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Differential expression analysis of a subset of *GmNAC* genes in shoots of two contrasting drought-responsive soybean cultivars DT51 and MTD720 under normal and drought conditions

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Abstract NAC transcription factors are known to be involved in regulation of plant responses to drought stress. In this study, the expression of 23 drought-responsive *GmNAC* genes was assessed in the shoot tissues of DT51 and MTD720, the two soybean varieties with contrasting drought-responsive phenotypes, by real-time quantitative PCR (RT-qPCR) under normal and drought conditions. Results indicated that expression profile of *GmNAC* genes was genotype-dependent, and six *GmNAC*s (*GmNAC019*, *043*, *062, 085*, *095* and *101*) had higher transcript levels in the shoots of the drought-tolerant DT51 in comparison with the drought-sensitive MTD720 under drought. Our study suggests a positive correlation between the higher drought tolerance degree of DT51 versus MTD720 and the up-regulation of at least these six drought-responsive *GmNAC*s in the shoot tissues. Furthermore, on the basis of our analysis, three genes, *GmNAC043*, *085* and *101*, were identified as promising candidates for development of drought-tolerant soybean cultivars by genetic engineering.

Keywords Comparative expression analysis · Drought stress · NAC transcription factor · Real-time quantitative PCR · Soybean

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Introduction

Drought is one of the main environmental constraints to crop productivity worldwide by affecting various plant physiological and biochemical processes, such as photosynthesis, respiration, translocation, ion uptake, carbohydrates and nutrient metabolism [1–3]. Detrimental impact of drought on soybean has been a matter of great concern since this important legume is considered as one of the world's leading economic oilseed crops, providing the largest source of vegetable oil, proteins, macronutrients and minerals for human consumption and animal feed with increasing demands year by year [4].

In the past two decades, great efforts have been made to elucidate the mechanisms of drought tolerance in plants through various molecular and genomic approaches, and a number of genes, which respond to drought, have been identified [5]. Occupying approximately 7 % of the total genes found in a plant genome, transcription factors (TFs) are important regulators of gene expression, thus controlling diverse biological processes including growth, development and responses to environmental stimuli [6]. Thanks

to the completion of the soybean genome sequencing project in 2010, more than 55 TF families have been identified by various TF databases, among which the NAC (NAM—no apical meristem, ATAF—*Arabidopsis* transcription activation factor, and CUC—cup-shaped cotyledon) TF family was estimated to consist of more than 180 members [7–11]. The NAC TFs, which share a preserved DNA-binding domain located at the N-terminal end, and a variable domain at the C-terminal end, were first described in *Petunia* more than a decade ago [12]. They are now known to comprise a large family of plant-specific TFs identified in many plant species, including crops, and have been reported to be involved in regulation of a number of biological processes, including plant responses to different environmental stresses [13–17].

Meng et al. [18] identified the first six soybean *GmNAC* genes called *GmNAC1-6*, and the expression of these genes in soybean under osmotic stress was thoroughly examined [6]. Later on, Tran et al. [19] proved that nine out of 31 *GmNAC* genes studied in soybean seedlings were induced by dehydration, high salinity, cold and/or ABA treatments. More recently, a comprehensive analysis of *GmNAC* family by Le et al. [20] identified 152 full-length GmNAC TFs in soybean, of which 11 members were found to be membrane-associated proteins. Expression analyses using real-time quantitative PCR (RT-qPCR) verified that out of 38 predicted abiotic stress-related *GmNAC* genes, twenty-five and six *GmNAC*s were induced and repressed, respectively, by twofold or more in roots and/or shoots of soybean seedlings subjected to a dehydration treatment [20]. In addition, a number of these genes displayed differential drought-responsive expression profiles in various tissues at the same development stage, or in the same tissue but at different development stages [21].

