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Molecular characterization of stress-inducible *PLATZ* gene from soybean (*Glycine max* L.)

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Abstract

PLATZ (plant AT-rich sequence and zinc-binding protein) is a novel class of DNA-binding proteins; however, the function of the *PLATZ* gene has not yet been identified in plants. This study aims to isolate, sequence, and analyse the *PLATZ* gene responsive to abiotic stress in soybean. We isolated a stress-inducible gene encoding the PLATZ from soybean (*Glycine max* L.). This gene, designated as *GmPLATZ1*, was specifically induced by drought, high salinity, or abscisic acid (ABA) in soybean. *GmPLATZ1* cDNA is composed of 711 bp nucleotide sequences encoding a PLATZ protein (236 amino acids) with 9.41 pI and 26.75 kDa. Multiple sequence alignment analysis showed that the N-terminal region of GmPLATZ1 shares the highly conserved zinc-finger motifs with other PLATZ proteins. The subcellular localization of the GmPLATZ1 protein was analysed via the green fluorescent protein (GFP)-GmPLATZ1 fusion protein in tobacco plant cell. The GFP-GmPLATZ1 protein was shown to be targeted to the nucleus. The function of the *GmPLATZ1* gene was further investigated using the overexpression transgenic *Arabidopsis*. The germination rate in transgenic plants overexpressing *GmPLATZ1* was significantly delayed in media supplemented with mannitol compared with that of wild-type (WT) plants. Moreover, cotyledon development in the *GmPLATZ1*-overexpression transgenic plants was remarkably retarded in the presence of ABA compared to WT. This shows that *GmPLATZ1* is implicated in developmental processes such as germination under osmotic stress conditions in plants.

Keywords: Abiotic stress; *Arabidopsis thaliana*, expression patterns, *Glycine max*, PLATZ, overexpression transgenic plant.
Abbreviations: ABA_abscisic acid; GFP_green fluorescent protein, PLATZ_plant AT-rich sequence and zinc-binding protein; WT_wild-type; ZF_zinc finger.

Introduction

Plants have evolved to cope with adverse environmental responses. Recently, many studies have aimed to understand the molecular mechanism of tolerance to abiotic stress responses. Among abiotic stresses, osmotic stress significantly affects crop yield loss. During osmotic stress responses, the biosynthesis of abscisic acid (ABA) increases as an adaptive response in plant. ABA is involved in seed development, germination, and responses to osmotic stresses including drought, high salinity, or low temperature. ABA induces stomatal closure, root development, and gene expression during osmotic stress responses. Gain-of-function or loss-of-function approaches have made it possible to identify the function of the genes involved in stress tolerance responses in plants. Nonetheless, the functions of most genes have not yet been identified in terms of the stress response mechanism in plants.

Zinc finger (ZF) proteins play a critical role in many cellular functions, including transcriptional regulation, RNA binding, apoptosis regulation, and protein-protein interactions (Ciftci-Yilmaz and Mittler, 2008; Sanchez-Garcia and Rabbitts, 1994; Takatsuji, 1998). Distinct ZF motifs composed of Cys and/or His coordinate a zinc atom(s) and form local peptide structures for their specific functions. They are classified into several different types including C2H2, C2C2, C2HC, C2C2C2C2, and C2HCC2C2 based on the number and order of the Cys and His residues essential for the zinc-binding secondary structure (Ciftci-Yilmaz and Mittler, 2008; Sanchez-Garcia and Rabbitts, 1994; Takatsuji, 1998). ZF motifs are present in many transcription factors (TFs) and play critical roles in interactions with other molecules. Some classes of ZF motifs, including TFIIIA and GATA types, represent DNA-binding domains of TFs and have been shown to be directly involved in the recognition of specific DNA sequences (Takatsuji, 1998). Other classes such as LIM- and RING-finger types are mostly implicated in protein-protein interactions (Takatsuji, 1998). In addition, some novel motifs such as WRKY and Dof motifs have been identified in plants. However, despite progress in this field, the functions of most plant ZF TFs remain poorly understood in relation to the stress tolerance response in plants.

