Genotypic variation in salinity tolerance and its association with nodulation and nitrogen uptake in soybean

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ABSTRACT
Saline soils hamper various physiological functions in soybean [Glycine max (L.) Merr.]. One example is the reduction in nitrogen (N) uptake capacity, a major dysfunction that limits soybean growth and yield under saline conditions. Previous studies have revealed that tolerance to salinity varies with cultivar; however, the cultivars used in these studies were selected solely based on agromorphological traits. In this study, we examined genotypic variation in salinity tolerance among 85 soybean genotypes which were selected based on an assessment of both single nucleotide polymorphisms (SNP) markers and agromorphological traits. Additionally, we examined whether salt tolerance is associated with nodulation and N uptake. We used a subset of the world soybean mini-core collection (80 cultivars) and an additional five cultivars/genetic lines (NILs72-T, NILs72-S, Enrei, En-b0-1, and En1282). All plants were grown in pots and treated with saline (final concentration of 150 mM NaCl) during the vegetative growth stage. To evaluate salinity tolerance, we used the ratio of saline-treated (S) to control (C) plant total dry weight [DW (S/C)]. The ratio differed markedly according to genotype. Furthermore, salinity-tolerant genotypes exhibited superior nodulation, leaf greenness, and N uptake under saline conditions. These results indicate that there is a marked genotypic variation in salinity tolerance, and that the tolerant genotypes exhibit greater nodulation and N uptake, although further studies are needed to clarify whether the superior nodulation and N uptake of salinity-tolerant genotypes are responsible for the observed tolerance.

Soil salinity is the primary environmental factor limiting crop production in arid and semi-arid regions (Neumann, 1997), with high salinity adversely affecting approximately 10% of the world’s cropland (Shannon, 1997). Affected soils contain high concentrations of soluble salts, particularly sodium and chloride, causing growth and yield reductions in most plant species (Flowers & Flowers, 2005; Niknam & McComb, 2000; Tuyen et al., 2010). Saline soil impairs physiological functions through multiple mechanisms, including water stress, specific ion toxicity, ion imbalance stress, induced nutrient deficiency, or a combination of these factors (Jones, 1981; Munns & Tester, 2008; Shannon, 1997; Zhang et al., 2010).

Genetic analyses using recombinant inbred lines (RIL) derived from crosses between salt-tolerant and susceptible genotypes have indicated that salt tolerance in soybean is linked to quantitative trait loci (QTLs) (Chen et al., 2008; Hamwieh & Xu, 2008; Hamwieh et al., 2011; Lee et al., 2004; Xu & Tuyen, 2012). Using a population derived from the salt-tolerant cultivar Tiefeng 8 and the salt-sensitive cultivar 85-140, Guan et al. (2014a) mapped a salt tolerance gene (GmSALT3) from Tiefeng 8, which is a dominant gene associated with limiting the accumulation of Na+ in shoots. In their subsequent study, they showed that salinity tolerance in soybean is modulated by natural variation in the GmSALT3 gene (Guan et al., 2014b). Recently, Do et al. (2016) isolated a gene (Ncl) that regulates ion (Na+, K+, Cl−) transport and accumulation, and demonstrated that higher expression of Ncl in the root led to lower ion accumulation in the shoot. Furthermore, when Ncl was incorporated into the ‘Kariyutaka’ soybean cultivar, the transformed genotype significantly enhanced salt tolerance. In another study, using an RIL population of a Glycine soja accession, Qi et al. (2014) identified the salt tolerance gene CHX1, which conferred the capacity of lowering the Na+ content in leaves. Using this gene, a subsequent study developed high-throughput SNP markers for salinity tolerance in soybean (Patil et al., 2016). These studies indicate that it is feasible to screen for or breed genotypes with an improved capacity of regulating the specific ions responsible for salt tolerance.

