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ORIGINAL ARTICLE

Overexpression of *AtYUCCA6* in soybean crop results in reduced ROS production and increased drought tolerance

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Abstract

Drought is a major abiotic stress in crop yield and its inevitable consequence is the increased production of reactive oxygen species (ROS) and cell damage. To reduce excessive ROS accumulation in soybean, *AtYUCCA6* gene was transformed via *Agrobacterium*-mediated transformation. About 3% of transformation efficiency was generated from five batches of the transformation experiment. Eighteen transgenic plants were produced with PPT resistance and analyzed for introgression of *AtYUCCA6*. T-DNA insertion and expression were confirmed by PCR, Southern blot and reverse transcriptase-PCR. In the drought tolerance tests with transgenic lines #2, #3, and #5, all three lines were less affected by drought treatment and survived in the water-deficit conditions while non-transgenic plants did not survive under the same drought condition. The physiological aspects of transgenic lines were also much stronger than NT plants by showing higher chlorophyll content and lower ion leakage during water-deficit conditions ($p < 0.01$), indicating the prevention of cell-membrane damage. Measurement of transpiration rate on detached leaves from transgenic plants showed nearly 10% less water loss. Finally, 3 transgenic lines (#2, #3, and #5) were investigated for ROS accumulation by DAB staining of detached leaves under water-deficit conditions. Unlikely NT plants with severe dark browning after 14 days of drought treatment, transgenic lines #2, #3, and #5 did not show significant browning.

Keywords Soybean · *Agrobacterium*-mediated transformation · *AtYUCCA6* · Drought tolerance · ROS

Jin Sol Park and Hye Jeong Kim contributed equally to this work.

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Introduction

As a sessile organism, plants are constantly exposed to external adverse environments, such as drought, salinity, heat, cold, and heavy metals. Drought is a major abiotic stress that causes severe problems in crop growth and yield (Santner and Estelle 2009; Manavalan et al. 2009; Zhang et al. 2010). One inevitable consequence of drought stress is the increase of toxic molecules, reactive oxygen species (ROS), in various cell compartments, such as chloroplasts, peroxisomes, and mitochondria (Cruz de Carvalho 2008; Blomster et al. 2011; Chan et al. 2016).

Plants produce various phytohormones, which play important roles in regulating growth and development as well as the response to diverse environmental stresses. Auxin, a plant hormone, regulates many aspects of physiological and developmental process in plants, including cell division, expansion, and differentiation, seed dormancy, lateral root formation, floral organ formation, and tropic responses (Kim et al. 2007; Iglesias et al. 2010; Park et al.

2013; Shi et al. 2014; Cha et al. 2015; Cheng et al. 2015). Indole-3-acetic acid (IAA), the major auxin, is synthesized via tryptophan (Trp)-dependent and Trp-independent pathways. In *Arabidopsis*, the Trp-dependent IAA biosynthetic pathway can proceed via four well-defined routes that produce indole-3-pyruvic acid (IPA), indole-3-acetaldoxime (IAOx), indole-3-acetamide (IAM), and tryptamine (TAM) as intermediates (Zhao 2012). YUCCA, belonging to plant flavin monooxygenase (FMO) family, directly catalyzes the conversion of IPA to IAA, which is considered as a predominant pathway (Zhao 2014). *Arabidopsis* possesses 11 *YUCCA* gene families, and the proteins play important roles in auxin (IAA) biosynthesis and plant development (Kim et al. 2011, 2013a, b; Dai et al. 2013; Park et al. 2013; Cha et al. 2015; Ke et al. 2015). Overexpression of *AtYUCCA6* in *Arabidopsis* and potato was found to improve IAA-related phenotypes and increase drought tolerance by controlling toxic ROS accumulation under drought stress (Kim et al. 2013a, b; Cha et al. 2015). Despite to these dominant agronomical traits of *YUCCA* family genes, genetic transformation has not been applied to crop soybeans so far. Therefore, the development of drought-tolerant soybean varieties will contribute not only to coping with environmental changes, but also to practical use in agriculture.