Pea PLATZ1 (plant A/T-rich sequence and zinc-binding protein 1) was first identified as a novel class of zinc-dependent DNA-binding proteins (Nagano et al., 2001). PLATZ contains two distantly located regions, C-x2-H-x11-C-x2-C-x(4-5)-C-x2-C-x(3-7)-H-x2-H and C-x2-C-x(10-11)-C-x3-C (Nagano et al., 2001). Pea PLATZ1 protein was shown to bind to the A/T-rich DNA sequence upstream of the DE1 element and to be responsible for A/T-rich sequence-mediated transcriptional repression (Nagano et al., 2001). The 12-bp *cis*-regulatory element DE1 was necessary for light downregulated expression of the pea *pra2* gene, which is a dark-inducible and light-repressible small GTPase that is mainly expressed in the growing zone of pea epicotyls (Inaba et al., 1999; Nagano et al., 1995). However, the function of *PLATZ* in plants has not yet been elucidated. Soybean (*Glycine max*) is one of the major crop plants grown worldwide. It has attracted considerable attention because of its high value in the agricultural and food industries. Once the soybean genome was sequenced via a

whole-genome shotgun approach (Schmutz et al., 2010), soybean transcription factors (TFs) were made available online (http://soybeantfdb.psc.riken.jp/; Schmutz et al., 2010; Wang et al., 2010). We were interested in the functional analysis of the TFs involved in the abiotic stress response in soybean. In this study, we identified a soybean *GmPLATZ1* (*G. max PLATZ1*) and analysed its RNA expressions under various abiotic stresses. Furthermore, we investigated germination processes altered by the ectopic expression of *GmPLATZ1* under osmotic stress conditions in Arabidopsis.

Results and Discussion

Expression analyses of soybean GmPLATZ1 induced by abiotic stresses

Based on a search of the soybean TF database website (http://soybeantfdb.psc.riken.jp/), 36 C2C2-type ZFs having an ABA-responsive *cis*-acting DNA element (ABRE) or drought-responsive *cis*-acting DNA element (DREB) in the upstream promoter region were further investigated for their RNA expression under drought stress conditions (data not shown). Among these genes, the RNA expression of a C2C2 gene encoding *GmPLATZ1* (*G. max PLATZ1*) was shown to be responsive to drought stress (Fig. 1A). Moreover, the transcription levels of *GmPLATZ1* were upregulated by high salinity or abscisic acid (ABA) treatment (Fig. 1A). Real-time quantitative RT-PCR analysis was performed to compare the time-course expression for *GmPLATZ1* genes with high salinity stress, ABA, and drought treatments (Fig. 1B). The RNA level of *GmPLATZ1* peaked 24 h after the high salinity treatment; however, the expression level during high salinity stress was quite lower than those under ABA or drought stress conditions (Fig. 1B). Exogenous ABA application resulted in a dramatic increase in the RNA expression of *GmPLATZ1* at 24 h (Fig. 1B). Under drought stress conditions, the mRNA level of *GmPLATZ1* steadily increased up to the maximum level 24 h after the stress (Fig. 1B).

Sequence analyses and multiple alignment of soybean GmPLATZ1

The full-length cDNA of *GmPLATZ1* was cloned, and its predicted amino acid sequences were analysed. *GmPLATZ1* cDNA is composed of 711 bp nucleotide sequences encoding a PLATZ protein (236 amino acids) with 9.41 pI and 26.75 kDa. Multiple alignments of GmPLATZ1 and other PLATZ proteins were carried out by T-Coffee (Fig. 2A). From the multiple alignment of PLATZ protein sequences, we observed a highly conserved region containing the zinc binding motif (Fig. 2A). Overall, the alignment shows that PLATZ proteins are alike with high homologies between 87% and 68% (Fig. 2A). Rice PLATZ protein contains a Gly-rich region between 173 and 194 aa, which is not found in other PLATZ proteins; this suggests that dicot PLATZ proteins may be divergent from monocot ones (Fig. 2A). We observed two putative NLSs (nuclear localization signals) rich in basic amino acids (K or R), implying that PLATZ proteins are predicted to be localized to the nucleus (Fig. 2A). As seen in Fig. 2A, the PLATZ protein is characterized by two putative zinc finger regions including 'C-x2-H-x11-C-x2-C-x(4-5)-C-x2-C-x(3-7)-H-x2-H' located in the N-terminal region and 'C-x2-C-x(10-11)-C-x3-C', in the central region. Both regions were shown to be required for DNA binding activities dependent on zinc ion binding (Nagano et al., 2001).

A phylogenetic tree was constructed using the MEGA6 neighbour-joining method, using multiple alignments of GmPLATZ1 and its homologues with a bootstrap analysis of