In the present study, we aimed to examine whether there was correlation between differential expression of *GmNAC* genes in the shoots and the differential drought-tolerant ability of two soybean cultivars, DT51 (a drought-tolerant variety) and MTD720 (a drought-sensitive variety), displaying contrasting drought-tolerant phenotypes that were observed in a previous study [22]. The results obtained in this work have enabled us to propose a list of *GmNAC* candidate genes that are deserved for further in-depth analyses, leading to development of drought-tolerant soybean varieties by improving shoot-related traits through genetic engineering.

Materials and methods

Plant growth and drought experiments

Growing conditions and the drought treatment of the drought-tolerant DT51 and drought-sensitive MTD720 soybean cultivars were described in a previous study [22]. Plants were well-watered and grown in plastic tubes (80 cm in height and 10 cm in diameter), containing a mixture of soil, coconut fiber and cow pat (6:2:2 w/w) from Southern Fertilizer Company, for 12 days under greenhouse conditions (30/28 °C day/night temperatures, photoperiod of 12/12 h, and 60–70 % humidity). The 12-day-old plants were subsequently subjected to a non-irrigation period of 15 days until the soil moisture content (SMC) was decreased to 5–6 %. Control plants were regularly watered once per day to maintain SMC at 65–70 %. After the drought treatment, control and drought-treated plants were carefully removed from soil by longitudinally cutting the plastic tubes. The shoot tissues (vegetative developmental stage V6), including leaves and stem, were collected from well-watered and drought-stressed plants in three biological replicates, and immediately frozen in liquid nitrogen for subsequent expression analyses using RT-qPCR.

Total RNA isolation and RT-qPCR

Total RNA isolation, cDNA synthesis and RT-qPCR were carried out as previously described [23]. Twenty-three dehydration-responsive *GmNAC* genes along with their specific primers were selected from the study conducted by Le et al. [20]. Detailed information about the selected *GmNAC* genes and their specific primers was provided in Table S1. *Fbox* was used as reference gene and $2^{-\Delta\Delta Ct}$ method was used in analysis of expression levels of the *GmNAC* genes [24]. The amplification efficiencies of 24 primer pairs in the shoot tissues (23 selected *GmNAC* and *Fbox* reference genes) were calculated using LinRegPCR software (version 2012.0).

In silico expression analysis

Expression data of *GmNAC* genes obtained under a wide variety of growth conditions, including biotic stress, alkaline stress and photoperiod change, as well as in different shoot growth stages, were extracted from Genevestigator (https://www.genevestigator.ethz.ch/) [25].

Statistical analysis

A *GmNAC* gene was considered as drought-responsive if its expression changed by at least twofold ($P < 0.05$) by drought treatment. For comparative expression analysis of *GmNAC*s between DT51 and MTD720, differential expression ratio with at least twofold was regarded as significant. Statistical significance of differential expression within a cultivar, or between two cultivars under either normal or drought treatment was assessed by Student's

Table 1 Expression of 23 *GmNAC* genes in shoots of DT51 and MTD720 upon 15-day-drought treatment

