DOI: 10.1007/s10535-017-0703-1 BIOLOGIA PLANTARUM 61 (3): 557-564, 2017

Physiological and molecular responses to drought and salinity in soybean

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Abstract

Drought and salinity are severe environmental stresses and limit soybean growth. In this study, a comparative analysis of physiological and molecular responses of two soybean (*Glycine max* L.) genotypes to these stresses was carried out. Plants of drought-tolerant genotype RD (cv. FD92) and sensitive genotype SD (cv. Z1303) were exposed to 15 % (m/v) PEG 6000, which simulated drought stress, or 150 mM NaCl. The RD plants maintained larger leaf area and higher net photosynthetic rate, chlorophyll content, stomatal conductance, and relative water content compared with the SD plants. Leaf proline content increased under both stresses more in RD than in SD. The drought tolerance of RD plants was also correlated with greater antioxidant activity and lower content of hydrogen peroxide and malondialdehyde under stress conditions. Amounts of abscisic acid, jasmonic acid, and salicylic acid under stress increased to a greater extent in RD than in SD plants. At the molecular level, the effects of 20-d stress treatments were manifested by relatively higher expression of drought- or salt-related genes: *GmP5CS*, *GmDREB1a*, *GmGOLS*, *GmBADH*, and *GmNCED1* in RD plants than in SD plants. These results form the basis for understanding the mechanisms of the drought- and salt-stress tolerance in soybean.

Additional key words: abscisic acid, chlorophyll, drought- and salt-tolerance, gene expression, *Glycine max*, jasmonic acid, malondialdehyde, photosynthetic rate, salicylic acid, stomatal conductance.

Introduction

Drought and salinity are the most important abiotic stresses that limit soybean yield (Radhakrishnan and Lee 2013). Low water potential makes difficult for plants to acquire water and nutrients (Al-Karaki 2006, Porcel *et al*. 2012) and the osmotic stress disturb the homeostasis of ion distribution in plant cells (Shabala and Cuin 2008, Pandolfi *et al*. 2012). However, a range of physiological mechanisms can be used to cope with such challenges, including stomatal control, osmotic adjustments, photoprotective effects (Valladares and Pearcy 2002), as well as the production of secondary metabolites and phytohormones (Thameur *et al*. 2011).

Responses to water stress and high salinity have mainly been investigated in terms of plant survival. However, it is possible to manipulate the expression of regulatory genes (Stolf-Moreira *et al.* 2010). Proline accumulation is modulated by the balance between its synthesis and catabolism. As the key rate-limiting enzyme, δ-1-pyrroline-5-carboxylate synthetase (P5CS) spontaneously converts glutamate to pyrroline-5-carboxylate (P5C). This reaction product is then reduced to proline by the P5C reductase (P5CR) enzyme (Verbruggen and Hermans 2008). In *Arabidopsis thaliana*, *Opuntia ficus-indica*, and *Brassica napus*, *P5CS* gene expression is related to proline accumulation under osmotic stress (Kubala *et al*. 2015). The dehydration-responsive element-binding (DREB1) protein is a transcription factor that binds to the promoter of genes such as *responsive dehydration 29A* (*RD29A*), thereby inducing its expression in response to drought, salinity, or low temperature. Likewise, in plants that naturally store glycinebetaine, the accumulation of it is induced by abiotic stresses such as

Submitted 7 March 2016, *last revision* 17 September 2016, *accepted* 14 October 2016.
Abbreviations: ABA - abscisic acid; BADH - betaine aldehyde dehydrogenase; Chl - chlorophyll; DREB1 - dehydration-responsive element-binding; g_s - stomatal conductance; JA - jasmonic acid; MDA - malondialdehyde; NCED - 9-*cis*-epoxy-carotenoid dioxygenase; P5CS - δ-1-pyrroline-5-carboxylate synthetase; P_N - net photosynthetic rate; RD - drought tolerant; REC - relative electric conductivity; RWC - relative water content; SA - salicylic acid; SD - drought sensitive.
Acknowledgments: This research was supported by the project (No. 11ZF096) from the Science and Technology Research and Development Guidance Plan of Baoding City, the Cutting-edge and Characteristic Disciplines of Biology (Botany), and the Key Subject of Biochemistry and Molecular Biology. The authors are grateful to Dr. Priscilla Licht for the critical reading of manuscript.
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high salinity, drought, and chilling. Betaine aldehyde dehydrogenase (BADH) catalyzes the conversion of betaine aldehyde to glycinebetaine (Jagendorf and Takabe 2001). Abscisic acid (ABA) plays a key role in the molecular signaling that is triggered by the onset of drought. The 9-cis-epoxycarotenoid dioxygenases (NCED) catalyze the rate-limiting step in ABA biosynthesis. Expression of the *NCED* gene not only induces but also determines the extent of ABA accumulation (Yamaguchi-Shinozaki and Shinozaki 2005). This ABA signal also affects the level of drought tolerance in soybean (Radhakrishnan and Lee 2013).