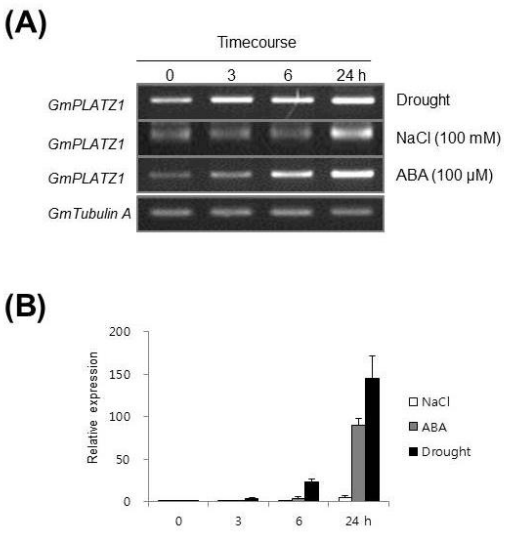

Fig 1. RNA expression analyses of soybean *GmPLATZ1* gene in response to dehydration, high salt or ABA treatment. (A) RT-PCR analyses were carried out for expression patterns of *GmPLATZ1* gene in soybean. Soybean plants were subjected to dehydration, high salt (100 mM NaCl) or ABA (100 μM) application, respectively. Each PCR product was analyzed on the agarose gel electrophoresis along with that of the housekeeping gene, *GmTubulin A*. (B) Real-time quantitative RT-PCR analyses of *GmPLATZ1* gene under the abiotic stress conditions as described above. Transcript levels of *GmPLATZ1* were quantified by real-time RT-PCR against those of *GmTubulin A*. The expression level was assigned a value of 1 under the unstressed condition. Each value is the mean ± SD of three independent experiments.

1,000 replicates to ensure the statistical reliability (Fig. 2B). In general, the phylogenetic analysis showed that the dicotyledonous PLATZ proteins are more distant from the monocotyledonous ones (Fig. 2B).

Subcellular localization of GFP-GmPLATZ1 fusion protein

The domain composed of basic amino acids (K or R) in the N-termini of GmPLATZ1 was predicted for nuclear localization (PSORT, http://psort.hgc.jp/form.html) (Fig. 2A). To test the nuclear targeting of GmPLATZ1 based on the prediction, the subcellular localization of GmPLATZ1 was examined by expressing the fusion protein with GFP in tobacco (Fig. 3). *Agrobacterium tumefaciens* cells transformed with DNA construct carrying GFP-GmPLATZ1 and GFP under the control of the 35S CaMV promoter were introduced into tobacco plant (Fig. 3). As shown in Fig. 3, the GFP-only controls were distributed uniformly throughout the whole cell, whereas the fusion protein of GmPLATZ1 was restricted to the nucleus (Fig. 3). This indicates that *GmPLATZ1* plays a role in the nucleus.

Retarded germination in GmPLATZ1-overexpression transgenic Arabidopsis in response to ABA and osmotic stress

To better understand the function of *GmPLATZ1* TF in plants, we attempted to generate overexpression (OE) transgenic Arabidopsis plants. Above all, transgenic Arabidopsis plants overexpressing *GmPLATZ1* were obtained, and their functional studies were described in this paper. *GmPLATZ1* OE construct driven by 35S CaMV promoter was transformed to Arabidopsis (Fig. 4A), and we were able to confirm that three OE plants