Nomenclature	Glyma ID	DT51	P-value	Regulation	MTD720	P-value	Regulation
GmNAC006	*Glyma02g07700.1*	**2.2**	**0.0015**	**Up**	2.0	0.1527	Unaltered
GmNAC011	*Glyma02g26480.1*	1.7	0.0959	Unaltered	5.1	0.0618	Unaltered
GmNAC019	*Glyma04g38990.1*	1.6	0.0693	Unaltered	2.1	0.0972	Unaltered
GmNAC038	*Glyma06g15990.1*	**2.9**	**0.0089**	**Up**	**7.9**	**0.0007**	**Up**
GmNAC043	*Glyma06g38410.1*	**4.1**	**0.0089**	**Up**	**3.0**	**0.0007**	**Up**
GmNAC044	*Glyma06g38440.1*	1.5	0.1412	Unaltered	**2.3**	**0.0233**	**Up**
GmNAC052	*Glyma07g35630.1*	1.1	0.4199	Unaltered	1.1	0.3683	Unaltered
GmNAC062	*Glyma08g19300.1*	1.0	0.4342	Unaltered	1.2	0.3108	Unaltered
GmNAC084	*Glyma12g22790.1*	1.3	0.1635	Unaltered	1.3	0.2505	Unaltered
GmNAC085	*Glyma12g22880.1*	**4.5**	**0.0021**	**Up**	**3.4**	**0.0002**	**Up**
GmNAC092	*Glyma12g35000.1*	**6.3**	**0.0431**	**Up**	2.7	0.1419	Unaltered
GmNAC095	*Glyma13g05540.1*	2.9	0.0602	Unaltered	1.9	0.0642	Unaltered
GmNAC099	*Glyma13g31660.1*	3.2	0.0524	Unaltered	**4.6**	**0.0470**	**Up**
GmNAC101	*Glyma13g35550.1*	**2.5**	**0.0334**	**Up**	**3.8**	**0.0045**	**Up**
GmNAC102	*Glyma13g35560.1*	1.4	0.4460	Unaltered	1.2	0.1617	Unaltered
GmNAC109	*Glyma14g24220.1*	1.5	0.1278	Unaltered	1.1	0.3267	Unaltered
GmNAC148	*Glyma20g04400.1*	1.3	0.2594	Unaltered	2.7	0.0539	Unaltered
GmNAC017	*Glyma04g33270.1*	1.4	0.2657	Unaltered	2.6	0.3150	Unaltered
GmNAC022	*Glyma04g42800.1*	1.4	0.0148	Unaltered	1.8	0.1157	Unaltered
GmNAC027	*Glyma05g24910.1*	**2.3**	**0.0151**	**Down**	2.6	0.1408	Unaltered
GmNAC071	*Glyma10g04350.1*	1.2	0.1454	Unaltered	**4.3**	**0.0329**	**Up**
GmNAC083	*Glyma12g13710.1*	**2.5**	**0.0438**	**Down**	4.6	0.0543	Unaltered
GmNAC113	*Glyma15g07620.1*	2.4	0.1834	Unaltered	**3.8**	**0.0255**	**Up**

The tested *GmNAC*s were gathered into dehydration-inducible (group A, gray) and dehydration-repressible (group B, white) groups according to [20]. Data in bold indicate gene regulation with statistically significant difference (≥2-fold up- or down-regulation, P value < 0.05)

t test (one tail, unpaired, equal variance) with the P-value < 0.05.

Results

In this study, we examined the potential contribution of *GmNAC* genes to shoot-related traits, which affect drought-tolerant capacity of soybean, by comparing differential expression of 23 drought-responsive *GmNAC* genes in shoots of two contrasting drought-responsive cultivars DT51 and MTD720. The contrasting drought-responsive phenotypes of DT51 (drought-tolerant) and MTD720 (drought-sensitive) were determined based on the shoot-related data and drought-tolerant index as summarized in Table S2 [22]. Seventeen (group A) up- and six (group B) down-regulated *GmNAC* genes, which exhibited the highest expression change in shoots and/or roots of soybean seedlings by dehydration [20], were selected for comparative expression analysis in shoots of unstressed and drought-stressed DT51 and MTD720 plants using RT-qPCR (Table S1). In DT51, out of 17 *GmNAC* genes of group A, we found 6 genes, including *GmNAC006*, *038*, *043*, *085*, *092* and *101*, which were induced more than twofold in the shoots under water deficit (Table 1). We recorded no significant alteration in expression of the remaining genes belonged to group A. As for the 6 *GmNAC* genes of group B, drought treatment significantly repressed the expression of two genes, *GmNAC027* and *083*, in DT51 shoots, but did not alter the expression of the 4 remaining *GmNAC* genes (Table 1).