Soybean (*Glycine max* L.) is one of the most significant sources of vegetable oil and plant-derived protein for food. Transformation is an optimal technique for developing soybean varieties with desired traits that are difficult to obtain from traditional breeding. *Agrobacterium*-mediated transformation has been efficiently used to introduce foreign genes into soybeans (Verma et al. 2014; Du et al. 2016; Li et al. 2017). Many researchers have used cotyledonary nodes by germinating seeds as the explants for soybean transformation (Hinchee et al. 1988; Di et al. 1996; Zhang et al. 1999). Instead of germinating soybean seeds, soybean transformation using "half-seed" explants has been improved (Paz et al. 2006). Using half-seed explants is a preferred method for soybeans, because it is less time-consuming, is simple to use, and allows easy explant preparation. There are important factors to increase the efficiency of the transformation process, such as simple wounding, treatment by sonication, vacuuming, and additional use of thiol compounds in the co-cultivation medium. Thiol compounds, including L-cysteine, sodium thiosulfate, and dithiothreitol (DTT), can inhibit the extensive tissue browning or cell death in the wounded area of a shoot pad and significantly improve T-DNA transfer and the frequency of transformed cells. (Olhoft et al. 2003; Dan 2008; Verma et al. 2014; Kim et al. 2013a, b, 2016, 2017a, b, 2018).

In this study, soybean plants overexpressing *AtYUCCA6* were produced by *Agrobacterium*-mediated transformation with the expectation they would be stress-tolerant because of the reduction of ROS. *AtYUCCA6* overexpression exhibited low amounts of ROS accumulation and better drought tolerance in transgenic soybean plants than in wild-type plants.

Materials and methods

Vector construction and *Agrobacterium* preparation

The *AtYUCCA6* cDNA open reading frame was amplified from its original vector (provided by Dr. D J Yun at Konkuk University, Republic of Korea) using *AtYUCCA6*-F primer (5′-ATGGAAGGTAAACTAGCACATGAC-3′) and *AtYUCCA6*-R primer (5′-TCAATTCCCACCACAATCACT CTC-3′). The desired destination vector, pPZP-3′PinII-*Bar* (provided by Dr. J K Kim at Myongji University, Republic of Korea) was used for the vector construction. The resultant plasmid, pPZP-3′PinII-*Bar*-*AtYUCCA6* (Fig. S1), was transformed into *Agrobacterium tumefaciens* strain EHA105 for soybean transformation and subsequently cultured on solid YEP media [10 g l^{-1} yeast extract, 5 g l^{-1} NaCl, 10 g l^{-1} peptic peptone, and 1.0% (w/v) plant agar, pH 7.0] containing 50 mg l^{-1} spectinomycin and 25 mg l^{-1} rifampicin at 28 °C for 2 days. A single colony was chosen and grown in 20 ml of liquid YEP medium containing 50 mg l^{-1} spectinomycin and 25 mg l^{-1} rifampicin at 28 °C for a day until OD_{600} reached between 0.6 and 0.8. Competent cells were then prepared by mixing equal volumes of 30% (v/v) glycerol. Aliquots of competent cells were frozen and kept at −70 °C until used.

Soybean transformation

Mature soybean seeds of Korean cultivar Kwangankong were used in *Agrobacterium*-mediated soybean transformation by following the method described by Kim et al. (2012, 2013a, b, 2016, 2017a, b, 2018). Five batches of the transformation experiment were carried out with 120–130 soybean seeds each time. To identify putative transformants expressing the *Bar* gene, two trifoliate leaves from T_0 plants were screened using an herbicide paint assay. The upper surface of a leaf was painted with the mixture of 100 mg l^{-1} PPT and Tween 20 using a brush. The response to this herbicide assay was screened after 3–5 days of PPT leaf painting. Plants with PPT resistance were grown in a greenhouse until maturity, and T_1 seeds were harvested (Fig. S2).

Confirmation of transgene in transgenic plants

Genomic DNA was extracted from leaf tissues of non-transgenic (NT) and transgenic plants using cetyltrimethylammonium bromide. The polymerase chain reaction (PCR) analysis was performed using KOD FX (TOYOBO, Osaka, Japan) according to the manufacturer's instructions with a thermal

cycler (Takara, Japan). The primer sets were designed in the regions of *AtYUCCA6* (5′-AGGTAAACTAGCACATGA CCACCG-3′/5′-TCAATTCCCACCACAATCACTCTC-3′) and *Bar* (5′-AGACAAGCACGGTCAACTTCCGTA-3′/5′-CCGGCAGGCTGAAGTCCAGC-3′) genes. To evaluate the T-DNA insertion into the plant genome, additional primers of DNA from *Bar* gene to left border (LB) (5′-AGACAA GCACGGTCAACTTCCGTA-3′/5′-TGGCAGGATATATTG TGGTGTAAA-3′) and from right border (RB) to *AtYUCCA6* gene (5′-GTTTACCCGCCAATATATCCTGTCA-3′/5′-TCA ATTCCCACCACAATCACTCTC-3′) were used to amplify both end regions of the vector.