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Saline conditions hamper nodule formation in soybean (Singleton & Bohlool, 1984; Yasuta & Kokubun, 2014), significantly reducing growth (Abel & Mackenzie, 1964; Wang & Shannon, 1999) and seed yield (Parker et al., 1983; Yang & Blanchar, 1993). Salt-induced limitation of nodule formation is linked to corresponding differences in legumes that appear to reduce growth and yield through reducing N uptake (Balasubramanian & Sinha, 1976; Lakshmi-Kumari et al., 1974; Lauter et al., 1981; Singleton & Bohlool, 1984; Tu, 1981; Wilson, 1970). In our previous study, we observed that the significantly improved nodule formation of En-b0-1 (a super-nodulating genotype) over its normal parent cultivar was associated with greater tolerance under saline conditions (Yasuta & Kokubun, 2014).

Although substantial research has demonstrated that soybean exhibits considerable genotypic differences in salt tolerance (Ghassemi-Golezani et al., 2009; Hakeem et al., 2012; Karim et al., 2012; Lee et al., 2008; Mannan et al., 2012; Parker et al., 1983; Yang & Blanchar, 1993), the cultivars used in these studies were selected solely based on agro-morphological traits. In the present study, we examined genotypic variation in salinity tolerance among 85 soybean genotypes which were selected based on an assessment of both single nucleotide polymorphisms (SNP) markers and agro-morphological traits, and may thus provide useful information for the genetic improvement of salt tolerance (Kaga et al., 2012).

Therefore, in this study, we aimed to examine the genotypic variation in salt tolerance among 85 representative soybean genotypes, and to identify whether differences in salt tolerance are linked to corresponding differences in nodulation and N uptake.

Materials and methods

Plant materials

For the purposes of this study, we used a subset of the world soybean mini-core collection (80 cultivars). This collection was developed by NIAS (National Institute of Agrobiological Sciences, Tsukuba, Japan), based on agro-morphological and SNP data from a global soybean collection maintained at the NIAS Genebank (Kaga et al., 2012). This sub-set has various seed traits, including color (yellow, green, brown, and black), size, and shape (round and oval). For comparison, we added a further five genotypes: Enrei, En-b0-1, En1282, NILs72-T, and NILs72-S. Enrei is among the major cultivars grown in the eastern part of Japan. En-b0-1 and En1282 are super-nodulating and non-nodulating genotypes, respectively, and are the progeny of Enrei × En6500, an ethyl methylsulfonate-induced Enrei mutant (Akao & Kouchi, 1992). Even under saline conditions, En-b0-1 forms significantly more nodules and thus exhibits higher N fixation activity than Enrei, traits that partly contribute to its higher salinity tolerance (Yasuta & Kokubun, 2014). Finally, NILs72-T and NILs72-S are salt-tolerant and -sensitive progenies, respectively, of an RIL population resulting from a cross between salt-tolerant FT-Abyara and salt-sensitive C01 (Do et al., 2016; Hamwieh et al., 2011).

The experiments were conducted at the Graduate School of Agricultural Science, Tohoku University (38°1′N, 140°50′E). Experiments were performed in 2013 and 2015, with the only difference being a 17-day shift in sowing time between the two years. Plants were potted in a greenhouse with four open sides. Prior to sowing, fertilizer was applied at fixed rates and mixed with soil: 0.05 g of N, 0.15 g of P2O5, 0.2 g of K2O, 1 g of fused phosphate, 2 g of superphosphate of lime, and 2 g of slaked lime per liter of low-humic Andosol field soil [N: 0.55%, cation-exchange capacity (CEC): 44.9 cmol kg⁻¹]. The seeds were inoculated with J1065, a strain of Bradyrhizobium japonicum obtained from Tokachi Nokyoren (Obihiro, Japan). On 16 May 2013 and 2 June 2015, 7–10 seeds per genotype were sown in paper pots (3.5 cm length × 3.5 cm width × 3.8 cm height), at a rate of one seed per pot. At the first trifoliate stage, medium-sized plants from each genotype were transplanted to plastic pots (10 cm length × 10 cm width × 15 cm height) for saline treatment, at a rate of one plant per pot. A total of 510 pots (3 plants × 85 genotypes × 2 treatments) were employed in each year. When not undergoing saline treatment, plants were regularly irrigated with tap water. Preventative insecticides were applied as needed during plant culture.