Although more information is now available concerning the mechanisms for drought and salt tolerance in soybean, less is known about the molecular mechanisms of defense against cell dehydration and osmotic stress (Ahuja *et al*. 2010). Here, we investigated to what extent drought and salinity affect growth parameters, the antioxidant machinery, proline content, phytohormones, and stress responsive genes in two soybean genotypes, either drought-resistant or drought-sensitive. The aim was to improve our understanding the stress resistance mechanisms that made certain cultivars more tolerant to such growing conditions.

Materials and methods

Two genotypes of *Glycine max* L. were compared: the drought tolerant cv. FD92 (RD) *versus* the sensitive cv. Z1303 (SD). Seeds were provided by the Agriculture Sciences Department of Cangzhou University, Hebei, China, and collected from a field in which soybean had been cultivated for eight consecutive years. Plants were grown in 170 cm^3 pots containing a 1:3 mixture of soil and *Vermiculite*. Conditions in the growth chamber included day/night temperatures of 28/20 °C, a 16-h photoperiod, an irradiance on 240 μmol m^{-2} s^{-1}, and a relative humidity of 75 %. After 35 d of growth under well-watered conditions (till the second vegetative stage), the irrigation water for selected plants was supplemented with either 15 % (m/v) polyethylene glycol (PEG-6000) or 150 mM NaCl to induce drought or salt stress, respectively. Control plants received water. When plants were grown to the third vegetative stage with 3 nodes and axillary buds (after 20 d), three expanded leaves were collected from control and stressed plants, placed in liquid nitrogen, and stored at -80 °C prior to RNA extraction and assays of enzyme activity. For hormone and glycinebetaine quantifications according to the method of Durgbanshi *et al*. (2005) and Moghaieb *et al*. (2004), the leaf samples were first lyophilized and then stored at -80 °C. Plant heights, root lengths, shoot and root dry masses, leaf areas, and indices of stress damage were examined.

For evaluation of plant morphology parameters, on day 20 of the stress period, height and leaf area were measured from individual plants. Entire root systems were scanned with an *EPSON Transparency* unit (*Seiko Epson Corp*., Tokyo, Japan) and then analyzed with *WinRHIZO v. 4.0b* (*Regent Instruments*, Quebec, Canada) to determine root diameters and surface areas. The relative electric conductivity (REC) was measured and calculated as described by Leopold and Toenniessen (1984). Based on the formula presented by Barrs and Weatherley (1962), relative water content (RWC) was determined by randomly sampling fresh leaves from at least three plants in three pots per replication. For determination of free proline (Pro) content, approximately 0.5 g of frozen leaf tissue from control and stressed-treated plants was homogenized in 10 cm^3 of 3 % (m/v) aqueous sulfosalicylic acid. Then it is measured as described by Bates *et al*. (1973).

After 20 d of drought or salt stress, the youngest fully expanded leaves were sampled from each treatment for evaluating their net photosynthetic rate (P_N) and stomatal conductance (g_s). Measurements were made between 10:00 and 13:00 at irradiance of 1 000 μmol m^{-2} s^{-1} with a *LI-6400* portable photosynthesis system (*Li-COR*, Lincoln, NE, USA). Other conditions in the leaf chamber included approximately 50 % relative humidity, a CO_2 concentration 350 to 400 μmol mol^{-1}, and an air temperature of 28 ± 2°C. The chlorophyll (Chl) *a* or *b* content was measured according to Arnon (1949).

Hydrogen peroxide was measured spectrophotometrically after reacting with potassium iodide (KI), which can be measured at 415 nm. The concentration of peroxide in the extracts was determined by analyzing the calibration curve representing different concentrations (from 1 to 10 mM) of the titanium-H_2O_2 complex (Alexieva *et al*. 2001). The malondialdehyde (MDA) was measured as follows: the complex containing 1 cm^3 of extract and 4 cm^3 of 0.5 % thiobarbituric acid in 20 % trichloroacetic acid, was incubated in water bath at 95 °C for 30 min, and then transferred to ice bath to stop the reaction. The liquid was extracted with isobutyl alcohol and measured by high-performance liquid chromatography (HPLC; *Agilent 1100 Series*; *Agilent Technologies*, Santa Clara, CA, USA) with fluorescence detection (Heath and Packer 1968).