For Southern blot analysis, 20 µg of genomic DNA from NT and transgenic plants were digested overnight using *Hind*III, fractionated on 0.8% (w/v) agarose gel by electrophoresis, then transferred onto Hybond N+ nylon membrane (Amersham Pharmacia, USA). Hybridization, washing, and detection were performed using a digoxigenin (DIG)-labeled DNA probe and a chemiluminescent system (Roche, Germany) according to the manufacturer's instructions. The DIG-labeled probe was prepared by PCR amplification with the *Bar* primers (5′- AACTTCCGTACCGAGCCGCA-3′/5′-TCGTAGGCGTTGCGTGCCTT-3′).

RNA analysis of transgenic plants

Total RNAs were isolated from both NT and transgenic T_0 plants using plant RNA purification reagent (Invitrogen, USA) according to the manufacturer's instructions. Reverse transcriptase-PCR (RT-PCR) was conducted using the RT-PCR Remix Kit (Genetbio, Korea) according to the manufacturer's instructions. The primer sets used in the RT-PCR were as follows: *AtYUCCA6*, 5′-AGGTAAACTAGCACA TGACCACCG-3′/5′-TCAATTCCCACCACAATCACT CTC-3′; *Bar*, 5′-AGACAAGCACGGTCAACTTCCGTA -3′/5′-CCGGCAGGCTGAAGTCCAGC-3′. The constitutive *TUB* (5′- TGAGCAGTTCACGGCCATGCT/5′-CTCGGC AGTGGCATCCTGGT-3′) was used as an internal control to normalize the amount of leaf RNA in the soybeans.

Drought-stress treatment in transgenic plants

To analyze the drought tolerance in *AtYUCCA6* transgenic plants, NT and transgenic plants (T_2) were grown in the same volume of soil and identical containers in a growth chamber with the conditions of 25 °C, 18 h light/6 h dark, and 60% humidity for 3 weeks, until the leaves on two nodes were fully expanded. Under the same conditions of plant growth, the phenotype of drought-stressed plants was monitored by the exposure of no-irrigation for 14 days and resuming irrigation for 3 days after the end of the drought treatment.

Measurement of total chlorophyll and relative ion leakage

Total chlorophyll from leaves of NT and transgenic plants after drought-stress treatment was isolated in 80% (v/v) acetone. The chlorophyll content was calculated using a spectrophotometer, as described by Wu et al. (2008). Statistical analysis was also performed using the Excel *t* test program to confirm significant differences.

The extent of ion leakage from NT and transgenic plants after the drought-stress treatment was measured by means of its conductivity. One gram of leaf samples was soaked in 10 ml of distilled water for 24 h at room temperature. The conductivity of the solution (L_t) was measured using an EC-400L conductivity meter (Istek, Korea). The leaf samples were then returned to the solution in the tubes, which were sealed and incubated at 95 °C for 20 min. The solution (L_0) was then cooled to room temperature, and conductivity was re-measured. The $L_t/L_0 \times 100$ values were calculated and used to evaluate the relative electrolyte leakage (Fan et al. 1997). Statistical analysis was also performed using the Excel *t* test program to confirm significant differences.

Examination of transpiration rate

NT and transgenic plants were grown under the same conditions, including identical containers, the same volume of soil, a long day photoperiod (18 h light/6 h dark), and 60% humidity in the growth chamber. The fully expanded leaves from two nodes were detached and weighed for 200 min at 40-min intervals and compared to the initial weight on a sterile bench in an extractor hood. Statistical analysis was performed using the Excel *t* test program to confirm significant differences.