Air temperature around the canopy of the plants was monitored every hour, using a thermorecorder (TR-52S; T and D, Matsumoto, Japan). The daily mean air temperature during the experiments (from sowing to harvest) was higher in 2015 (22.7 °C) than in 2013 (19.9 °C). Data regarding solar radiation during the experiments were obtained from the website of Sendai Regional Headquarters, Japan Meteorological Agency, which is located approximately 3 km apart from the experimental site. The daily mean solar radiation was 18.3 and 17.4 MJ m⁻² in 2013 and 2015, respectively. Solar radiation inside the greenhouse was 10% lower than outside the house.

Saline treatment

Pots containing plants were placed in quadrilateral boxes (135 cm length × 91 cm width × 20 cm depth) containing saline water of 10 cm depth, which allowed plants to take up water through the hole at the bottom of each pot. Saline treatments were initiated at the second trifoliate compound leaf expansion stage. During the first week, 50 mM NaCl was added, followed by 100 mM in the second week, and 150 mM in the third week. The final NaCl concentration was determined based on previous studies.
that clearly distinguished cultivar differences in salinity tolerance after treatments with 100–150 mM NaCl (Lee et al., 2008; Valencia et al., 2008; Yasuta & Kokubun, 2014). Control plants were supplied with tap water using the same box setup as the saline treatments. Saline and tap water were renewed every few days.

**Measurements and data analyses**

Leaf chlorophyll content of the last expanded leaf was measured at several-day intervals during the saline treatment, using a chlorophyll meter (SPAD 502; Minolta Inc., Tokyo, Japan). After the 150 mM NaCl treatment, three or four plants per genotype were sampled, and separated into shoots and roots + nodules. Samples were oven-dried at 80 °C for 3 days, and then weighed. Subsequently, nodules were detached from roots, counted, and weighed. Dried samples of shoots were ground in a mill and N content was analyzed using an automated CN analyzer (JM1000CN; J-Science Lab Co., Ltd., Kyoto, Japan).

To evaluate salinity tolerance, we used the ratio of saline-treated (S) to control (C) plant total dry weight [DW(S/C)]. Correlations between total DW(S/C) and other traits associated with nodulation and N uptake (nodule number, nodule weight per plant/nodule, root weight) were determined.

Significant differences between genotypes, saline treatments, and years in traits were tested with ANOVA in JMP Statistical Discovery software (SAS Institute, Cary, NC, U.S.A.); individual means were compared with the Tukey’s test. The genotypic ranking for total DW(S/C) between the two years was determined with Spearman’s rank correlations.

**Results**

**Genotypic variation in salinity tolerance**

Analysis of variance (ANOVA) revealed that genotype, saline treatment, and year had a significant effect on total DW and the traits associated with nodulation (nodule number, nodule weight per plant/nodule) (Table 1), indicating that there exists a substantial genotypic variation among the diverse genetic resources in the responses of these traits responding to saline treatment. In addition, the analysis revealed interactive effects (genotype x treatment or year) on these traits, suggesting that the magnitude and manner of the genotypic variations are affected by saline condition and year.

A marked genotypic variation was observed in the total DW(S/C) in both years (2013, 2015). The data for the two years showed similar frequency distributions, but the values were generally greater in 2013 than in 2015 (Figure 1).

| Source of variation | Total DW | Nodule number per plant | Nodule weight per plant | Nodule weight per nodule |
|---------------------|----------|-------------------------|-------------------------|--------------------------|
| Genotype (G)        | ***      | ***                     | ***                     | ***                      |
| Saline treatment (T)| ***      | ***                     | ***                     | ***                      |
| Year (Y)            | ***      | ***                     | ***                     | ***                      |
| G x T               | ***      | ***                     | ***                     | ***                      |
| T x Y               | ***      | ***                     | ***                     | ***                      |
| Y x G               | ***      | ***                     | ***                     | ***                      |

*Significant at the p < 0.05 level.  
**Significant at the p < 0.001 level.

Table 1. Summary of ANOVA for total dry weight (DW) and traits associated with nodulation of 85 soybean genotypes.

Total DW(S/C), were stable across years, in which sowing times and climatic conditions differed (Spearman’s rank correlations; p < 0.01).