Leaf samples were homogenized at 4 °C in 100 mM K-phosphate buffer (pH 7.8) with 10 mM $MgCl_2$, and 0.2 mM EDTA. The homogenate was centrifuged at 17 000 *g* for 30 min to yield a crude enzyme extract. Then, the activities of superoxide dismutase (SOD) and peroxidase (POD) were monitored as described Beyer and Fridovich (1987).

For measurements of potassium and sodium content, leaf and root tissues were collected from each treatment type, rinsed with distilled water, and then oven-dried at 80 °C for 20 h. The dried samples (approximately 1.0 g each) were boiled in a mixture of nitric acid and perchloric acid (4:1, v/v) in the presence of glass beads for 5 h. The digested plant material was filtered, diluted with distilled water, and analyzed for Na^+ and K^+ content with an atomic

flame photometer *FP6410* (*Shanghai Precision & Scientific Instrument Co.*, Shanghai, China).

For analysis of gene expression, total RNA was extracted from root tissues sampled from control and stress-treated plants. The cDNA synthesis was conducted with a *Prime-Script* RT-PCR kit (*TaKaRa*, Tokyo, Japan). To monitor differential expression, all primers were designed with the *Primer 5.0* program (*Premier Biosoft International*, Palo Alto, CA, USA). The primer sequences (Table 1 Suppl.) were determined for genes with putative amplicons of 100 to 200 bp, and expression was analyzed by real-time quantitative (q) PCR, using a *LightCycler® 1.5* (*Roche*, Mannheim, Germany) and a *SYBR® Premix Ex TaqTM II* kit (*TaKaRa*). Each 0.025 cm^3 of reaction mixture contained 500 nmol of primer and 100 ng of cDNA. Thermal-cycling conditions included an initial 95 °C for 10 s; then 45 cycles at 95 °C for 10 s, 59 °C for 15 s, and 72 °C for 15 s; followed by a final melting curve step from 59 to 95 °C (ramp rate 0.05 °C s^{-1}). Expression was monitored by real-time qPCR for 20 d during the treatment period. All data were normalized to constitutively expressed *Gm18SrRNA* (GenBank accession No. X02623.1) according to the $2^{-\Delta\Delta CT}$ method (Schmittgen and Livak 2008). Each experiment was repeated at least three times.

The physiological indices and relative expressions of genes are shown as means ± standard deviations (SDs) from three independent experiments. Statistical analysis was performed using the *Origin* software (*v. 7.5*). One-way analysis of variance was used to detect the effect of salt and drought on gene expression. Values were considered significantly different at $P < 0.05$ or $P < 0.01$ for all experiments. Treatment results were compared by three tests for least significant differences.

Results

Under well-watered conditions, the plant morphology or development were similar in the RD and SD genotypes (Fig. 1). However, after 15 d of drought treatment, the leaf tips from RD plants were somewhat etiolated whereas some of SD leaves were discolored. After 20 d of stress, SD leaves were severely wilted whereas those of the RD plants showed only slight wilting. The results also demonstrated that leaves of RD plants maintained a higher RWC than those of the SD plants under either drought or salinity (Fig. 2*A*).

Under both stresses, the RD plants were significantly taller than the SD plants (Table 1). Root and shoot biomass accumulation and root/shoot ratio also differed between the genotypes. For example, in response to PEG treatment, shoot and root dry masses after 20 d were 72.4 and 160.7 % greater, respectively, when compared with the SD plants. Finally, root/shoot ratios of RD were 48.8 and 23.3 % higher than of SD under drought and salt stress, respectively.