Detection of ROS content in transgenic plants

Drought treatment was applied to NT and transgenic plants for 14 days under the same conditions as mentioned above. The leaves of similar developmental stages were then detached from randomly chosen sites, immersed in 3,3′-diaminobenzidine (DAB) staining solution (1 mg ml^{-1}, pH 3.8; Sigma, USA) for 4 h, incubated in 100% ethanol at 95 °C for 15 min until chlorophyll was cleared, and then observed with a stereomicroscope. Quantitative analysis of DAB staining was performed using image analysis software (ImageJ 1.52a; Java 1.8.0_112) (Sekulska-Nalewajko et al. 2016).

Results

Production of transgenic soybean plants using *Agrobacterium*-mediated transformation

To produce transgenic soybean plants, pPZP-3′PinII-*Bar*-*AtYUCCA6* plasmid (Fig. S1) was used for the soybean transformation with half-seed explants of the Korean soybean cultivar Kwangankong (Fig. S2), following the modified protocol described by Kim et al. (2012, 2013a, b, 2016, 2017a, b). About 3% of transformation efficiency was generated from five batches in the transformation experiment, in which 100–120 soybean seeds were used each time. Eighteen transgenic plants were produced with PPT resistance. Among them, eight well-grown and early harvested transgenic plants (lines #2, #3, #4, #5, #9, #10, #12, and #14) were selected to confirm the integration of the transgene, and these lines were examined using PCR with *AtYUCCA6* and *Bar* primers to amplify the DNA fragments of 1281 bp and 552 bp in size, respectively. In addition, T-DNA insertion was also confirmed by amplifying both end regions of the vector construct (Fig. 1). All eight transgenic lines showed the expected amplification of transgene sequences. To analyze the transcription level of *AtYUCCA6* and *Bar* genes, reverse transcriptase-PCR (RT-PCR) was conducted with RNAs extracted from those eight transgenic plants (Fig. 2). The transformed *AtYUCCA6* and *Bar* genes were expressed in all transgenic lines as expected, while those were not detected in non-transgenic (NT) plants.

To investigate the number of transgene insertions in the selected eight transgenic lines, genomic Southern blot analysis was carried out using leaf samples from T_2 seedlings (Fig. 3). Genomic DNAs from NT and transgenic plants were digested with *Hind*III and hybridized with *Bar* probe. All eight transgenic lines (#2, #3, #4, #5, #9, #10, #12, and #14) showed multiple insertion events. Lines #4 and #5 showed similar patterns in the result; they seem to be clones generated from the same shoot pad.

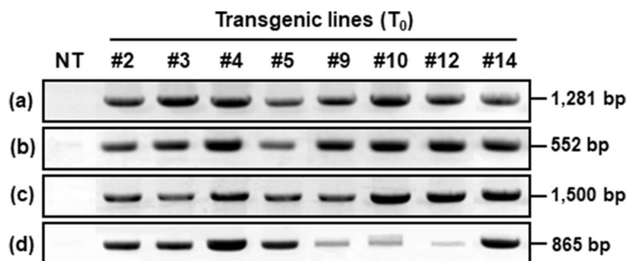

Fig. 1 Confirmation of introduced genes from *AtYUCCA6* transgenic soybean plants (T_0) using PCR. Genomic DNAs were extracted from transgenic plants (T_0). **a** *AtYUCCA6* gene. **b** *Bar* gene. **c** The DNAs between *Bar* gene and left border (LB). **d** The DNAs between right border (RB) and *AtYUCCA6* gene. NT, non-transgenic plant; #2, #3, #4, #5, #9, #10, #12, and #14, *AtYUCCA6* transgenic lines (T_0)

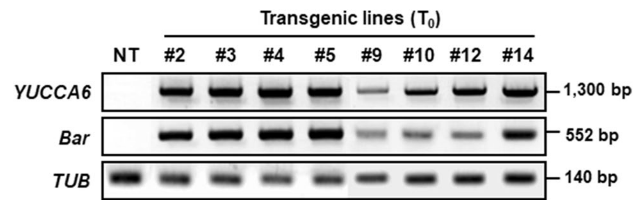

Fig. 2 Transcript level of *AtYUCCA6* and *Bar* genes in transgenic plants (T_0) using reverse transcriptase-PCR (RT-PCR). Total RNAs were extracted from *AtYUCCA6* transgenic plants (T_0), and RT-PCR was used to confirm gene expressions. *TUB* gene was used as a quantitative control. NT, non-transgenic plant; #2, #3, #4, #5, #9, #10, #12, and #14, *AtYUCCA6* transgenic lines (T_0)