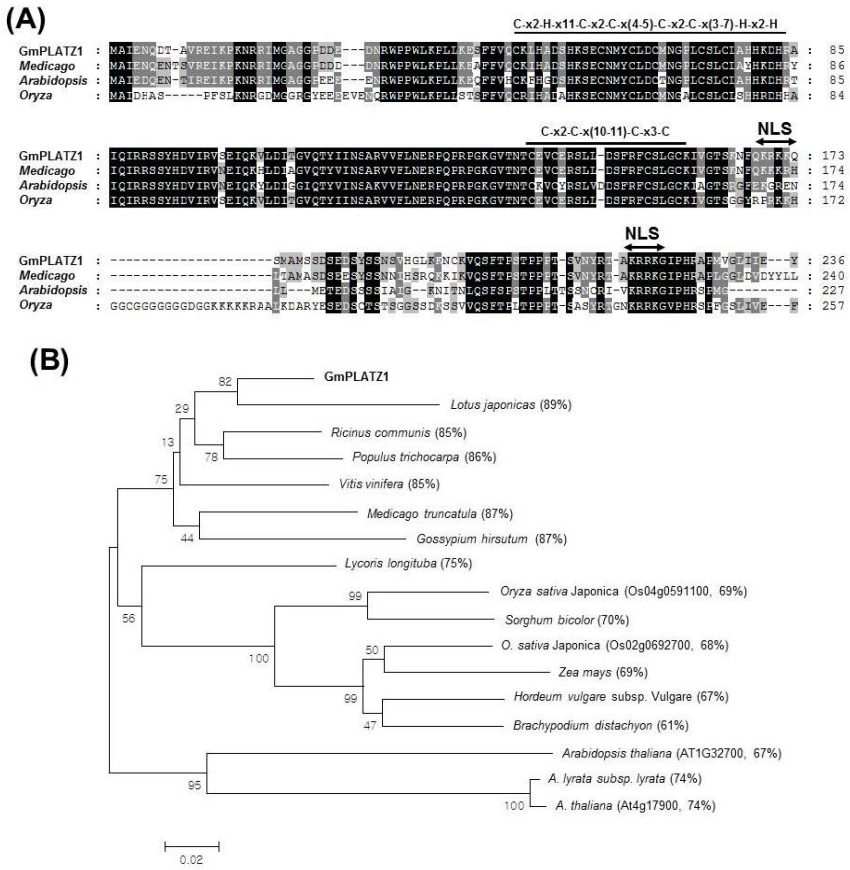

Fig 2. Multiple sequence alignment and neighbor-joining phylogenetic tree for GmPLATZ1 and its homologues from other plants. (A) Multiple sequence alignments of GmPLATZ1 and PLATZ proteins from other plants. The multiple alignment results clearly show the highly conserved DBD domains among maize *NAC* genes. Proteins were aligned using CLUSTALW at the T-coffee website. Protein sequences were as follows; *Medicago truncatula* (ACJ84547), *Arabidopsis thaliana* (ABK32199, At4g17900), *Oryza sativa* Japonica (NP_001047798, Os02g0692700). (B) The neighbor-joining phylogenetic tree was constructed with MEGA6.0, was generated using the amino acid sequences coding for the entire protein from soybean *GmPLATZ1* gene and other *PLATZ* genes. PLATZ sequences from other plants were retrieved from databases and were used to construct the tree. The confidence level of monophyletic groups was estimated by bootstrap analysis of 1000 replicates. Bootstrap values are shown for each node based on a bootstrap analysis of 1,000 replicates. Protein sequences were as follows; *M. truncatula* (ACJ84547), *Vitis vinifera* (CBI33878), *Ricinus communis* (EEF34742), *Lotus japonicas* (AFK44214), *Populus trichocarpa* (EEE72327), *Gossypium hirsutum* (AFH57272), *A. lyrata* subsp. Lyrata (EFH46337), *Lycoris longituba* (ADG58020), *A. thaliana* (ABK32199, At4g17900; AEE31518, AT1G32700), *O. sativa* Japonica (NP_001047798, Os02g0692700; NP_001053711, Os04g0591100), *Zea mays* (NP_001183696), *Sorghum bicolor* (EES12755), *Hordeum vulgare* subsp. Vulgare (BAJ90368), *Brachypodium distachyon* (XP_003570139).

constitutively express *GmPLATZ1* transcript (Fig. 4B). Homozygous T4 transgenic seeds were used for their phenotype and germination test. The germination rates of the OE lines were examined compared to those of WT in either normal MS media or supplemented with ABA or mannitol (Fig. 4C). In the presence of ABA in the media, the OE transgenic seeds germinated more slowly than the WT seeds during the period between 2 and 4 DAG (Fig. 4C). In the presence of osmoticum such as mannitol in the media, the germination rates of OE plants were much lower between 3 and 5 DAG compared to those of WT (Fig. 4C). These results indicate that the ectopic expression of *GmPLATZ1* may be involved in the repression of germination during the early germination process, especially with the addition of mannitol in Arabidopsis.

A number of genetic studies showed that ABA is essential for the developmental arrest process (Gazzarrini and McCourt, 2001). To evaluate the effect of *GmPLATZ1* overexpression on the cotyledon development by ABA, *GmPLATZ1*-overexpressed plants were germinated on the media containing ABA (Fig. 5A). On a medium containing 1.0 μM ABA, the shoot growth of the WT plants was much higher compared with that of the *GmPLATZ1* OE transgenic lines (Fig. 5A). Exposure to different concentrations of ABA (either 1 or 2.5 μM) led to a significant reduction in cotyledon development in the *GmPLATZ1* OE transgenic lines compared to the WT (Fig. 5B). In the presence of ABA (1 μM), the cotyledon greening efficiency of WT seedlings (81%) was significantly higher than those of the *GmPLATZ1* OE transgenic lines (47.8%, 65.3%, 68.7%) (Fig. 5B). At a higher concentration of ABA (2.5 μM), there was a significantly higher difference in the cotyledon greening efficiency of WT (9%) compared with those of the *GmPLATZ1* OE transgenic lines (1.2%, 2.5%, 1.4%) (Fig. 5B). These results indicate that *GmPLATZ1* is involved in early developmental processes such as germination and cotyledon development under ABA or osmotic stress conditions.