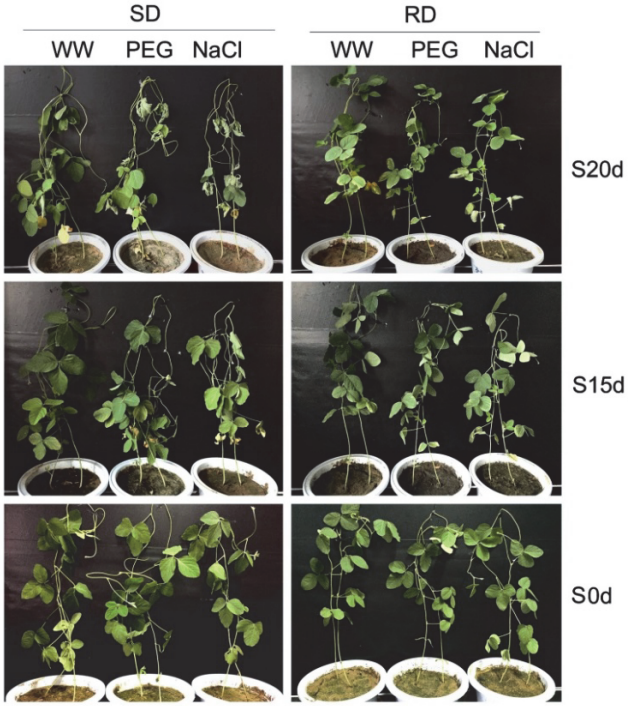

Fig. 1. Effect of drought (15 % PEG) and salinity (150 mM NaCl) on drought sensitive (SD) and drought tolerant (RD) soybean plants. S0d - seedlings grown for 35 d under well-watered (WW) conditions, S15d and S20d - plants treated with drought or salinity for 15 and 20 d, respectively.

Table 1. Biomass accumulation, morphological traits, and photosynthetic parameters in sensitive (SD) and resistant (RD) soybean plants grown under well watered conditions (WW), drought (15 % PEG), and salinity (150 mM NaCl) for 20 d. SDM - dry mass of shoots [g plant^{-1}], RDM - dry mass of roots [g plant^{-1}], PH - plant height [cm], R/S - root/shoot ratio, TRL - total root length [cm], RSA - root surface area [cm^2], RD - root diameter [cm], SRL - specific root length [m g^{-1}], LA - leaf area [cm^2], P_N - net photosynthetic rate [μmol m^{-2} s^{-1}], g_s - stomatal conductance [mmol m^{-2} s^{-1}], Chl - chlorophyll content [mg g^{-1}(f.m.)]. Means ± SDs, n = 3, significant differences between SD and RD at * - $P < 0.05$ or ** - $P < 0.01$.

Parameters	Well-watered		Drought		Salinity	
	SD	RD	SD	RD	SD	RD
SDM	2.35±0.42	2.29±0.14	1.38±0.13	2.38±0.21*	1.08±0.11	2.58±0.31*
RDM	2.27±0.35	2.17 ±0.41	0.56 ±0.10	1.46±0.12**	0.46± 0.09	1.46±0.15**
PH	40.90±1.21	39.21±1.45	25.80±1.64	35.02±1.03*	26.25±1.72	38.62±1.92*
R/S	0.97±0.03	0.95±0.07	0.41±0.05	0.61±0.04	0.43±0.06	0.53±0.04
TRL	1207.20±70.32	1204.83±40.50	842.87±41.44	1401.63±40.58**	898.87±45.46	1321.63±45.38
RSA	166.32±12.42	165.75±30.35	74.78±8.44	122.08±9.91**	84.78±8.44	142.98±9.81*
RD	0.57±0.04	0.45±0.05	0.65±0.02	0.49±0.15	0.55±0.02	0.35±0.16
SRL	284.32±6.02	293.21±8.02	181.32±10.42	235.36±10.02**	179.12±10.02	235.32±10.32**
LA	236.21±11.12	230.52±15.01	145.23±10.53	186.02±12.23*	135.31±16.03	175.36±10.05*
P_N	16.36±2.2	15.36±1.1	8.23±1.06	13.23±0.96*	7.03±1.26	12.23±1.6*
g_s	445.56±18.26	462.36±21.70	201.23±19.04	331.26±20.96*	192.73±20.15	352.13±18.06*
Chl	3.26±0.45	3.06±0.13	1.98±0.56	2.65±0.36**	2.03±0.43	2.43±0.16*