Table 2 lists all the genotypes examined in order of total DW(S/C) with their geological origin. We found that genotypes with both superior and inferior total DW(S/C) originated from vast geographical regions, including India, China, South-East Asia, and eastern Asia (Table 2). The ranking for NILs72-T, which was previously evaluated as salt-tolerant, was 9th, whereas that of NILs72-S was 46th. When comparing the morphology of tolerant versus sensitive genotypes under saline conditions, leaf wilting, and plant growth retardation were more pronounced in the latter (Figure 2).

**Relationship between salinity tolerance and traits involved in nodulation and N uptake**

Across both years, the ratios of nodule number and nodule dry weight per plant/nodule (S/C) were positively correlated with total DW(S/C), except for nodule number in 2013 (Figure 3). Furthermore, root DW (S/C) was positively correlated with shoot DW(S/C) across both years (Figure 4).

Regarding N uptake, total DW (actual value) was negatively correlated with shoot N concentration (actual value), but positively correlated with shoot N content (actual value) under both control and saline conditions (Figure 5). Significant correlations were observed regardless of treatment. Leaf N content (S/C) was positively correlated with SPAD reading (S/C) (Figure 6). Table 3 compares total DW and shoot N content [actual value and its ratio (S/C)] between salinity-tolerant and -sensitive genotypes, which ranked among the top 20 and bottom 20 in total DW(S/C) for the two years (refer to Table 2). Under saline conditions, salt-tolerant genotypes exhibited superior shoot N content [in both actual value and its ratio (S/C)].

**Discussion**

That saline stress during the vegetative growth stage can markedly reduce dry matter production and yield in
Table 2. The ratio of saline-treated (S) to control (C) plant total dry weight [DW (S/C)] of all the genotypes subjected to saline treatment (final concentration of 150 mM NaCl) for two years (2013, 2015) and their country of origin.

