, these results indicate that the deficiency of bioactive GAs in transgenic soybean plants is mainly because of the inhibition of stepwise oxidation catalyzed by \textit{C_{20}}-GA2ox.

### Discussion

In this study, we demonstrated that overexpression \textit{AtDREB1A} in soybean caused a dwarfism phenotype, probably by the up-regulation of the \textit{GmGA2ox4} gene, which resulted in decreasing levels of active GAs and conferred dwarfism phenotypes. Overexpression of \textit{AtDREB1A} transcription factor in soybean caused dwarfism (Fig. 2, Table 1). Similar phenomena was also found when DREB members were overexpressed in other plant species, such as \textit{Arabidopsis} [12,13], \textit{tobacco} [18,19] and \textit{chrysanthemum} [20]. The dwarfism caused by overexpression of \textit{DREB1B} and \textit{DREB1F} in \textit{Arabidopsis} can be reversed by exogenous GA$_3$ treatment [14,16,17]. However, the dwarfism caused by overexpression of \textit{DREB1A} in \textit{Arabidopsis} and \textit{tobacco} cannot be reversed by GA treatment [15,18,19]. In this research, exogenous GA$_3$ treatment restored the plant height (Fig. 3, Fig. S4). This suggested that the different DREB-like transcription factors or the same DREB transcription factors but in different transgenic plant backgrounds may contribute to plant growth differentially [26].

We detected the expression of GA-20-oxidase and GA-3-oxidase genes which involve in GA biosyntheses and GA2-oxidase which convert bioactive GAs into deactivated forms [27]. In contrast, the transcripts of \textit{GmGA20ox5} and \textit{GmGA3ox6} were both up-regulated in transgenic soybean plants compared with that in wide type (Fig. 4). It seems that the inhibition of GA biosynthesis does not account for the transcriptional repression of GA-20-oxidase or GA-3-oxidase genes. Similarly, previous studies found that the expression of \textit{AtGA20ox1}, \textit{AtGA20ox2}, \textit{AtGA20ox3} and \textit{AtGA3ox1} were all up-regulated in \textit{35S::DREB1F} dwarf plants [14]. Meanwhile, the expression of \textit{Gh20ox1-4}, \textit{Gh3ox1} and \textit{Gh3ox2} were also increased in \textit{35S::GhDREB1 Arabidopsis} [28]. In addition, the up-regulation of GA-20-oxidase and GA-3-oxidase genes has been reported in GA-deficient and GA-insensitive mutants [4,29]. These suggested that the up-regulation of \textit{GmGA20ox5} and \textit{GmGA3ox6} in \textit{35S::DREB1A} soybean plants may due to the negative feedback regulation of endogenous GAs levels. However, the transcriptional expression of one GA deactivating gene of \textit{GmGA20ox4} was up-regulated, while the transcriptional expression of other GA deactivating genes were down-regulated compared to those of wide type (Fig. 4). Previous study predicted that \textit{GmGA20ox4} only hydrolylates \textit{C_{20}}-GA rather than the \textit{C_{19}}-GA substrates in soybean, which is clustered into the same subgroup of \textit{C_{20}}-GA2ox with \textit{spinach} \textit{GA2ox3}, \textit{AtGA2ox7}, \textit{AtGA2ox8}, and \textit{OsGA2ox4-5,-6} [5,27]. Amino acid sequence alignment showed that \textit{GmGA20ox4} was closed to \textit{AtGA20ox7} and \textit{AtGA20ox8} than any other GA-2 oxidase in soybean and has the conserved motifs for binding GAs and other common cofactors (Fig. S5). However, a unique region (at the positions 115 to 143 of \textit{AtGA2ox8}) in \textit{C_{20}}-GA subgroup may define the specificity of the reactions performed by these enzymes. It has been reported that overexpression of \textit{AtGA2ox7} and \textit{AtGA2ox8} decreased the levels of active GAs and conferred dwarf phenotypes both in \textit{Arabidopsis} and \textit{tobacco} [7]. Consistent with this observation, homologous and heterologous over-expression of rice \textit{GA2ox5} and \textit{GA2ox6} resulted in typical GA-deficient dwarfism [9]. Similarly, transgenic \textit{tobacco} of over-

### Table 2. The bioactive GAs contents in transgenic and WT plants.

| GAs               | WT    | L1    |
|-------------------|-------|-------|
| Non-13-hydroxylated | 0.39±0.08 | ND     |
| GA12              | 1.39±0.17 | 0.99±0.12 |
| GA9               | 7.10±0.29 | ND     |
| GA4               | 5.00±0.15 | ND     |
| 13-Hydroxylated   |       |       |
| GA53              | 0.61±0.10 | ND     |
| GA19              | 5.64±0.23 | 1.58±0.19 |
| GA20              | 4.12±0.48 | 0.24±0.08 |
| GA1               | 6.55±0.33 | 1.61±0.14 |

3-week-old soybean seedlings of transgenic and wide type were sampled for GA content measurement. WT: wide type; L1: AtDREB1A transgenic line 1. All values are ng/g fresh weight, data represents four biological replicates. ND, not detectable.