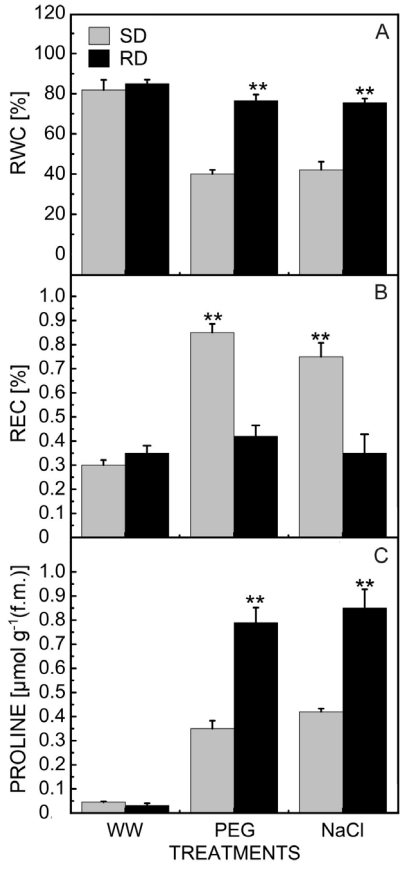

Fig. 2. Relative water content (RWC; *A*), relative electrical conductivity (REC; *B*), and proline content (*C*) in drought sensitive (SD) and drought tolerant (RD) soybean genotypes exposed for 20 d to well-watered conditions (WW), drought stress (15 % PEG), or 150 mM NaCl. Means ± SDs, n = 3, ** - differences significant at $P < 0.01$.

Development of an adequate root system is critical for plants to take up sufficient amount of water at low soil moisture. Here, the roots of RD plants were 66.4 % longer from those of SD plants under drought conditions, and were also longer under salt stress (Table 1). Values for specific root length and root surface area were also higher in stressed RD plants but their average root diameters were smaller under such conditions. Also leaf area was 28.1 and 29.6 % higher in RD plants under drought and salt stresses, respectively, when compared with the SD plants.

Both genotypes tested here showed similar patterns of response to stresses in terms of their leaf gas exchange (Table 1). In general, those parameters declined under drought and salinity. Nevertheless, P_N and g_s were higher in RD plants under both stresses than in SD plants (Table 1). In addition, Chl content was reduced in stressed plants and more in the SD than in the RD genotype.

Under stress conditions, RWC declined for both genotypes. However, in RD leaves RWC was 72.4 and 63.8 % higher than in SD leaves under drought and salt stresses, respectively (Fig. 2*A*). This implied that the RD was able to absorb water more efficiently or had greater capacity to prevent water loss. The REC values were similar in both genotypes when plants were well-watered. Although REC increased in both genotypes under stress conditions, the values were 50.6 and 53.3 % lower in the RD plants than in SD plants after drought and salt treatments, respectively (Fig. 2*B*). In addition, Pro content in RD drought- and NaCl-stressed plants was 1.26-times and 1.02 times higher when compared with the SD plants (Fig. 2*C*).

As indicators of oxidative stress, MDA and H_2O_2 content was determined in leaves of both genotypes. When compared with the well-watered control, the content of MDA and H_2O_2 in SD plants was 119.8 and 76.4 % higher

under drought and 62.3 and 121.7 % higher under salinity (Fig. 3*A*,*B*). In the RD genotype, MDA and H_2O_2 content remained lower than in SD plants after 20 d of either stress treatment. For assessment of the antioxidant response, SOD and POD activities were examined. Under either type of stress, activities increased for each enzyme in both genotypes, although those increments were greater in the RD plants (Fig. 3*C*,*D*).

The content of Na^+ in leaves and roots was similar in both genotypes under well-watered conditions. However, under drought and salt stresses, Na^+ content rose in all leaves and roots, but more in leaves of SD plants and roots of RD plants (Fig. 4*A*). When either stress treatment was applied, K^+ content decreased by approximately 50 % in both the leaves and roots of SD plants, whereas values in the RD tissues remained mostly similar to those measured in controls (Fig. 4*B*). Furthermore, the K^+/Na^+ ratio was similar for the leaves and roots of control plants from both genotypes (Fig. 4*C*). However, when 15 % PEG or 150 mM NaCl was applied, that ratio was 1.73-fold and 2.05-fold higher, respectively, in the RD leaves than in the SD leaves.