| Ranking | Genotype          | 2013 DW (S/C) | 2015 DW (S/C) | Origin     | Ranking | Genotype          | 2013 DW (S/C) | 2015 DW (S/C) | Origin     |
|---------|-------------------|---------------|---------------|------------|---------|-------------------|---------------|---------------|------------|
| 1       | Sandek Sieng      | 0.99          | 0.71          | Cambodia   | 42      | Mashokutou (Kou 503) | 0.77          | 0.52          | China      |
| 2       | Tegineneng        | 0.91          | 0.74          | Indonesia  | 46      | Nezumi Meta       | 0.58          | 0.71          | Korea      |
| 3       | Cheongye Myong-tae | 1.01          | 0.64          | Korea      | 46      | L 2A              | 0.76          | 0.52          | Philippines|
| 4       | M 44              | 0.97          | 0.67          | India      | 46      | NILS72-S          | 0.77          | 0.50          | *          |
| 5       | Hakuchikou        | 1.02          | 0.61          | China      | 49      | PK 73–54          | 0.62          | 0.65          | India      |
| 6       | L 317             | 0.92          | 0.70          | China      | 49      | M 581             | 0.60          | 0.67          | India      |
| 7       | Ringgit           | 0.87          | 0.73          | Indonesia  | 49      | Bhatmas           | 0.66          | 0.60          | Nepal      |
| 8       | EC 112,828        | 0.89          | 0.71          | India      | 49      | Merapi            | 0.74          | 0.52          | Indonesia  |
| 9       | Keumdu            | 0.85          | 0.74          | Korea      | 49      | Seita             | 0.60          | 0.66          | Korea      |
| 10      | U 1416            | 0.84          | 0.74          | Nepal      | 49      | Aoki Mame         | 0.69          | 0.56          | China      |
| 11      | NILS72-T          | 0.93          | 0.64          | *          | 55      | Chuuho 2          | 0.68          | 0.55          | Korea      |
| 12      | Chousenshu (Cha)  | 0.74          | 0.79          | Korea      | 56      | Oudu              | 0.69          | 0.53          | Korea      |
| 13      | U-7141–2 NO.3     | 0.86          | 0.67          | Nepal      | 57      | KS 1034           | 0.72          | 0.49          | Malaysia   |
| 14      | Choyoutou         | 0.87          | 0.66          | China      | 57      | Bishu Daizu       | 0.76          | 0.44          | Korea      |
| 15      | Peking            | 0.75          | 0.78          | China      | 59      | Anto Shokokutokut | 0.66          | 0.50          | China      |
| 16      | Kadi Bhatto       | 0.94          | 0.59          | Nepal      | 59      | Enrei             | 0.70          | 0.45          | Japan      |
| 17      | Karasumame (Heitou) | 0.76         | 0.76          | Taiwan     | 61      | M 918             | 0.70          | 0.44          | India      |
| 18      | San Sai           | 0.82          | 0.69          | Thailand   | 61      | Karasumame        | 0.69          | 0.44          | China      |
| 19      | U 1155–4          | 0.86          | 0.65          | Philippines| 61      | Jeokgak           | 0.77          | 0.37          | Korea      |
| 20      | MISS 33 DIXI      | 0.88          | 0.61          | Philippines| 64      | Songchunbaekjgam  | 0.69          | 0.44          | China      |
| 21      | Baritou 3A        | 0.91          | 0.56          | Indonesia  | 64      | N 2491            | 0.57          | 0.55          | Nepal      |
| 22      | M 652             | 0.89          | 0.57          | India      | 64      | JAVA 5            | 0.62          | 0.50          | Indonesia  |
| 23      | Manshuu Massho-kutou | 0.68         | 0.77          | China      | 64      | Pekin Dai Outou   | 0.55          | 0.57          | China      |
| 24      | Ichiguiuhou       | 0.89          | 0.55          | Japan      | 64      | Col/Pak/1989/ IBPGR/ | 0.63        | 0.48          | Pakistan   |
| 25      | Karasumame (Naihou) | 0.86         | 0.57          | Taiwan     | 64      | 2323(2)           | 0.61          | 0.45          | Taiwan     |
| 26      | U 1042–1          | 0.78          | 0.65          | Nepal      | 69      | Hakubi            | 0.69          | 0.41          | China      |
| 27      | Senyoutou         | 0.70          | 0.72          | China      | 69      | HM 39             | 0.65          | 0.45          | India      |
| 28      | Fiskeby V         | 0.82          | 0.57          | Sweden     | 69      | Heukdaelp         | 0.58          | 0.51          | Korea      |
| 29      | Col/Thai/1986/ Thai-78 | 0.76      | 0.63          | Thailand   | 69      | Kongnamul Kong    | 0.50          | 0.59          | Korea      |
| 30      | Chineuem Kong     | 0.71          | 0.67          | Korea      | 69      | Shirosota         | 0.65          | 0.44          | Korea      |
| 31      | En 1282           | 0.84          | 0.54          | Korea      | 74      | Karasumame (Shin-chiku) | 0.61        | 0.45          | Taiwan     |
| 32      | Massokutou (Kou 502) | 0.67         | 0.70          | China      | 74      | U 1290–1         | 0.71          | 0.35          | Nepal      |
| 33      | Seputhi Raman     | 0.69          | 0.67          | Indonesia  | 74      | Petek             | 0.57          | 0.49          | Indonesia  |
| 34      | Uronkon           | 0.80          | 0.56          | Korea      | 77      | N 2392            | 0.60          | 0.45          | Nepal      |
| 35      | Manshuu           | 0.81          | 0.54          | China      | 78      | N 2295            | 0.49          | 0.53          | Nepal      |
| 36      | Chienigmai Palmetto | 0.84         | 0.51          | Thailand   | 79      | Heamnam           | 0.70          | 0.31          | Korea      |
| 37      | Col/Pak/1989/ IBPGR/ | 0.82         | 0.52          | Pakistan   | 80      | M 42              | 0.47          | 0.41          | India      |
| 38      | JAVA 7            | 0.68          | 0.65          | Indonesia  | 74      | En-b0-1           | 0.76          | –             | *          |
| 39      | U 8006–3          | 0.79          | 0.53          | Nepal      | 82      | Williams 82       | 0.65          | –             | U.S.A.     |
| 40      | Hakka Zashi       | 0.74          | 0.58          | China      | 86      | Rigai Seioutou    | –             | 0.71          | China      |
| 41      | Col/Thai/1986/ Thai-80 | 0.69         | 0.62          | Thailand   | 86      | KE 32             | –             | 0.66          | Philippines|
| 42      | Pochal            | 0.67          | 0.63          | Taiwan     | 86      | Okjo              | –             | 0.56          | Korea      |
| 43      | Gapsanjaelae (1)  | 0.74          | 0.56          | Korea      | 86      | Mean              | 0.74          | 0.58          | Korea      |

Notes: All the genotypes examined are listed in order of the means of DW (S/C). The last five genotypes were excluded from the genotype ranking, because the data for one of the two years were not available. The values in italics and bold are ranked top 20 and bottom 20 genotypes for each year, respectively. *Experimental lines.