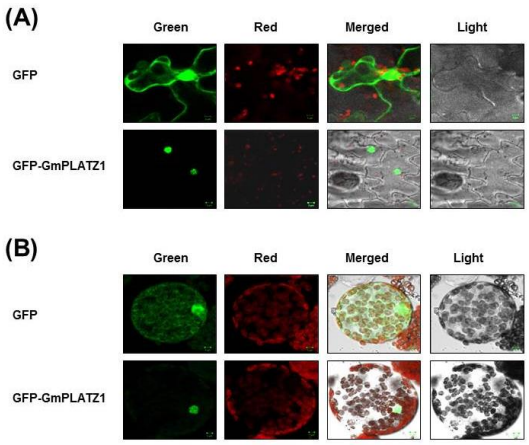

Fig 3. Subcellular localization of GFP-GmPLATZ1 fusion protein in the tobacco epidermis (A) and protoplast (B). GFP-GmPLATZ1 was transiently expressed in *Nicotiana benthamiana* using an *Agrobacterium*-mediated transformation method. The leaf epidermis and isolated protoplasts were observed with a Zeiss LSM700 confocal microscope 2 days after Agro-infiltration. The scale bar represents 5 μm. For each panel, light represents the actual image, the red channel represents chloroplast fluorescence, and the green channel represents GFP fluorescence. The merged image is combined with the light, red and green images.

A number of ZF proteins were shown to be involved in environmental stress responses (Cifcti-Yilmaz et al., 2007; Huang et al., 2009; Li et al., 2010; Luo et al., 2012; Mittler et al., 2006; Sakamoto et al., 2000, 2004). The plant ZF proteins are thought to recognize target sequences and to regulate gene expression in a plant-specific manner (Takatsuji, 1999; Sakamoto et al., 2004). In this study, we tried to analyse the function of *GmPLATZ1* responsive to abiotic stress. In the mature stages, overexpression of *GmPLATZ1* did not significantly contribute to improved tolerance in response to drought, high salinity, or freezing stress tolerance (data not shown). The role of *GmPLATZ1* is not clear in response to abiotic stress in mature plants. The effect of *GmPLATZ1* overexpression in the mature tissues may not be as strong as in the early development stage. It may be possible that the role of *GmPLATZ1* can be different depending on the tissue types or developmental stages in plants. Phylogenetic analysis shows that GmPLATZ1 is homologous to Arabidopsis PLATZ genes (At4g17900, At1g32700) (Fig. 2B). As *in silico* expression data is available for these two genes, the RNA expression patterns of these two genes were investigated using eFP browser or AtGenExpression Visualization Tool database (Supplementary Table S1-S3). It was shown that the RNA level of these genes is highly abundant in the root, cotyledon, and senescing leaf tissues (Supplementary Table S1). In response to abiotic stress treatments, the RNA expression of Arabidopsis PLATZ genes was induced by low temperature, osmosis, high salinity, drought, or UV in either shoots or roots (Supplementary Tables S2 and S3). In the case of pea *PLATZ1*, the RNA level is more abundant in the root tip and terminal buds rather than in the mature leaf, stem, and root tissues (Nagano et al., 2001). As the root tip and terminal buds are characteristic of the active cell division region in plants, it is speculated that *PLATZ1* may be related to cell-cycle regulation in cells undergoing cell division (Nagano et al., 2001).

Materials and Methods

Plant materials, growth conditions and stress treatments

Soybean (*Glycine max* L. Gwangan) and *Nicotiana benthamiana* were grown in a in a growth chamber at 28°C/24°C with a photoperiod of 16 h at a photosynthetic flux of 70 μmol photons m^{-2} s^{-1}. Soybean plants (3-4 week-old) were treated with dehydration, wounding, high salinity stress (100 mM NaCl), and with abscisic acid (ABA) (100 μM) solution as described previously (Chung et al., 2013). After the treatments, the leaves were taken for the sampling and were frozen in liquid nitrogen and stored at 75ºC. Arabidopsis (*Arabidopsis thaliana* ecotype Columbia, Col-0) and transgenic plants were grown at 22°C under long-day conditions (16 h light/8 h dark) or short-day conditions (8 h light /16 h dark).

Database search, multiple sequence alignment, and phylogenetic analysis for PLATZ proteins

Soybean Transcription Factor Database website (http://soybeantfdb.psc.riken.jp/) was utilized to retrieve 36 C2C2-type zinc finger transcription factors, of which promoter regions contain any ABA responsive *cis*-acting element (ABRE) or drought-responsive *cis*-acing element (DREB). Alignments of protein sequences were performed by the ClustalW program (Higgins et al., 1996) with default parameters. The neighbor-joining tree was constructed based on the obtained sequences of GmPLATZ1 and their most homologous PLATZ proteins from other plants. Phylogenetic analysis was based on the neighbor-joining tree using the MEGA6.0 web-based alignment program, with 1000 bootstrap replications (http://align.bmr.kyushu-u.ac.jp/mafft/online/server/index.html).