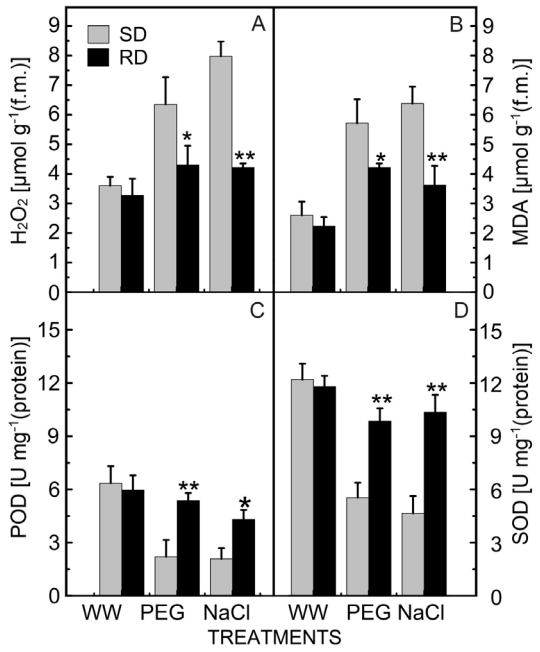

Fig. 3. Content of hydrogen peroxide (*A*) and malondialdehyde (MDA; *B*), and activities of peroxidase (POD; *C*) and superoxide dismutase (SOD; *D*) in drought sensitive (SD) and drought tolerant (RD) soybean genotypes exposed for 20 d to well-watered conditions (WW), drought stress (15 % PEG), or 150 mM NaCl. Means ± SDs, $n = 3$, *,** - differences significant at $P < 0.05$ and $P < 0.01$, respectively.

Under well-watered conditions, ABA content was similar in RD and SD plants. However, ABA content increased 8.02-fold (PEG) and 7.02-fold (NaCl) in RD plants, and 4.16-fold (PEG) and 2.98-fold (NaCl) in SD plants when compared with well-watered plants (Fig. 5*A*). In addition, comparing between genotypes, ABA content was 96.5 and 126.3 % higher in the RD plants than in the SD plants under drought and salt stress, respectively. The amount of SA declined slightly in both RD and SD upon stress treatments, with greater reduction in the SD genotype (Fig. 5*B*). The content of JA was higher in RD leaves than in SD leaves, and it was minimally affected by these stresses in RD plants (Fig. 5*C*). Further, the RD plants accumulated 5.0- to 4.14-fold more glycinebetaine in their leaves under both stresses compared to the SD plants (Fig. 5*D*).

We monitored the differential expression of genes related to abiotic-stress responses. Under drought and salt stress, expression of *GmP5CS* was 4.21- and 3.58-times higher, respectively, in RD than in SD (Fig. 6*A*) whereas that of *GmDREB1a* increased by 2.97- and 2.67-times, respectively, in RD plants when compared with SD (Fig. 6*B*). Expression profiles for *GmGOLS*, *GmBADH*, and *GmNCED1* tended to be similar in SD genotype under stress treatments. Comparing between well-watered conditions and exposure to drought or salinity, expression in RD plants was up-regulated by 1.83-fold (PEG) and 2.44-fold (NaCl), 6.03-fold (PEG) and 6.84-fold (NaCl), and 2.05-fold (PEG) and 2.20-fold (NaCl) for *GmGOLS*, *GmBADH*, and *GmNCED1*, respectively (Fig. 6*C*-*E*).

Discussion

The tolerance to drought and/or salt enables plants to grow in environments where water is limited or soils have high amount of salts. However, little is known about the adaptive mechanisms. Although research has been conducted to understand the response to drought stress in several tolerant genotypes of wheat, rice, and soybean (Thameur *et al*. 2011, Fan *et al*. 2013, Mutava *et al*. 2015), few have examined their responses to salinity. Here, we compared the physiological traits and molecular characteristics of soybean genotypes with contrasting stress tolerance. Our assessment was based on changes in root and shoot growth, leaf gas exchange, oxidative stress and antioxidants, phytohormones, and stress-induced gene expressions.

Here, the RWC values under both stresses remained significantly higher in the RD leaves than in the SD leaves. This was the evidence that the RD genotype efficiently absorbed water and prevented water loss. Similar results have been reported previously (Chaparzadeh and Mehrnejad 2013). In many species, Pro is accumulated in response to salinity (Molla *et al*. 2014). We also noted here that, compared with the SD plants, Pro content in RD plants was enhanced more under drought or salt stress. Previous researchers have also shown that higher RWC or Pro content and lower REC, induced by drought or salt stress, help to maintain the activity of antioxidant enzymes (Hoque *et al*. 2007, Kumar *et al*. 2010). The induction of cellular antioxidant machinery is critical for protecting

