Research article

Effects of moderate soil salinity on osmotic adjustment and energy strategy in soybean under drought stress

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ABSTRACT

Under drought and soil salinity, plants usually respond to accumulate inorganic and organic osmolytes for adaptation, that would induce changes in energy consumption strategy of plants. Moderate soil salinity would enable plants to lower energy consumption for osmotic adjustment by passively absorbing more Na^+. This action would keep more energies for growth of drought-stressed plants. Thus, Na^+ accumulation might be an energy-efficient strategy for plants to cope with drought was speculated. To support this speculation, we assessed the effects of soil salinity on osmotic adjustment and energy utilization under drought in this study. Our results indicated that the ratio and content of inorganic osmolytes was significantly higher under drought-saline stress (D + S) than those under single drought stress (D), while the osmolarity and contents of organic osmolytes of D + S were significantly lower than those of D. This indicated that moderate soil salinity could enable soybean seedlings to consume relatively lower energies to produce less organic osmolytes and accumulate more inorganic ions for osmotic adjustment coping with drought. Meanwhile the water content, cell turgor, ash content, and specific leaf area and biomass of D + S were significantly higher than those of D, but the leaf construction cost of D + S was significantly lower than those of D. This suggested that moderate soil salinity could enhance water retention, and reduce the photoassimilate and energy consumption of droughty soybean seedlings. This work would help to understand the positive effects of moderate soil salinity on plant growth on the level of osmotic adjustment and energy consumption strategy.

1. Introduction

Drought and salinity are considered as two most serious abiotic stresses for plant growth and productivity, resulting in a sequence of morphological, biochemical and molecular alterations (Boyer, 1982; Sahin et al., 2018). Particularly in the arid and semi-arid regions, evaporation and upward water extraction by plant roots cause the soil solution to become concentrated and salt levels in near-surface soil to increase. Thus, drought routinely overlaps with soil salinity, and these both affect plant growth and productivity together.

Osmotic adjustment is an important feature allowing plants to adapt in osmotic stress conditions. Under osmotic stress, plants can accumulate osmotically compatible solutes to lower osmotic potential, and maintain cell turgor and physiological processes (Gebre et al., 1994; Turner, 2017; Dutta et al., 2018). In plants, there are two kinds of osmolytes mediating osmotic adjustment, organic solutes, such as sucrose, polyols, glycine betaine, and proline; and inorganic ions, such as K^+, Ca^{2+}, Na^+, Mg^{2+}, and Cl^- (Chen and Jiang, 2010). Among them, the synthesis of organic osmolytes consumes a considerable amount of

energy in plants. Furthermore, the synthesis, accumulation and translocation of massive organic osmolytes would interfere with and reorient the metabolic processes of plants (Sun et al., 2015), and then interrupt plant growth and reduce productivity.

The energy consumption required for transferring and accumulating inorganic ions as osmotic adjustment substances was known to be far lower than that required for synthesizing and accumulating of organic osmolytes in plants (Yeo, 1983; Parida and Das, 2005; Zeng et al., 2015). Under salt stress with drought, plants could quickly passively absorb some inorganic ions like Na^+ and accumulate some organic molecules, such as proline and betaine to maintain the turgor (Munns and Tester, 2008). In view of energy consumption, under drought stress with salinity, plants need to consume more energy for accumulating inorganic ions and organic osmolytes, and accordingly the energy able to be used for plant growth is relatively decreased, that results in reduced biomass accumulation and crop production of plants under drought stress with salinity. However, if the salinity level in soil is affordable and appropriate, some plant species under drought stress could make greater use of inorganic ions to achieve osmotic adjustment, so

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that they would save more residual photoassimilate and energy for plant growth and productivity. Therefore, this could easily explain that low to moderate Na^+ concentrations are commonly found to be beneficial, and even stimulate the growth of many plant species under drought conditions (Ikeda et al., 2007; Schulze et al., 2012; Kang et al., 2015). However, the salt-tolerance threshold values of different plant species under drought conditions varies largely (Wang et al., 2017), and the degree of salinity stress which could stimulate plant growth and development depends on plant species.