RT-PCR and quantitative real-time RT-PCR analyses

Total RNA was isolated from the samples using an RNA extraction kit (Ambion, Republic of Korea) and treated with DNase I (Promgea, USA). First strand cDNA was synthesized using 2 μg of total RNA, oligo d(T) primer, and M-MLV reverse transcriptase (Invitrogen, USA) according to the manufacturer instructions. Samples from each reaction (1 μL) were used in a 20-μL premix PCR mixture containing *Taq* polymerase (Bioneer, Republic of Korea), with gene-specific primers: GmTublin, F-GmTublin (5'-TTCTCCATTATTCAA-ACTGT-3') and R-GmTublin (5'-CACCAAAATAGAAGCA-TAAT-3'); GmPLATZ1, F-GmPLATZ1 (5'-TCCGCTTCTGT-TCTCTTGGT-3') and R-GmPLATZ1 (5'-TCTTCTCTTGG-CCGTTCTGT-3'). For real-time RT-PCR, amplification was performed for 26 cycles at 94ºC for 30 s, 55ºC for 30 s, and 72ºC for 30 s. PCR product was analyzed by agarose gel electrophoresis and staining with ethidium bromide. Quantitative real-time PCR was performed for 40 cycles using 1 μL cDNA as a template and CFX-96^{TM} Real Time system with SYBR Premix (Bio-Rad, USA). Real-time RT-PCR data were analyzed with CFX Manager v2.1 software (Bio-Rad). Data was normalized to *Tubulin* mRNA levels. All the primers used for RT-PCR analysis are listed as above.

Construct and subcellular localization of GFP fusion protein

The entire ORF (open reading frame) of *GmPLATZ1* was PCR-amplified with primers such as 5-GmPLATZ1 (5'-CACCATGGCAATTGAAAACCAAGA) and 3-GmPLATZ1 (5'-CTAATATTCTATGATTAGTCCCACCATTGGG-3') and the PCR products were cloned to the pENTR-TOPO vector

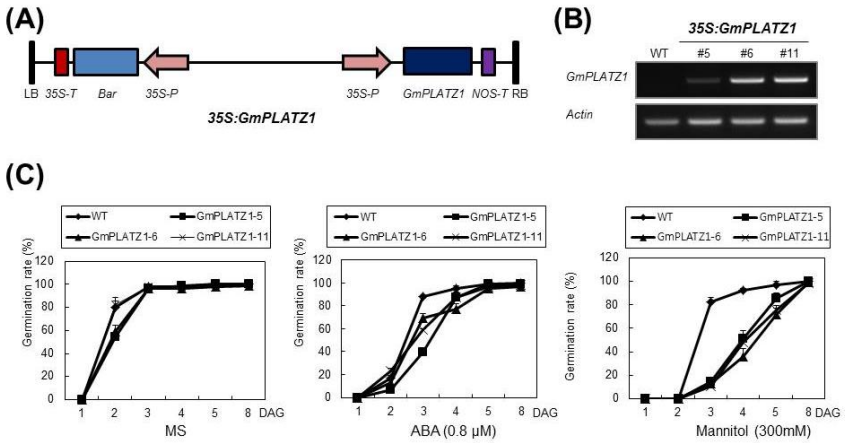

Fig 4. Delayed germination rates under the osmotic stress conditions by the ectopic expression of GmPLATZ1 in Arabidopsis. (A) Schematic representation of the overexpression construct of GmPLATZ1. (B) RT-PCR analysis of GmPLATZ1 in the WT and the 35S:GmPLATZ1 overexpression plants. It was shown that GmPLATZ1 was constitutively expressed in the overexpression transgenic plant. (C) Seed germination rates of the WT and GmPLATZ1 overexpression lines were analyzed in the media supplemented with ABA (0.8 μM) or mannitol (300 mM). Seeds were germinated on MS medium containing ABA (0.8 μM), or mannitol (300 mM) and were incubated at 4ºC for the stratification (3 d). Seed germination percentage of the indicated lines was recorded at 3 d after the end of stratification. Data show the mean ± SD of three replicates. At least 100 seeds per line were measured in each replicate.