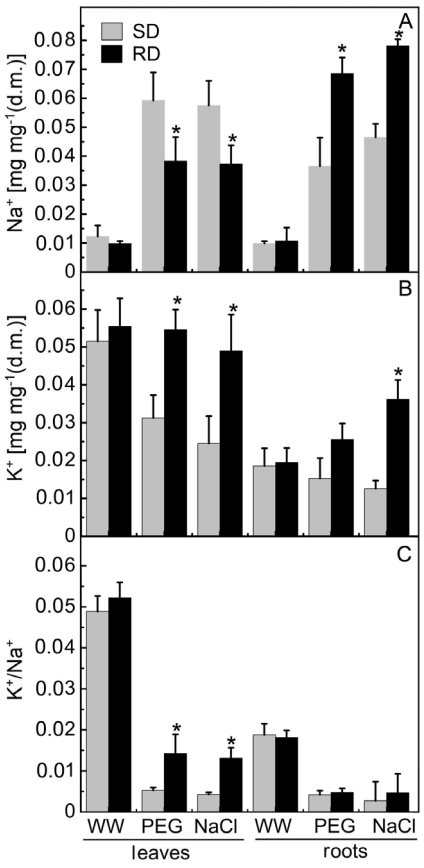

Fig. 4. Comparison of Na^+ (*A*) and K^+ content (*B*), and K^+/Na^+ ratio (*C*) in drought sensitive (SD) and drought tolerant (RD) soybean genotypes exposed for 20 d to well-watered conditions (WW), drought stress (15 % PEG), or 150 mM NaCl. Means ± SDs, $n = 3$, * - differences significant at $P < 0.05$.

plants against the adverse effects of abiotic stresses (Hameed *et al*. 2011). Our data indicated that SOD and POD activities were significantly increased in stressed RD plants whereas their content of H_2O_2 and MDA was lower than in SD plants. Moreover, we found a significant negative correlation between SOD/POD activities and MDA/H_2O_2 content. This suggested that the antioxidant defense machinery activated under stress remained operative throughout that challenging period, enabling plants to adapt to such conditions (Pérez-Clemente *et al*. 2013). Similarly, Khanna-Chopra and Selote (2007) have reported that drought-resistant wheat genotypes acclimate better than sensitive genotypes by minimizing oxidative damage through a well-coordinated defense that includes the upregulation of antioxidant enzymes.

The phytohormones ABA, SA, and JA regulate plant defenses, generating a network of signal-transduction pathways leading to a cascade of events responsible for the physiological adaptation of plants to numerous stresses (Pieterse *et al*. 2009). In our study, ABA content in RD plants increased under both drought and salt stresses, in accordance with results previously reported (Aimar *et al*. 2011). Under various abiotic stresses, SA can help plants acclimate and enhance their degree of tolerance by

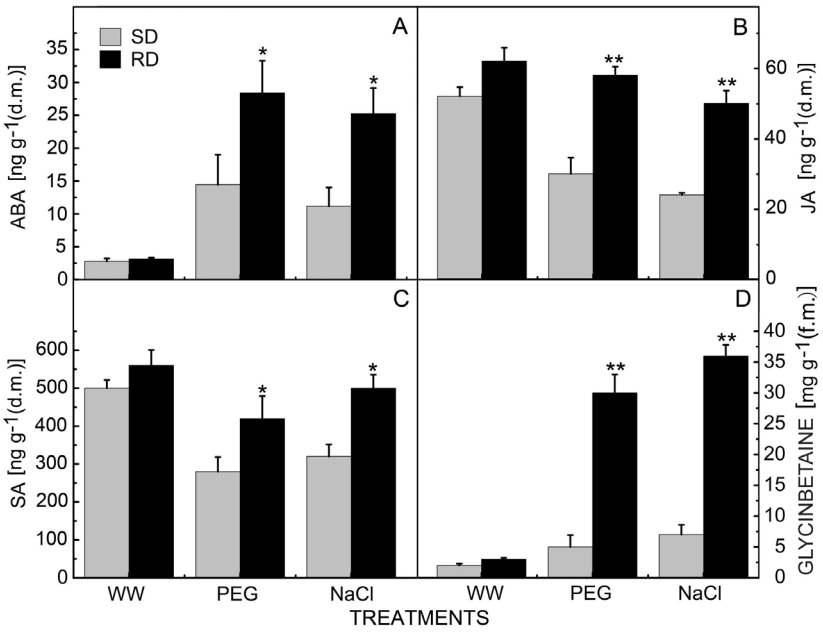

Fig. 5. Content of abscisic acid (ABA; *A*), jasmonic acid (JA; *B*), salicylic acid (SA; *C*), and glycinebetaine (*D*) in leaves of drought sensitive (SD) and drought tolerant (RD) soybean genotypes exposed for 20 d to well-watered conditions (WW), drought stress (15 % PEG), or 150 mM NaCl. Means ± SDs, $n = 3$, *,** - differences significant at $P < 0.05$ and $P < 0.01$, respectively.

improving their antioxidant capacity (Yang *et al*. 2004). We also found that, JA content in the RD leaves was higher than in the SD leaves. Taken together, these results suggested that the great increase in the ABA, SA and JA content is due to the drought or salt stress. These are important characteristics to demonstrate that the RD soybean is tolerant to drought and salt.