In comparison with the drought-tolerant DT51, in the drought-sensitive MTD720, six genes of group A (*GmNAC038*, *043*, *044*, *085*, *099* and *101*) was up-regulated in shoots by drought stress (Table 1). Hence, four genes (*GmNAC038, 043, 085* and *101*) shared similar response in both cultivars upon the drought treatment. The expression of the rest of the *GmNAC* genes belonged to group A did not significantly alter in MTD720 shoots under drought stress. Regarding the *GmNAC* genes in group B,

Table 2 Comparison of the expression levels of 23 *GmNAC* genes in shoots of DT51 and MTD720

Nomenclature	Glyma ID	Shoots				Regulation	
		Normal	P-value	Drought	P-value	DT51	MTD720
GmNAC006	*Glyma02g07700.1*	**-4.2**	**0.0006**	-3.8	0.1005	Up	Unaltered
GmNAC027	*Glyma05g24910.1*	1.7	0.4138	-3.3	0.0709	Down	Unaltered
GmNAC038	*Glyma06g15990.1*	1.4	0.2310	**-2**	**0.0084**	Up	Up
GmNAC043	*Glyma06g38410.1*	**3.5**	**0.0496**	**4.9**	**0.0037**	Up	Up
GmNAC044	*Glyma06g38440.1*	1.6	0.0787	**-2.2**	**0.0399**	Unaltered	Up
GmNAC071	*Glyma10g04350.1*	**2.2**	**0.0090**	-2.4	0.0587	Unaltered	Up
GmNAC083	*Glyma12g13710.1*	**2.4**	**0.0395**	**-4.6**	**0.0050**	Down	Unaltered
GmNAC085	*Glyma12g22880.1*	**3.9**	**0.0414**	**5.2**	**0.0005**	Up	Up
GmNAC092	*Glyma12g35000.1*	1.9	0.1976	4.5	0.1113	Up	Unaltered
GmNAC099	*Glyma13g31660.1*	1.2	0.2631	**-11.9**	**0.0323**	Unaltered	Up
GmNAC101	*Glyma13g35550.1*	**5.6**	**0.0189**	**3.7**	**0.0113**	Up	Up
GmNAC113	*Glyma15g07620.1*	2.1	0.1204	**-4.4**	**0.0497**	Unaltered	Up
GmNAC011	*Glyma02g26480.1*	1.4	0.1681	-2.1	0.1003	Unaltered	Unaltered
GmNAC019	*Glyma04g38990.1*	**4.9**	**0.0049**	**3.8**	**0.0356**	Unaltered	Unaltered
GmNAC017	*Glyma04g33270.1*	1.9	0.3838	-1.9	0.1641	Unaltered	Unaltered
GmNAC022	*Glyma04g42800.1*	1.2	0.3044	1	0.3148	Unaltered	Unaltered
GmNAC052	*Glyma07g35630.1*	**-11**	**0.0010**	-9.3	0.0929	Unaltered	Unaltered
GmNAC062	*Glyma08g19300.1*	2.2	0.0647	**2.7**	**0.0240**	Unaltered	Unaltered
GmNAC084	*Glyma12g22790.1*	1	0.4669	-1.7	0.1480	Unaltered	Unaltered
GmNAC095	*Glyma13g05540.1*	**20.7**	**0.0254**	**3.9**	**0.0085**	Unaltered	Unaltered
GmNAC102	*Glyma13g35560.1*	-2.9	0.0579	-1.7	0.0717	Unaltered	Unaltered
GmNAC109	*Glyma14g24220.1*	-1.3	0.2892	1.2	0.0757	Unaltered	Unaltered
GmNAC148	*Glyma20g04400.1*	**3.4**	**0.0367**	1.6	0.1502	Unaltered	Unaltered

The tested *GmNAC*s were gathered into drought-responsive in DT51 and/or MTD720 (gray) and drought-unresponsive (white) groups according to the data from Table 1. The analyses were performed for data obtained at both normal and drought conditions. Data in bold indicate statistically significant differential expression ratios (at least 2-fold change, P value < 0.05) in DT51 versus MTD720 comparisons under normal or drought conditions. Lower expression levels in DT51 compared with MTD720 were specified by negative fold changes

data in Table 1 indicated that out of six *GmNAC*s, *GmNAC071* and *113* were significantly induced in the shoots of MTD720 by drought treatment, while the remaining four *GmNAC*s displayed insignificant change in drought-stressed MTD720 shoots.