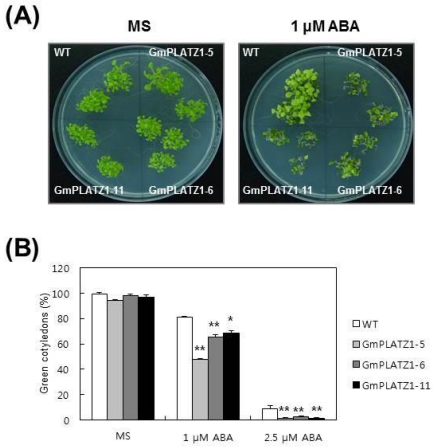

Fig 5. ABA sensitivities of *35S:GmPLATZ1* plants. (A) Photographs of 14-day-old seedlings. Seeds were germinated on MS medium with or without ABA (1 μM) and were incubated at 4ºC for the stratification. (B) Seeds were germinated and grown on MS medium containing ABA (1 or 2.5 μM) for 12 days, and seedlings with green cotyledons were counted (triplicates, n = 50 each). Asterisks indicate the significance of the difference from the values between the WT and the *35S:GmPLATZ1* Arabidopsis transgenic plants as determined by Student's *t* test (* $0.01 \leq P < 0.05$, ** $P < 0.01$).

(Invitrogen, USA). After its sequencing confirmation, pENTR-GmPLATZ1 was then recombined as a C-terminal fusion of GFP into the Gateway destination binary vector, pK7FGW2 (Plant Systems Biology, Belgium; http://www.psb.ugent.be/) yielding *35S:GFP-GmPLATZ1* by a LR recombination reaction. The GFP fusion construct was transformed into *Agrobacterium* sp. strain C58c1, respectively. For the transient expression of GFP proteins in plant, the positive transformants were inoculated into the leaves of *benthamiana* respectively as described previously (Chung et al., 2004). The protoplasts were isolated from the leaves as described by Abel and Theologis (1994). A Zeiss LSM700 (Zeiss, Germany) confocal microscope was used to observe the fluorescence as described previously (Chung et al., 2009).

Transformation vectors and construction of transgenic plants

To produce the *35S:GmPLATZ1* transgenic plants, the ORF region of *Gm*PLATZ1 (236 aa) of pENTR-GmPLATZ1 was cloned into the pENTR vector (Invitrogen) and then recombined into the gateway destination binary vector, pB7WG2D (Plant Systems Biology, Belgium; http://www.psb.ugent.be/), in which transgene expression is under the control for the CaMV 35S promoter. Transformation of Arabidopsis was performed by the vacuum infiltration method using *Agrobacterium tumefaciens* strain C58c1 (Abel and Theologis, 1994). Transgenic plants were sprayed with basta and the resistant plants were transferred to soil to obtain homozygous T4 seeds. For the phenotypic analysis, T4 homozygous lines were used.

Germination tests

Each plant was grown in the same conditions, and seeds were collected at the same time. Germination (full emergence of radicles) of the WT and the *35S:GmPLATZ1* transgenic seeds was scored on MS medium (2% Suc and 0.8% agar) without or with different concentrations of ABA (0.8 μM), or mannitol (300 mM) as indicated. Plates were chilled at 4ºC in the dark

for 3 days (stratified) and moved to 22°C with a 16-h-light/8-h-dark cycle. The percentage of seed germination was scored after 3-day stratification with 3 repetitions. The numbers of green cotyledons were counted with the WT and the *35S:GmPLATZ1* transgenic seedlings 12 days after germination without ABA or in the presence of ABA (1 or 2.5 μM). This experiment was carried out at least 3 replications.

Data analysis

Three independent biological replicates were performed in all experiments. Data were analyzed using Student's t-test with significance being defined as $P \leq 0.05$, or $P \leq 0.01$ and values represent the mean of three biological replicates.

Conclusion

In conclusion, this study presents molecular characterization of soybean *GmPLATZ1* gene upregulated by abiotic stresses such as drought, salt or ABA applications. Based on the structural characteristics and the phylogenetic relationships of soybean GmPLATZ1 and other proteins, PLATZ proteins share the highly conserved region containing the zinc binding motif. Based on GFP-fusion expression system in plant, GmPLATZ1 was localized in the nucleus. Constitutive expression of *GmPLATZ1* led to the significant changes such as delayed germination rates and cotyledon development during early development stages, but we could not find any significant improved in osmotic stress tolerance in mature Arabidopsis. As for now, it is presumed that *GmPLATZ1* is more likely to be involved in the germination process under osmotic conditions.

Acknowledgements

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References

Abel S, Theologis A (1994) Transient transformation of *Arabidopsis* leaf protoplasts: a versatile system to study gene expression. Plant J. 5: 421–427.

Chung E, Seong E, Kim Y-C, Chung EJ, Oh S-K, Lee S, Park JM, Joung YH, Choi D (2004) A method of high frequency virus-induced gene silencing in chili pepper (*Capsicum annum* L. cv. Bukang). Mol Cells. 17: 377–380.