Soybean has been well documented (Abel & MacKenzie, 1964; Parker et al., 1983; Wang & Shannon, 1999; Yang & Blanchar, 1993). In this study, we therefore evaluated salinity tolerance via the ratio of saline-treated to control plant total dry weight [DW(S/C)] during the vegetative growth stage.
results revealed a marked genotypic variation in the total DW(S/C), with the ratios of the top several tolerant genotypes being more than 0.8, whereas those of the most sensitive genotypes were less than 0.5 (Figure 1, Table 2). The tolerant genotypes screened in the present study will have applications as genetic resources for breeding programs, whereas both tolerant and sensitive genotypes could be used as experimental materials in future physiological studies on salinity tolerance. Importantly, we were able to identify several genotypes more tolerant than NILs72-T and En-b0-1 (Table 2), both of which were previously identified as salt-tolerant (Do et al., 2016; Hamwieh et al., 2011; Yasuta & Kokubun, 2014), suggesting the possibility of identifying further tolerant cultivars.

We had expected that tolerant genotypes would originate from regions where salinity is a serious issue hampering soybean production. However, there was a considerable overlap in the countries of origin of tolerant and sensitive genotypes, ranging from India, through several South-Eastern Asian nations, to Korea. In addition, the agro-morphological traits (e.g. growth habit, seed shape/
uptake by the root system might contribute to the greater total DW(S/C). Thus, our data do not allow us to conclude whether symbiotic N₂ fixation or non-symbiotic N₂ absorption is more important for the enhanced N uptake of salt-tolerant cultivars. Nonetheless, the close correlation between total DW and shoot N content under saline conditions (Figure 5) and the greater shoot N content (actual value and S/C) of salt-tolerant genotypes (Table 3) suggest that superior N uptake is crucial to better dry matter production under saline conditions. However, there is a possibility that salt tolerance resulted in superior N uptake, rather than superior N uptake being responsible for the tolerance. Thus, on the basis of the results of the present study, it is not possible to conclusively determine the causal relationship between salt tolerance and N uptake in soybean.

In our previous study, we found that En-b0-1, a super-nodulating genotype, exhibits both greater N₂-fixing capacity and better salinity tolerance than its parental genotype Enrei, suggesting that nodulation capacity is partly responsible for salinity tolerance in soybean (Yasuta & Kokubun, 2014). In the present study, the non-nodulating genotype En1282 ranked higher than the super-nodulating genotype En-b0-1 and the normal nodulating genotype (Enrei). The reason for the inconsistency between the results of our present and previous studies is currently not clear.

Root and shoot DW(S/C) were closely correlated among genotypes (Figure 4), suggesting that non-symbiotic N uptake by the root system might contribute to the greater total DW(S/C). Thus, our data do not allow us to conclude whether symbiotic N₂ fixation or non-symbiotic N₂ absorption is more important for the enhanced N uptake of salt-tolerant cultivars. Nonetheless, the close correlation between total DW and shoot N content under saline conditions (Figure 5) and the greater shoot N content (actual value and S/C) of salt-tolerant genotypes (Table 3) suggest that superior N uptake is crucial to better dry matter production under saline conditions. However, there is a possibility that salt tolerance resulted in superior N uptake, rather than superior N uptake being responsible for the tolerance. Thus, on the basis of the results of the present study, it is not possible to conclusively determine the causal relationship between salt tolerance and N uptake in soybean.
Thus, the salinity tolerance of both wild and cultivated soybean is largely dependent on the ability to control ion (i.e. $\text{Na}^+$ and $\text{Cl}^-$) distribution in plant parts. Regarding the mechanisms regulating Na/Cl distribution in plants, Do et al. (2016) isolated a gene ($\text{Ncl}$) from a salt-tolerant soybean cultivar (FT-Abyara), which synchronously regulates ion ($\text{Na}^+$, $\text{K}^+$, and $\text{Cl}^-$) transport and accumulation. They showed that higher $\text{Ncl}$ expression in the root decreased ion accumulation in the shoot under saline conditions. We are currently investigating whether this $\text{Ncl}$ mechanism is associated with the genotypic variation in salinity tolerance among our studied genotypes.