The RT-qPCR data also allowed us to compare the expression levels of the selected *GmNAC* genes between DT51 and MTD720 shoots under the same conditions. Data analysis indicated that under normal growing condition, eight *GmNAC* genes (*GmNAC019*, *043*, *071*, *083*, *085*, *095*, *101* and *148*) had higher expression levels in DT51 shoots than in MTD720 shoots (Table 2). Meanwhile, only two genes, *GmNAC006* and *052*, exhibited significantly higher expression levels in MTD720 shoots relative to DT51 shoots (Table 2). Under drought stress, we also detected more *GmNAC* genes with greater expression levels in DT51 shoots than in MTD720 shoots. Specifically, six genes, namely *GmNAC019*, *043*, *062, 085*, *095* and *101*, expressed more highly in DT51 shoots relative to MTD720 shoots, whereas five genes, *GmNAC038*, *044*, *099*, *083* and *113*, accumulated higher amounts of transcript in MTD720 shoots in comparison with DT51 shoots (Table 2). These results together indicated that *GmNAC043*, *085* and *101* were induced by drought in both culivars and had higher induction levels in DT51 shoots than in MTD720 shoots under both normal and drought conditions.

In addition, in silico analysis using expression data housed in Genevestigator [25] demonstrated that among 23 examined *GmNAC*s, twelve genes, including *GmNAC011, 019, 022, 043, 052, 071, 085, 092, 101, 102, 109* and *148*, showed altered expression in soybean under various stressful conditions, such as biotic stress, alkaline stress, photoperiod change, and in different growth stages, suggesting their involvement in soybean responses to various types of stresses, as well as in regulation of soybean growth and development (Fig. S1).

Discussion

It is well established that breeding or genetic engineering for appropriate shoot-related traits could enhance tolerance of crops to drought [26]. NAC TFs have been shown to control many shoot-related traits in plant responses to drought. For example, in rice, *SNAC1* was induced mainly in guard cells by drought treatment, and its overexpression resulted in an increase in drought tolerance under field conditions which was associated with improved shoot-related traits. Specifically, transgenic *SNAC1* rice plants showed delayed leaf-rolling and reduced rate of water loss resulted from increased stomatal closure as compared with the wild-type plants [27]. In another research, drought tolerance of transgenic rice overexpressing *OsNAC6* was significantly enhanced at least by restricting shoot growth rate when only limited resource was available during stress [28]. Our previous morphological and physiological investigations demonstrated that the higher drought-tolerant degree of DT51 versus MTD720 was associated with improvement of both shoot-related and root-related traits [22], whose regulations are quite complex and involve many molecular and biochemical processes [26, 29]. In present study, to examine whether drought-responsive *GmNAC* genes might be involved in improvement of drought tolerance of DT51 versus MTD720 in association with shoot-related traits, we performed a differential expression analysis of the selected 23 drought-responsive *GmNAC* genes in DT51 and MTD720 under both well-watered and drought conditions. Our results revealed that there were more *GmNAC* genes induced by drought treatment in MTD720 than in DT51 (Table 1). This observation might indicate that more *GmNAC* genes should be induced and/or activated in response to stress in susceptible plants to enable them to adapt better to adverse environmental conditions.