Drought tolerance of *YUCAA6* transgenic soybean plants

Drought tolerance tests were done with transgenic lines #2, #3, and #5, from which we were able to harvest T_2 seeds relatively quickly out of the eight transgenic lines that underwent proliferation in the greenhouse. We investigated the response to drought stress in these three lines and compared them with NT plants. All plants were drought-treated for 14 days under the same conditions. Among them, NT plants started to lose vigor on the 11th day of drought, and all had withered completely at the end of the 14-day drought treatment. On the other hand, transgenic lines #2, #3, and #5 were less affected by drought treatment and survived in the water-deficit conditions. When re-watered for 3 days, NT plants did not survive and eventually died, whereas the

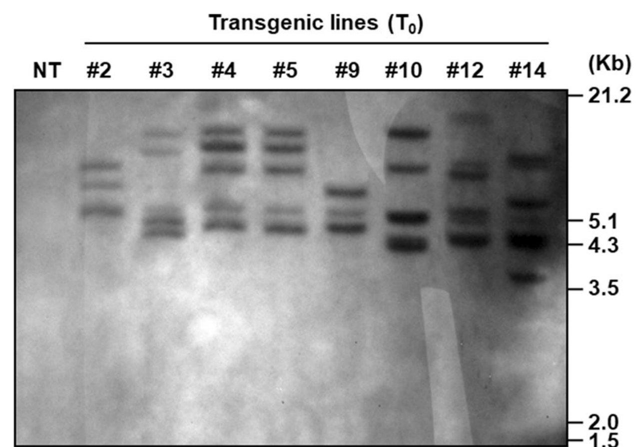

Fig. 3 Genomic Southern blot analysis of *AtYUCCA6* transgenic soybean. Twenty micrograms of genomic DNAs were digested with *Hind*III and hybridized with *Bar* probe. The approximate DNA size markers are indicated on the right. NT, non-transgenic plant; #2, #3, #4, #5, #9, #10, #12, and #14, *AtYUCCA6* transgenic lines (T_2)

transgenic lines #2, #3, and #5 fully recovered and continued to grow (Fig. 4a). And then, we confirmed the relative expression level of *AtYUCCA6* at 14 days after drought stress by qRT-PCR and found that the expression was confirmed in all transgenic lines #2, #3, and #5 (Fig. 4b).

The physiological aspects of transgenic lines were investigated by measuring changes in chlorophyll content and ion leakage during water-deficit conditions. The chlorophyll contents of transgenic lines #2, #3, and #5 were significantly higher than those of NT plants after 14 days of drought treatment ($p < 0.01$); the increased drought tolerance may have resulted from the maintenance of chlorophyll content (Fig. 4c). Moreover, ion leakage was increased in transgenic lines #2, #3, and #5, but significantly less ($p < 0.01$) than in NT plants; these transgenic lines were less affected by drought treatment because of the prevention of cell- membrane damage (Fig. 4d).

The water loss by transpiration was investigated by weighing detached leaves from transgenic and NT plants for 200 min at intervals of 40 min (Fig. 5). The leaves in NT started to curl up as a withered phenotype from 120 min after detached, while those in transgenic lines did not (Fig. 5a). After 200 min of drought treatment, the water content of NT plant leaves was only 47% of the initial leaf weight (Fig. 5b). In contrast, those of transgenic lines #2, #3, and #5 were about 5 to11% greater than that of the NT plants ($p < 0.05$ in lines #3 and #5). This result suggests that the *AtYUCCA6* gene appeared to have a positive effect on drought tolerance by slowing water loss.

Low ROS accumulation of *AtYUCCA6* transgenic soybean plants

ROS is known to accumulate under drought stress, and transgenic leaves of *Arabidopsis* and potato overexpressing the *AtYUCCA6* gene have been reported to have levels of ROS lower than those in leaves of the wild-type plants (Park et al. 2013; Cha et al. 2015; Chan et al. 2016). Thus, we analyzed ROS accumulation using DAB staining of detached leaves from transgenic soybean plants under water-deficit conditions (Fig. 6). By observing DAB stained leaves under the stereomicroscope, ROS accumulation could be visualized by the brown color in leaves of NT plants at the 11th day of drought treatment, and severe dark browning shown after