In conclusion, we found that our two soybean genotypes show distinct differences in physiological, biochemical, and molecular responses to drought or salinity stress. Firstly, the RD genotype has better genetic potential for presenting morphological and photosynthetic traits with larger leaf area and P_N, Chl content, and g_s, which might be advantageous for water use efficiency. Secondly, the drought-tolerant genotype maintained a higher water status than the sensitive genotype under either drought or saline conditions. Thirdly, the RD plants invoked a drought or salt tolerance by maintaining a high K^+/Na^+ ratio in a stressed environment. Finally, the tolerant genotype utilized a more efficient antioxidant system that counteracted the negative effects of oxidative stress under such stresses. All above results could partially explain the strategy by which RD plants are tolerant to these environmental challenges: the changes of physiological and biochemical indexes as well as of the hormones and the expression of related genes.

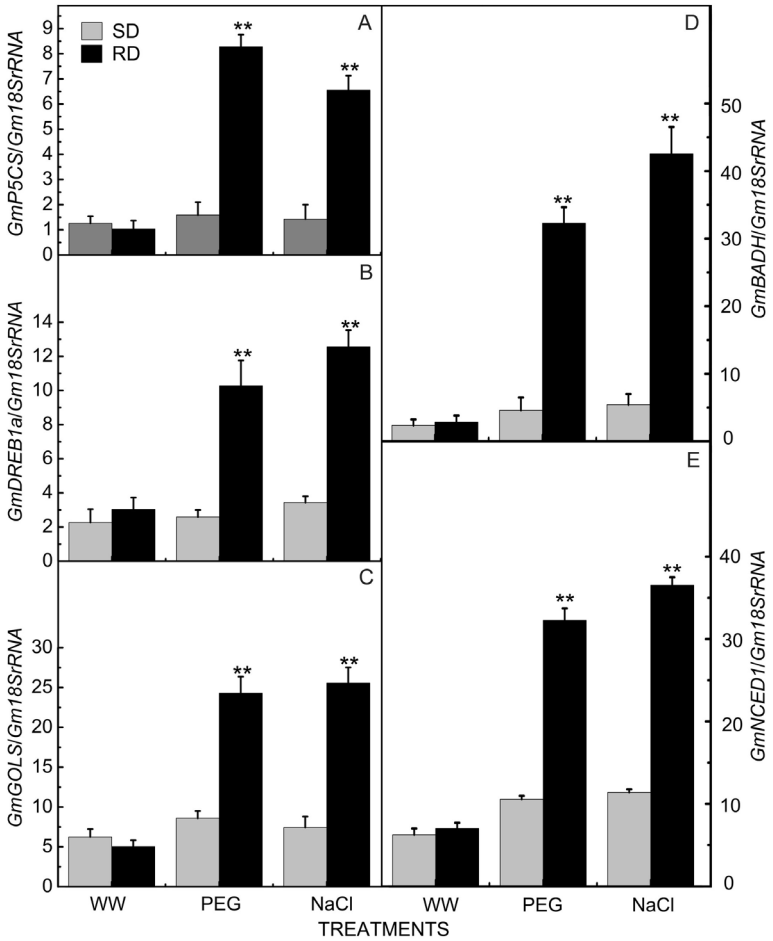

Fig. 6. Expression analysis of *GmP5CS* (*A*), *GmDREB1a* (*B*), *GmGOLS* (*C*), *GmBADH2* (*D*), and *GmNCED1* (*E*) in leaves from drought sensitive (SD) and drought tolerant (RD) soybean genotypes exposed for 20 d to well-watered conditions (WW), drought stress (15 % PEG), or 150 mM NaCl. Transcript abundance was calculated and normalized relative to that of *Gm18SrRNA*. Means ± SDs, $n = 3$, ** - differences significant at $P < 0.01$.

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