Chung E, Cho CW, Yun BH, Choi HK, So HA, Lee SW, Lee J-H (2009) Molecular cloning and characterization of the soybean DEAD-box RNA helicase gene induced by low temperature stress. Gene. 443: 91–99.

Chung E, Cho C-W, So H-A, Kang J-S, Chung YS, Lee J-H (2013) Overexpression of *VrUBC1*, a mung bean E2 ubiquitin-conjugating enzyme, enhances osmotic stress tolerance in *Arabidopsis*. PLoS One. 8: e66056.

Ciftci-Yilmaz S, Mittler R (2008) The zinc finger network of plants. Cell Mol Life Sci. 65: 1150–1160.

Ciftci-Yilmaz S, Morsy MR, Song L, Coutu A, Krizek BA, Lewis MW, Warren D, Cushman J, Connolly EL, Mittler R (2007) The EAR-motif of the Cys2/His2-type zinc finger protein Zat7 plays a key role in the defense response of Arabidopsis to salinity stress. J Biol Chem. 282: 9260–9268.

Gazzarrini S, McCourt P (2001) Genetic interactions between ABA, ethylene and sugar signaling pathways. Curr Opin Plant Biol. 4: 387–391.

Higgins DG, Thompson JD, Gibson TJ (1996) Using CLUSTAL for multiple sequence alignments. Methods Enzymol. 266: 383–402.

Huang X, Chao D, Gao J, Zhu M, Shi M, Lin H (2009) A previously unknown zinc finger protein, DST, regulates drought and salt tolerance in rice via stomatal aperture control. Genes Dev. 23: 1805–1817.

Inaba T, Nagano Y, Sakakibara T, Sasaki Y (1999) Identification of a cis-regulatory element involved in phytochrome down-regulated Expression of the pea small GTPase gene pra2. Plant Physiol. 12: 491–499.

Li S, Xu CH, Yang YN, Xia GM (2010) Functional analysis of TaDi19A, a salt-responsive gene in wheat. Plant Cell Environ. 33: 117–129.

Luo X, Cui N, Zhu Y, Cao L, Zhai H, Cai H, Ji W, Wang X, Zhu P, Li Y et al. (2012) Over expression of CsZFP1, an ABA-responsive C2H2-type zinc finger protein lacking a QALGGH motif, reduces ABA sensitivity and decreases stomata size. J Plant Physiol. 169: 1192–1202.

Mittler R, Kim Y, Song L, Coutu J, Coutu A, Ciftci-Yilmaz S, Lee H, Stevenson B, Zhu JK (2006) Gain- and loss-of-function mutations in Zat10 enhance the tolerance of plants to abiotic stress. FEBS Lett. 580: 6537–6542.

Nagano Y, Okada Y, Narita H, Asaka Y, Sasaki Y (1995) Location of light-repressible, small GTP-binding protein of the YPT/rab family in the growing zone of etiolated pea stems. Proc Natl Acad Sci USA. 92: 6314–6318.

Nagano Y, Furuhashi H, Inaba T, Sasaki Y (2001) A novel class of plant-specific zinc-dependent DNA-binding protein that binds to A/T-rich DNA sequences. Nucleic Acids Res. 29: 4097–4105.

Sakamoto H, Araki T, Meshi T, Iwabuchi M (2000) Expression of a subset of the Arabidopsis Cys(2)/His(2)-type zinc-finger protein gene family under water stress. Gene. 248: 23–32.

Sakamoto H, Maruyama K, Sakuma Y, Meshi T, Iwabuchi M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Arabidopsis Cys2/His2-type zinc finger proteins function as transcription repressors under drought, cold, and high-salinity stress conditions. Plant Physiol. 136: 2734–2746.

Sanchez-Garcia I, Rabbitts TH (1994) The LIM domain: A new structural motif found in zinc-finger-like proteins. Trends Genet. 10: 315–320.

Schmutz J, Cannon SB, Schlueter J, Ma J, Mitros T, Nelson W, Hyten DL, Song Q, Thelen JJ, Chen J et al. (2010) Genome sequence of the palaeopolyploid soybean. Nature. 463: 178–183.

Takatsuji H (1998) Zinc-finger transcription factors in plants. Cell Mol Life Sci. 54: 582–596.

Takatsuji H (1999) Zinc-finger proteins: the classical zinc finger emerges in contemporary plant science. Plant Mol Biol. 39: 1073–1078.

Wang Z, Libault M, Joshi T, Valliyodan B, Nguyen HT, Xu D, Stacey G, Cheng J (2010) Soy DB: a knowledge database of soybean transcription factors. BMC Plant Biol. 10: 14.