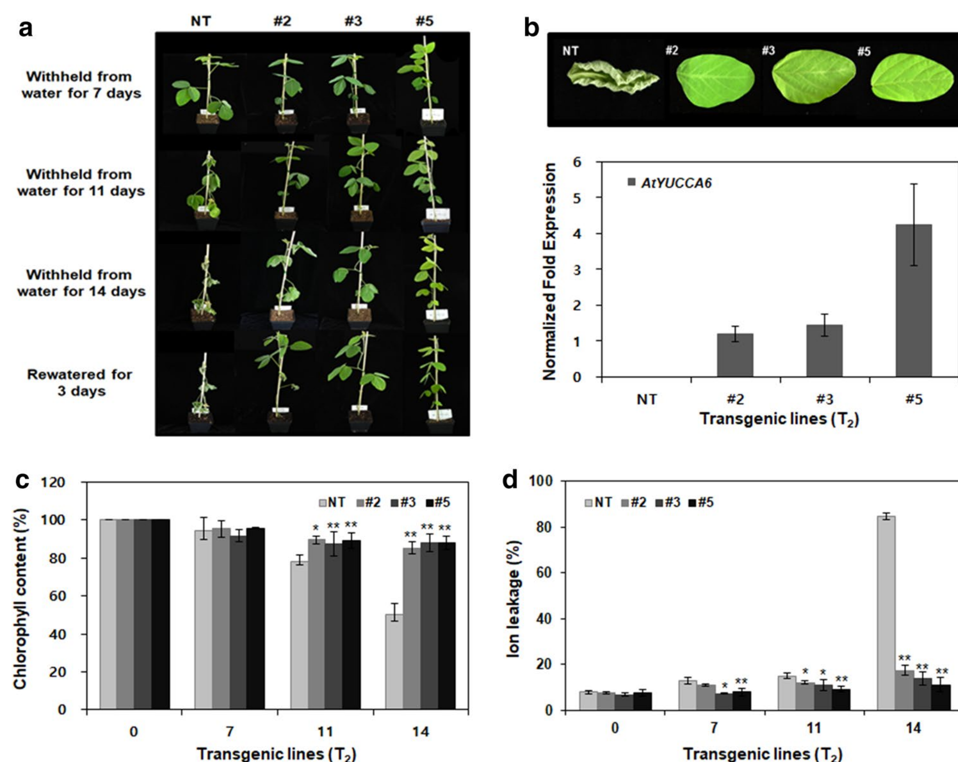

Fig. 4 Drought tolerance of *AtYUCCA6* soybean transgenic plants (T_2). **a** Analysis of drought tolerance of *AtYUCCA6* transgenic plants compared with NT plants. Plants were grown on soil until leaves were fully expanded on two nodes under the same conditions. Plants were deprived of water for 14 days, and then re-watered for 3 days ($n = 12$ each). The photographs were taken 7, 11, and 14 days after drought stress and 3 days after re-watering. **b** *AtYUCCA6* gene expression with detached leaves at 14 days after drought stress using real-time PCR (qRT-PCR). **c, d** Calculation of chlorophyll content and ion leakage at the indicated days after drought treatment from two-node leaves ($n = 6$ each). NT, non-transgenic plant; #2, #3, #4, #5, #9, #10, #12, and #14, *AtYUCCA6* transgenic lines (T_2). Error bars indicate mean ± standard deviation. Asterisks indicate significant changes compared with NT (*$p < 0.05$; **$p < 0.01$)