For this postulation of energy consumption, there has been no direct experimental evidence to confirm it. In this study, we assessed the effects of moderate soil salinity on the accumulation of Na^+, K^+, proline, betaine, soluble sugars, and other osmolytes-induced osmotic adjustment and energy utilization in plants under drought stress. Soybean [*Glycine* max (L.) Merr. variety Jiyu 5] seedlings considered as a salt-sensitive to moderately salt-tolerant crop (Umezawa et al., 2000), was selected as the research object. To comprehensively explain the effects on osmotic adjustment and energy utilization, the parameters related to water status, osmotic adjustment, leaf construction cost, biomass were quantified in this experiment.

2. Materials and methods

2.1. Plant growth conditions

The soybean [*Glycine* max (L.) Merr. variety Jiyu 5] seeds were sown in pots (38.5 cm internal diameter × 34.0 cm tall) filled with 26.5 kg of cultivated soil (haplic luvisols), with periodic watering. A total of 20 pots were used in this experiment. After germination, soybean seedlings were thinned to 10 seedlings per pot and then to 5 seedlings per pot at the 4-leaf stage. The cultivated soil had a bulk density of 0.91 g cm^{-3}, a field water capacity of 50.3%, a pH of 6.8, an electrical conductivity of 0.89 mS cm^{-1}, and available N, P, and K of 48.12, 21.27 and 31.09 mg kg^{-1}, respectively. The potted seedlings were grown in a greenhouse with a long-day photoperiod (16 h light/8 h dark), and the highest and lowest temperatures were 18.7 °C and 34.1 °C, respectively. The maximum photosynthetic photon flux density was approximately 910 μmol m^{-2} s^{-1}, and the relative humidity ranged from 40.7 to 81.2%.

2.2. Stress treatments

The soybean seedlings were subjected to stress treatments when they reached a height of approximately 20 cm (50 days old). We established four different treatments: control (well watered and salt free, Ctrl), salt stress alone (S), drought stress alone (D), and combined drought and salt stress (D + S), and each treatment included 5 pots of soybean seedlings as 5 biological replications. More specifically, the Ctrl and D-soybean seedlings were first fully irrigated with untreated water, and the S and D + S-soybean seedlings were fully irrigated with a 100 mM NaCl solution (our laboratory work had indicated that this salt concentration was equivalent to mild to moderate salinity stress in soybean seedlings). Thereafter, the Ctrl and S treatments were periodically watered to maintain a 75–80% relative soil water content. Irrigation was withheld in the D and D + S treatments to achieve natural water loss and thus induce drought stress. On the 30th day after treatment (permanent wilting had appeared in some leaves of the seedlings treated with drought stress), the aerial part of soybean seedlings was harvested, and related measurements were carried out.

2.3. Measurement methods

2.3.1. Leaf water potential, osmotic potential and water content

Leaf pre-dawn water potential was measured with a pressure bomb following the method (Miller, 1985). Leaf osmotic potential was measured with a dew point microvolt-meter (HR-33T, Wescor, USA), as outlined in Song et al. (2006). Leaf disks (12.4 mm in diameter) were punched from fully developed leaves and placed into an Eppendorf tube, capped, and stored in a freezer at −20 °C for 12 h. The frozen leaf disks were allowed to thaw at room temperature and osmotic potential was measured after thawing. The cell turgor pressure was calculated using the following formula: cell turgor pressure = water potential − osmotic potential. Water content of leaves was calculated using the following formula: water content (%) = (fresh weight − dry weight)/fresh weight × 100%.

2.3.2. Osmolarity of leaf sap

Osmolarity in leaf sap was measured according to Senguttuvel et al. (2014). Fresh functional leaf samples (approximately 10 g) were taken and kept at −80 °C overnight. The next day, the leaves were removed and allowed to thaw at room temperature for 30 min. The sap was removed by applying pressure to the leaves in a 10-ml syringe. The total osmolarity (osmolarity of the expressed sap) was measured with a dew point microvolt-metre (HR-33T, Wescor, USA). The expressed sap was then centrifuged at 3500 × g for 10 min, and the supernatant was filtered with a 0.45 μm filter membrane into a 10-ml vial. The 3 ml filtrate was moved into a crucible, desiccated at 70 °C, and burnt in a muffle furnace for 8 h at 600 °C. The mineral residue was dissolved in distilled water (3 ml), and the osmolarity of inorganic ions was then measured with a dew point microvolt-metre (HR-33T, Wescor, USA) and used in the following equation: osmolarity of organic osmolytes = total osmolarity − osmolarity of inorganic ions.