In addition, we found that in the shoot tissues, there were eight and six *GmNAC* genes with higher expression levels in DT51 than in MTD720 under non-stressed and stressed conditions, respectively, whereas there were only two and four genes, respectively, exhibiting higher expression levels in MTD720 than in DT51 under well-watered and drought conditions (Table 2). This result implies that the higher number of *GmNAC* genes, which showed higher expression levels in the shoots of DT51 versus MTD720, under both normal and water deficit conditions, might contribute to better shoot-related traits of DT51, and thus higher drought-tolerant capacity, in comparison with MTD720 (Table S2). Thus, the results of our study suggest a positive correlation between the number of *GmNAC* genes, which showed higher expression levels, and the improved drought-tolerant degree, at least in the case of these two contrasting drought-responsive cultivars. In fact, this correlation was not only observed in shoots but also in roots of DT51 and MTD720 as shown by our previous study [23]. These two studies together have clearly shown that several *GmNAC* genes act in a tissue-specific manner and/or genotype-dependent under both normal and drought conditions [23]. Specifically, our results revealed that (i) the numbers of drought-inducible *GmNAC* genes in roots and shoots of each cultivar are different. In DT51, eleven genes (*GmNAC011*, *022*, *027*, *043*, *085*, *092*, *095*, *099*, *101*, *102* and *109*) were up-regulated in drought-treated roots, whereas only six genes showed induction in drought-stressed shoots (*GmNAC006*, *038*, *043*, *085*, *092* and *101*). In MTD720, nine genes (*GmNAC022*, *038*, *043*, *062*, *085*, *095*, *099*, *101* and *109*) and eight genes (*GmNAC038*, *043*, *044*, *071*, *085*, *099*, *101* and *113*) were up-regulated by drought in roots and shoots, respectively; (ii) in both two cultivars, several *GmNAC* genes were induced in shoots, but not in roots, and vice versa some *GmNAC*s are induced in roots but not in shoots by drought. For instance, *GmNAC011* was found to be induced in roots but not in shoots of DT51, whereas *GmNAC006* was of DT51 by water withholding treatment; (iii) different *GmNAC* genes were found to be repressed in shoots and roots of each cultivar by drought. For instance, only *GmNAC148* was down-regulated in drought-treated DT51 roots, whereas *GmNAC027* and *083* were repressed in drought-stressed DT51 shoots. On the other hand, eight drought-repressive genes (*GmNAC006*, *027*, *011*, *019*, *071*, *083*, *113* and *148*) were found in MTD720 roots, while none was found in MTD720 shoots during drought treatment; and (iv) our data also showed that the numbers of *GmNAC* genes, which exhibited higher expression levels in DT51 roots and shoots under both normal and drought conditions relative to MTD720, were different. Specifically, seven (*GmNAC017, 085, 092, 095, 101, 109* and *148)* and thirteen (*GmNAC006*, *011, 017, 019, 022, 027, 071, 083, 085, 092, 095, 101* and *109) GmNAC* genes displayed higher transcript levels in DT51 roots than in MTD720 roots under normal and drought treatments, respectively. On the other hand, with respect to well-watered and drought-treated shoots, eight (*GmNAC019, 043, 071, 083, 085, 095, 101* and *148)* and six (*GmNAC019, 043, 062, 085, 095* and *101) GmNAC* genes showed higher expression levels in DT51, respectively, than in MTD720.

Our findings revealed that the drought-responsive *GmNAC* genes might play particular role in regulating drought responses in different soybean cultivars that might have their unique defense strategies against drought. In agreement with this observation, a number of *GmNAC* genes also displayed different stress-responsive expression patterns in W82 [20] when compared with DT51 and/or MTD720 ([23] and this work). Alternatively, the difference in age of the plants and/or in treatment methods applied

might result in the difference in gene expression profile found in W82 versus DT51 and/or MTD720. In study of Le et al. [20], 12-day-old W82 seedlings were dehydrated on a filter paper for 10 h, whereas in our study, drought treatment was performed on 12-day-old soil-grown plants for a period of 15 days (thus, the age of the plants used in expression analysis in this study was 27-day-old) by withholding water. Taken together, our data suggested that drought-responsive expression of a significant number of *GmNAC* genes is genotype- and/or tissue-dependent.