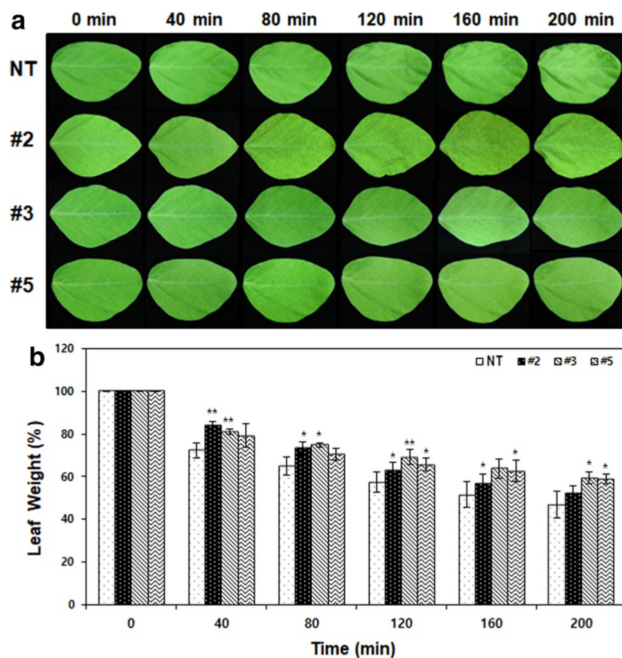

Fig. 5 The water loss content of *AtYUCCA6* transgenic plants (T_2). Plants were grown on soil until leaves were fully expanded on two nodes; leaves were detached ($n=6$ each) and weighed at the indicated times after drought treatment (**a, b**). NT, non-transgenic plant; #2, #3, #4, #5, #9, #10, #12, and #14, *AtYUCCA6* transgenic lines (T_2). Error bars indicate mean ± standard deviation. Asterisks indicate significant changes compared with NT (*$p<0.05$; **$p<0.01$)

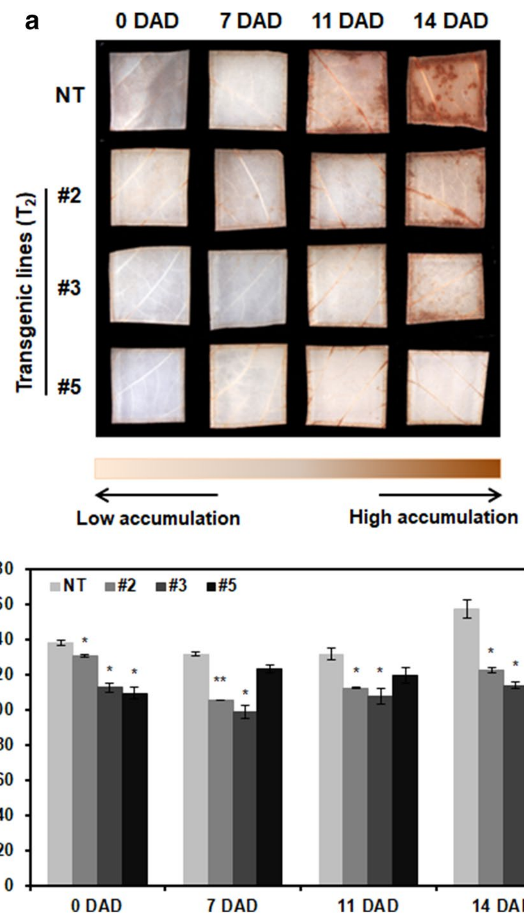

Fig. 6 Low ROS accumulation in *AtYUCCA6* soybean transgenic plant leaves (T_2). **a** ROS accumulation visualized by DAB staining. Plants were grown on soil until leaves were fully expanded on two nodes and deprived of water for 14 days. Detached leaves subjected to drought stress were stained with DAB (1 mg ml^{-1}, *p*H 3.8) to examine ROS accumulation. **b** Quantitative analysis of DAB staining. ROS accumulation in detached leaves shown in **a** were quantified by analyzing the image analyzer. Data represent that mean ± SE from two biological replicates. NT, non-transgenic plant; #2, #3, #4, #5, #9, #10, #12, and #14, *AtYUCCA6* transgenic lines (T_2). DAD, day after drought treatment. Asterisks indicate significant changes compared with NT plants (*$p<0.05$; **$p<0.01$)

14 days of drought treatment. However, transgenic lines #2, #3, and #5 did not show significant browning until day 14 of drought treatment (Fig. 6a). To confirm the intensity of DAB staining in drought-treated transgenic plants, ROS accumulation was quantified by the image analyzing program. In transgenic lines #2, #3, and #5, accumulation of ROS was significantly less than NT plants after 14 days of drought treatment ($p<0.05$ in lines #2 and #3) (Fig. 6b).

Discussion

Auxin, an essential regulator of plant growth and development, plays an important role in responses to environmental stress, such as drought, salinity, and pathogens. ROS is known to be accumulated under drought stress in plants (Miller et al. 2010). YUCCA6 as a flavin monooxygenase enzyme converts IPA to auxin which is a downstream step of Trp aminotransferase (TAA1/TAR1/TAR2) converting Trp to IPA (Zhao 2012, 2014). Overexpression of the *AtYUCCA6* gene in *Arabidopsis* and potato plants exhibited auxin overproduction and drought tolerance. These transgenic plants also showed less ROS than did wild-type plants under drought stress conditions (Park et al. 2013; Cha et al. 2015; Chan et al. 2016).