2.3.3. Proline, betaine, and total soluble sugars

Proline content was determined spectrophotometrically following the ninhydrin method (Bates et al., 1973). Approximately 1.0 g of ground-dried samples was homogenized in 10 ml of 3% (w/v) aqueous sulphosalicylic acid and filtered. 1 ml filtrate was mixed with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid and boiling for 60 min, the tubes were cooled and 5 ml toluene were added, shaking well and letting stand for 3 h. The absorbance of the upper phase was spectrophotometrically determined at 520 nm. The proline content was calculated using a standard curve.

Glycinebetaine (Betaine) was determined according to Li et al. (2013) with some modifications. Approximately 0.5 g of fresh leaf samples was homogenized in 2 ml of 80% (v/v) methanol and shaken at 60 °C for 30 min. The mixture was centrifuged at 10,000 × g for 15 min, and the supernatants were mixed with Reineckesalt and generated red precipitates, which was dissolved in 70% (v/v) acetone and the absorbance was determined using a spectrophotometer at 525 nm. The betaine content was calculated using a standard curve.

Total soluble sugars were determined based on the anthrone method (Irigoyen et al., 1992). Approximately 0.5 g of fresh leaf samples was homogenized in 10 ml of 80% (v/v) ethanol and stirred at 80 °C for 40 min. The homogenate was centrifuged at 3000 × g for 10 min and the supernatant was decanted. The residue was resuspended in 5 ml of 80% ethanol and repeatedly extracted twice, the supernatant was combined with the original extract and made up to 25 ml with distilled water. 1 ml extract was mixed with 4 ml of 0.15% (v/v) anthrone and boiling for 10 min. The reaction was terminated by incubating the mixture on ice for 5 min. The absorbance of the mixture was determined using a spectrophotometer at 620 nm. Total soluble sugars were calculated by creating a standard curve using a standard glucose.

The contents of proline, betaine and total soluble sugars were expressed as μmol g^{-1} DW and mg g^{-1} DW, respectively.

2.3.4. Water-soluble substances

The extraction of water-soluble substances followed the methods (Gebre et al., 1994). Approximately 3.0 g of fresh leaf sample (the mass was converted to dry weight according to water content and recorded as M) was homogenized with 10 ml distilled water in a 15-ml centrifuge tube and incubated in a water bath at 70 °C for 25 min. The sample was

Table 1
The water status of soybean seedlings under different treatments (mean ± SD, n = 5).

Treatment	Water potential (MPa)	Osmotic potential (MPa)	Cell turgor (MPa)	Osmotic adjustment (MPa)	Water content (%)	Leaf state
Ctrl	−0.74 ± 0.06a	−0.96 ± 0.05a	0.22 ± 0.02a	0c	74.24 ± 0.41a	Normal
S	−0.86 ± 0.03b	−1.01 ± 0.03a	0.16 ± 0.03b	0.05 ± 0.02c	73.77 ± 0.50a	Normal
D	−1.69 ± 0.06c	−1.67 ± 0.06b	−0.02 ± 0.02d	0.71 ± 0.06b	64.59 ± 0.89c	Severe wilting
D + S	−1.71 ± 0.05c	−1.76 ± 0.06c	0.05 ± 0.02c	0.80 ± 0.06a	67.68 ± 0.94b	Mild wilting

Ctrl: control; S: salt stress alone; D: drought stress alone; D + S: combined drought and salt stress. Osmotic adjustment is the difference between the osmotic potential of unstressed leaves and the osmotic potential of stressed leaves. Different letters in the columns represent significant differences at P < 0.05.

then centrifuged at $3500 \times g$ for 10 min, and the supernatant was filtered with a 0.45 μm filter membrane into a 20-ml test tube. The extraction was repeated 2 times for each sample. The filtered extracts were combined, frozen and lyophilized. The lyophilized extract was moved into a pre-weighed crucible (M_0), desiccated at 70 °C until its mass stabilized, and weighed (M_1). The extract was then burnt in a muffle furnace for 8 h at 600 °C and weighed (M_2) after cooling. The contents of total water-soluble substances, water-soluble organic substances and water-soluble inorganic substances were calculated using the following formulas:

The content of total water-soluble substances ($g\,g^{-1}DW$) = $(M_1 − M_0)/M$;

The content of water-soluble organic substances ($g\,g^{-1}DW$) = $(M_1 − M_2)/M$; and

The content of water-soluble inorganic substances ($g\,g^{-1}DW$) = $(M_2 − M_0)/M$.