Among the examined genes, three genes, *GmNAC043*, *085* and *101*, were in our particular interest since these genes showed (i) a similar up-regulation tendency in both cultivars under drought, and (ii) exhibited higher expression levels in DT51 shoots than in MTD720 shoots not only under stressed but also non-stressed conditions (Tables 1, 2). In a phylogenetic analysis, *GmNAC043* (previously named *GmNAC3*) was assigned to the stress-responsive NAC subgroup [6], containing three well-known positive regulators ANAC019, ANAC055 and ANAC072 that have been demonstrated to enhance drought tolerance in *Arabidopsis* transgenic plants [30]. *GmNAC085* is another attractive candidate gene, which encodes a protein sharing 39 % amino acid identity with SNAC1, one of the most well-known *NAC* genes subjected to field test in transgenic rice as previously discussed [27]. Additionally, a search in Genevestigator database indicated that all three genes *GmNAC043, 085* and *101* were inducible by various treatments, such as biotic stress, alkaline stress and photoperiod change, as well as showed development stage-related expression (Fig. S1). These findings suggest that these three genes play roles not only in soybean responses to drought but also to other kinds of stresses, broadening their potential use in genetic engineering for improved tolerance to various types of stresses.

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References

1. Tran LSP, Urao T, Qin F, Maruyama K, Kakimoto T, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of AHK1/ATHK1 and cytokinin receptor histidine kinases in response to abscisic acid, drought, and salt stress in *Arabidopsis*. Proc Natl Acad Sci USA 104(51):20623–20628
2. Nishiyama R, Watanabe Y, Leyva-Gonzalez MA, Van Ha C, Fujita Y, Tanaka M, Seki M, Yamaguchi-Shinozaki K, Shinozaki K, Herrera-Estrella L (2013) *Arabidopsis* AHP2, AHP3, and AHP5 histidine phosphotransfer proteins function as redundant negative regulators of drought stress response. Proc Natl Acad Sci USA 110(12):4840–4845
3. Thao NP, Tran L-SP (2012) Potentials toward genetic engineering of drought-tolerant soybean. Crit Rev Biotechnol 32(4):349–362
4. Ha CV, Le DT, Nishiyama R, Watanabe Y, Tran UT, Dong NV, Tran L-SP (2013) Characterization of the newly developed soybean cultivar DT2008 in relation to the model variety W82 reveals a new genetic resource for comparative and functional genomics for improved drought tolerance. Biomed Res Int 2013:1–8
5. Qin F, Shinozaki K, Yamaguchi-Shinozaki K (2011) Achievements and challenges in understanding plant abiotic stress responses and tolerance. Plant Cell Physiol 52(9):1569–1582
6. Pinheiro GL, Marques CS, Costa MDBL, Reis PAB, Alves MS, Carvalho CM, Fietto LG, Fontes EPB (2009) Complete inventory of soybean NAC transcription factors: sequence conservation and expression analysis uncover their distinct roles in stress response. Gene 444(1):10–23
7. Wang Z, Libault M, Joshi T, Valliyodan B, Nguyen H, Xu D, Stacey G, Cheng J (2010) SoyDB: a knowledge database of soybean transcription factors. BMC Plant Biol 10(1):14
8. Guo A-Y, Chen X, Gao G, Zhang H, Zhu Q-H, Liu X-C, Zhong Y-F, Gu X, He K, Luo J (2008) PlantTFDB: a comprehensive plant transcription factor database. Nucleic Acids Res 36(suppl 1):D966–D969
9. Mochida K, Yoshida T, Sakurai T, Yamaguchi-Shinozaki K, Shinozaki K, Tran L-SP (2009) In silico analysis of transcription factor repertoire and prediction of stress responsive transcription factors in soybean. DNA Res 16(6):353–369
10. Mochida K, Yoshida T, Sakurai T, Yamaguchi-Shinozaki K, Shinozaki K, Tran L-SP (2010) LegumeTFDB: an integrative database of *Glycine max*, *Lotus japonicus* and *Medicago truncatula* transcription factors. Bioinformatics 26(2):290–291
11. Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Cheng J (2010) Genome sequence of the palaeopolyploid soybean. Nature 463(7278):178–183
12. Souer E, van Houwelingen A, Kloos D, Mol J, Koes R (1996) The no apical meristem gene of petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. Cell 85(2):159–170
13. Nuruzzaman M, Manimekalai R, Sharoni AM, Satoh K, Kondoh H, Ooka H, Kikuchi S (2010) Genome-wide analysis of NAC transcription factor family in rice. Gene 465(1):30–44
14. Yoo SY, Kim Y, Kim SY, Lee JS, Ahn JH (2007) Control of flowering time and cold response by a NAC-domain protein in Arabidopsis. PLoS ONE 2(7):1–10
15. Zhong R, Richardson EA, Ye Z-H (2007) Two NAC domain transcription factors, SND1 and NST1, function redundantly in regulation of secondary wall synthesis in fibers of Arabidopsis. Planta 225(6):1603–1611
16. Olsen AN, Ernst HA, Leggio LL, Skriver K (2005) NAC transcription factors: structurally distinct, functionally diverse. Trends Plant Sci 10(2):79–87
17. Puranik S, Sahu PP, Srivastava PS, Prasad M (2012) NAC proteins: regulation and role in stress tolerance. Trends Plant Sci 17(6):369–381
18. Meng Q, Zhang C, Gai J, Yu D (2007) Molecular cloning, sequence characterization and tissue-specific expression of six NAC-like genes in soybean (*Glycine max* (L.) Merr.). J Plant Physiol 164(8):1002–1012
19. Tran LSP, Quach TN, Guttikonda SK, Aldrich DL, Kumar R, Neelakandan A, Valliyodan B, Nguyen HT (2009) Molecular characterization of stress-inducible GmNAC genes in soybean. Mol Genet Genomics 281(6):647–664