Another physiological process that is probably involved in soybean salt tolerance is osmotic adjustment capacity under saline conditions. Under saline treatment, the salt tolerant AGS313, screened from 170 cultivars/lines, was able to maintain higher leaf water potential than other genotypes (Karim et al., 2012; Mannan et al., 2012). This cultivar also produced more biomass under saline conditions, and the authors ascribed its superior biomass production to the plant’s enhanced osmotic adjustment, which may enable the maintenance of higher photosynthetic activity. Thus, the mechanisms regulating soybean salinity tolerance are multifaceted. Although further studies are needed to clarify the overall processes underlining soybean salinity tolerance, this study has uncovered multiple salt-tolerant genotypes that can be employed as suitable materials for both breeding programs and future research.

Table 3. Total dry weight (DW) and shoot nitrogen content (NC) of salinity-tolerant and -sensitive genotypes subjected to saline conditions.

| Genotype       | Total DW (g plant$^{-1}$) | Shoot NC (mg plant$^{-1}$) | Shoot NC (S/C) |
|----------------|---------------------------|-----------------------------|----------------|
| Tolerant genotypes |                           |                             |                |
| Sandek Sieng    | 4.52bc                     | 91.4bc                      | 0.84           |
| Tegineneng      | 4.04c                      | 94.8ab                      | 0.97           |
| M 44            | 4.60bc                     | 82.4bc                      | 0.73           |
| L 317           | 4.33c                      | 78.0c                       | 0.80           |
| Ringgit         | 3.69c                      | 91.0bc                      | 0.94           |
| EC 112,828      | 5.25b                      | 107.9a                      | 0.94           |
| Keumdu          | 4.75bc                     | 92.6b                       | 1.04           |
| U 1416          | 6.26a                      | 88.5bc                      | 0.71           |
| U-1741–2-2 No.3 | 4.68bc                     | 89.7bc                      | 0.93           |
| Mean            | 4.68***                    | 90.7***                     | 0.88***        |

| Genotype       | Total DW (g plant$^{-1}$) | Shoot NC (mg plant$^{-1}$) | Shoot NC (S/C) |
|----------------|---------------------------|-----------------------------|----------------|
| Sensitive genotypes |                           |                             |                |
| M 42            | 2.18c                      | 48.7b                       | 0.73           |
| N 2392          | 2.80b                      | 61.9ab                      | 0.59           |
| Petek           | 2.51bc                     | 60.0ab                      | 0.71           |
| Karasumame (Shinchiku) | 3.34ab                     | 77.4a                       | 0.78           |
| Shiranawa       | 3.26ab                     | 64.0ab                      | 0.69           |
| HM 39           | 2.98ab                     | 51.0b                       | 0.65           |
| Col/Pak/1989/IB-PGR/2323(2) | 2.60bc                     | 58.5ab                      | 0.61           |
| JAVA 5          | 3.30ab                     | 75.7ab                      | 0.78           |
| Anto Shouko-Kutou | 3.53a                      | 73.6ab                      | 0.68           |
| Mean            | 2.94                       | 63.4                        | 0.69           |

Notes: The top 20 (tolerant) and bottom 20 (sensitive) ranked genotypes in both years are listed (refer to Table 2. Total DW (g plant$^{-1}$), shoot NC (mg plant$^{-1}$): Actual values for plants subjected to saline conditions. Shoot NC (S/C): Ratio of saline-treated (S) to control (C) plant NC. The data obtained in 2015 are shown. Values followed by the same letter within respective groups are not significantly different at $p < 0.05$ as determined by Tukey’s means comparison test. ***Significantly different between tolerant and sensitive genotypes at $p < 0.001$. **
Disclosure statement
No potential conflict of interest was reported by the authors.

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