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expression of *spinach* GA2ox3 showed dwarf phenotype [27]. In this research, overexpression of *AtDREB1A* in soybean increased expression level of *GmGA2ox4*, resulted in typical GA-deficient dwarfism and decreased the active GAs levels (GA1 and GA4). What’s more, the bioactive levels of C20-GAs (GA12, GA53 and GA24) in transgenic plants were lower than limit of detection (Table 2). Recently reports showed that GA2ox7 and GA2ox8 in *Arabidopsis* and GA2ox3 in *spinach* hydroxylate C20-GA precursors (GA12 and GA53) [7,15,27]. In addition, GA24 could metabolize by GA2ox7 in *vinca*, suggesting GA29 is another substrate of GA2ox7 [15]. Therefore, we tentatively propose that GA29, GA53 and GA24 were substrates of GmGA2ox4 in soybean and C20-GA oxidation plays an important role in resulting GA deficiency in transgenic soybean plants.

Overexpression of *AtDREB1A* modulates plant growth through regulating C20-GA deactivation genes with a similar mechanism as found in other plants. In 35S:DDF1 transgenic plants, the expression of *AGI2ox7* were dramatically increased (223-fold) compared to control plants [15]. DDF1 protein can bind to DRE-L motifs in the GA2ox promoter, suggesting that GA2ox7 is a direct target of DDF1 transcriptional activator [15]. In addition, the expression of GA2ox3 was up-regulated in CBF1 overexpression plant while there is no CRT/DRE-like cis-element in the promoter region of GA2ox3, implying that CBF1 up-regulated GA2ox3 gene expression indirectly [16]. In this study, no CRT/DRE-like cis-element was found in the promoter region of GmGA2ox4. Alternatively, there exist two ERE (ethylene-responsive element) elements with a core sequence of AGCCGCC, and some DREBs such as TINY2, BnDREBIII-1 and CBF1/DREB1B were demonstrated to bind to ERE element [30–32]. However, CBF2/DREB1C and CBF3/DREB1F/3/DREB1H have been demonstrated without binding to ERE due to 15th-Cys other than Ser like in TINY, TINY2, BnDREBIII-1, which is crucial for the specific binding of ERE element [33]. Therefore, the results suggested that *AtDREB1A* regulates GmGA2ox4 gene expression through an indirectly way.

It was widely reported that overexpressing DREBs in plants increased the transgenic plants tolerance to abiotic stresses [12–20]. It was reported that soybean lines transformed with an *rd29A::AtDREB1A* construct improved the tolerance to drought [34], suggesting that *AtDREB1A* is involved in stress tolerance in soybean. In this study we demonstrated that overexpression of *AtDREB1A* gene up-regulated the expression of the only C20-GA-oxidase *GmGA2ox4*, which decrease the active GAs and corresponding for dwarf phenotype in soybean.

Taken together, we showed that overexpression *AtDREB1A* in soybean could result in a typical phenotype of GA-deficient mutants including severe dwarfism, small and dark-green leaves, and late flowering in transgenic plants. The dwarfism phenotype could be rescued by the application of exogenous GA3 with the concentrations of 60 μM or 144 μM. The dwarfism of 35S:AtDREB1A reveals *AtDREB1A* can mediate GA metabolism and regulate some GA-responsive genes involved in the GA synthase genes and GA deactivated genes, which were further confirmed by the contents of endogenous bioactive GAs. The gained information suggested that *AtDREB1A* causes soybean dwarfism mediated by GA biosynthesis pathway.

Supporting Information

Figure S1 The phenotype of transgenic and wide-type shoots during the period of tissue culture. A: wild type; B: regenerate shoots. (TIF)

Figure S2 The phenotypes of transgenic plants after transferred into pots. A: wide type; B–H: transgenic plants. (TIF)

Figure S3 The phenotypes of transgenic plants during the growth and development. A, E and I: wild type at vegetable stage, flowering and podding stage. C, G and L are the magnified pictures of wild type plants corresponding to A, E and I, respectively. B, F and J: *AtDREB1A* transgenic plants at vegetable, flowering and podding stage. D, H and K are the magnified pictures taken from the top of *AtDREB1A* transgenic plants corresponding to B, F and J, respectively. (TIF)

Figure S4 Effects of GA3 on phenotypic restoration. A: Phenotypes of transgenic plants under 60 μM and 144 μM GA3 treatments. B: The plant height of transgenic and WT soybean plants after treated with or without GA3 treatment for two weeks. WT: wide type; L1: *AtDREB1A*-transgenic line 1; L2: *AtDREB1A* transgenic line 2; Values are the mean of six biological replicates ± SE, the same letter on each column set indicates no significant difference and different letters are significantly different by the analysis of variance (ANOVA), p<0.05. (TIF)

Figure S5 Sequence alignment of predicted proteins of C20-oxidase group. Black shading indicates identical amino acid residues, and gray shading indicates similar residues. GenBank accession numbers of proteins are (in parentheses): AtGA2ox7 (At1g50960), AtGA2ox3 (At4g21200), OsGA2ox5 (Os07g01340), OsGA2ox6 (Os04g44150), SoGA2ox3 (Os02g14954), GmGA2ox4 (Glyma11g00550). (TIF)

Table S1 Primers used for real-time quantitative RT-PCR. (TIF)

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Author Contributions

Conceived and designed the experiments: HCS QBM KXY. Performed the experiments: HCS QBM KXY JLY QYF. Analyzed the data: HCS QBM. Contributed reagents/materials/analysis tools: CYQ QBM. Wrote the paper: HCS QBM XZY JLY. Soybean transformation and gained the soybean transgenic lines: HCS KXY YJT JH.

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