20. Le DT, Nishiyama R, Watanabe Y, Mochida K, Yamaguchi-Shinozaki K, Shinozaki K, Tran L-SP (2011) Genome-wide survey and expression analysis of the plant-specific NAC transcription factor family in soybean during development and dehydration stress. DNA Res 18(4):263–276
21. Le DT, Nishiyama R, Watanabe Y, Tanaka M, Seki M, Yamaguchi-Shinozaki K, Shinozaki K, Tran L-SP (2012) Differential gene expression in soybean leaf tissues at late developmental stages under drought stress revealed by genome-wide transcriptome analysis. PLoS ONE 7(11):1–10
22. Thu NBA, Nguyen QT, Hoang XLT, Thao NP, Tran LSP (2014) Evaluation of drought tolerance of the Vietnamese soybean cultivars provides potential resources for soybean production and genetic engineering. Biomed Res Int 2014:1–9
23. Thao NP, Thu NBA, Hoang XLT, Ha VC, Tran LSP (2013) Differential expression analysis of a subset of drought-responsive *GmNAC* genes in two soybean cultivars differing in drought tolerance. Int J Mol Sci 14(12):23828–23841
24. Le DT, Aldrich DL, Valliyodan B, Watanabe Y, Van Ha C, Nishiyama R, Guttikonda SK, Quach TN, Gutierrez-Gonzalez JJ, Tran L-SP (2012) Evaluation of candidate reference genes for normalization of quantitative RT-PCR in soybean tissues under various abiotic stress conditions. PLoS ONE 7(9):1–10
25. Hruz T, Laule O, Szabo G, Wessendorp F, Bleuler S, Oertle L, Widmayer P, Gruissem W, Zimmermann P (2008) Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. Adv Bioinform 2008:420747–420752
26. Manavalan LP, Guttikonda SK, Tran L-SP, Nguyen HT (2009) Physiological and molecular approaches to improve drought resistance in soybean. Plant Cell Physiol 50(7):1260–1276
27. Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. Proc Natl Acad Sci USA 103(35):12987–12992
28. Nakashima K, Tran L-SP, Van Nguyen D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. Plant J 51(4):617–630
29. Wu Y, Cosgrove DJ (2000) Adaptation of roots to low water potentials by changes in cell wall extensibility and cell wall proteins. J Exp Bot 51(350):1543–1553
30. Tran L-SP, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Isolation and functional analysis of Arabidopsis stress-inducible NAC transcription factors that bind to a drought-responsive cis-element in the early responsive to dehydration stress 1 promoter. Plant Cell 16(9):2481–2498