Our stable *Agrobacterium*-mediated soybean transformation has been established based on the half-seed (Paz et al. 2006) and cotyledonary-node (Hinchee et al. 1988) methods. This method included a mixture of thiol compounds, such as L-cysteine, sodium thiosulfate, and dithiothreitol, in a co-cultivation medium, which inhibited the activity of enzymatic browning and cell death in the wounded area. These additional treatments resulted in significantly increased T-DNA delivery into cotyledonary cells (Olhoft and Somer 2001; Olhoft et al. 2003). Thus, our modified transformation protocol enabled us to produce stable transgenic soybean plants with agronomically important genes for practical use in agriculture. Based on the evidence from previous studies,

the *AtYUCCA6* gene was transformed into soybean via *Agrobacterium*-mediated transformation to identify increased drought tolerance. Transgenic soybean plants showed normal growth under drought stress, whereas NT plants withered and died eventually. Drought stress resulted in a significant increase in ion leakage and low chlorophyll contents from leaves of NT plants. However, the phenotypic changes of transgenic plants were less affected by drought conditions, their cell membranes were protected, and chlorophyll content was also maintained. These physiological responses showed that drought tolerance was significantly increased by introducing the *AtYUCCA6* gene into transgenic soybeans.

A correlation between *AtYUCCA6* overexpression and low ROS accumulation was also investigated in soybean leaves. ROS, the result of the partial reduction of atmospheric O_2, is continuously produced in plants under normal conditions with a relatively low level in organelles, such as chloroplasts, mitochondria, and peroxisomes, that function as components of a stress-signaling pathway. When plants are exposed to environmental stresses, such as drought, the production of ROS is significantly increased. A certain level of ROS toxicity results in oxidative damage to cellular membranes and other cellular components, including membrane lipids, chlorophyll, nucleic acid, and proteins. ROS can cause membrane lipid peroxidation, ion leakage, and chlorophyll loss that lead to cell death. Chloroplasts are particularly susceptible to ROS because of their damage in the photosynthetic electron-transfer system. In addition, when the concentration of ROS exceeds a certain range, it activates a programmed cell-death response in cells (Wang et al. 2005; Cruz de Carvalho 2008; Yasar et al. 2008; Gill and Tuteja 2010; Miller et al. 2010; Wu et al. 2008). When ROS accumulation was visualized by DAB staining in soybean leaves subjected to drought stress, the severe brown color related to ROS accumulation was detected in non-transgenic leaves. In contrast, lower ROS accumulation was exhibited by transgenic leaves. Among the transgenic plants (lines #2, #3, and #5), line #5 showed the greatest drought tolerance and the lowest ROS accumulation, which seemed to be associated with the highest level of *AtYUCCA6* expression. Our results suggest that the level of *AtYUCCA6* expression was correlated with the change of ROS accumulation. Low ROS accumulation resulted in the protection of cell membranes and the maintenance of chlorophyll content, and finally increased drought tolerance in *AtYUCCA6* transgenic plants under drought treatment.

In our previous study, soybean transgenic plants overexpressing the *AtABF3* gene reduced stomatal opening and increased drought tolerance under water-stress conditions (Kim et al. 2018). However, closure of stomata had a negative effect on plant height. *AtYUCCA6* overexpressing soybean transgenic plants are not likely to have a negative impact on plant growth, since they are associated with ROS scavenging instead of stomatal closure. Under the natural condition, a field test of *AtYUCCA6* transgenic soybeans will be carried out in the near future.

Soybean, a drought-sensitive crop, is severely affected by drought stress, which is the major yield-limiting factor in crop plants. In this study, we introduced the *AtYUCCA6* gene from *Arabidopsis* into soybean using *Agrobacterium*-mediated transformation to increase drought tolerance with low ROS accumulation. As always, the modest significance of our work is to test any available gene from a model plant in field-crop soybeans to evaluate its potential. The valuating gene via genetic transformation is urgently needed in the era of outpouring of genomics.

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