In this experiment, the contents of organic and inorganic osmolytes were represented by the content of water-soluble organic and inorganic substances, respectively, because all water-soluble substances in cells can contribute to osmotic adjustment.

2.3.5. Na⁺, and K⁺

Dried finely-ground samples were ashed in a muffle furnace for 6 h at 550 °C. The ash was dissolved in nitric acid, diluted in distilled water and filtered through a sheet of filter paper. Na^+ and K^+ contents were determined by atomic-absorption spectrophotometer (GBC-932B, GBC, AU). Blank control was distilled water with equal amount of nitric acid.

2.3.6. Shoot biomass

Dry weight was obtained from samples oven-dried at 70 °C until their mass stabilized. The increase in shoot biomass was calculated from the difference between the final and initial shoot biomass.

2.3.7. Specific leaf area (SLA) and leaf construction cost

SLA was measured according to the method (Ackerly et al., 2002).

Leaf construction cost per unit mass (CC_{mass}) was calculated according to Williams et al. (1987), with the equation CC_{mass} (g glucose g^{-1}) = $[(0.06968\ AFCV − 0.065)(1 − AC) + 7.5(k\ N_{mass}/14.0067)]/EG$, where AFCV is the ash-free caloric value ($kJ\,g^{-1}$), AC is the ash content ($g\,g^{-1}DW$), k is the oxidation state of the nitrogen substrate (+5 for nitrate or −3 for ammonium), N_{mass} is the nitrogen content ($g\,g^{-1}DW$) and EG is the growth efficiency, estimated to be 0.87. CC_{mass} was estimated as the mean of the construction cost values calculated with k = +5 for NO_3^- and −3 for NH_4^+, because the relative proportions of these forms of N utilized by the plants are unknown (Song et al., 2007).

Leaf construction cost per unit area (CC_{area}) was calculated according to Song et al. (2007), with the equation CC_{area} (g glucose·m^{-2}) = CC_{mass}/SLA.

2.3.8. Ash content

Approximately 1 g of ground sample was placed in a pre-weighed crucible. The sub-sample was burnt in a muffle furnace for 8 h at 600 °C. The mineral residue was then weighed. Ash content (%) was expressed as a dry mass.

2.3.9. Caloric value

Before being measured, the plant samples (powders) were dried again at 80 °C for 3 h. The gross caloric value (GCV) was measured using an oxygen bomb calorimeter (C200, IKA, Germany). The measurement was done at a laboratory temperature of 20 °C. The ash-free caloric value (AFCV) was calculated according to Zhou et al. (2010), using the equation AFCV = $GCV/(1 − AC)$, where AC is the ash content ($g\,g^{-1}DW$).

2.4. Data and statistical analysis

All measurements were repeated 5 times in this experiment. The data are presented as the mean ± SD (n = 5). The differences between treatments were analyzed by one-way ANOVA and the significant differences among means were identified by Duncan's test. All statistical analyses were performed using SPSS software (SPSS Inc. Chicago, Illinois, USA).

3. Results

3.1. Water status

The leaf water status reflects the metabolic activity and drought resistance of plants (Yan et al., 2016). To assess the water status of leaves, the water potential, osmotic potential, cell turgor pressure, and water content of leaves of soybean seedlings under the different treatments were measured (Table 1). D and D + S could induce a remarkable decrease in the leaf water potential, osmotic potential, cell turgor, and water content compared with the control, and the leaf growing status of D, and D + S was found to have a certain degree of wilting (Table 1). Moreover, S could only induce a slight decrease in the leaf water potential, and cell turgor pressure, but not in the leaf osmotic potential, water content, and the leaf growing status (Table 1). These results indicated that saline stress investigated in this experiment could only slightly affect the growth status of soybean seedlings, and drought stress could significantly affect the plant growth. In terms of the descent degree of water potential, osmotic potential, cell turgor pressure, and water content, drought stress could cause more serious effects than the salinity stress implemented in this experiment.

However, under drought conditions, the presence of salinity stress kept the water potential of soybean leaves at the similar level as that of D, and made the cell turgor pressure, and water content significantly increased compared to those of D (Table 1). This indicated that the D + S treatment seedlings were superior to the D treatment seedlings in terms of water absorption and retention, and the leaf growth status was also better in the D + S treatment than that in the D treatment.

3.2. The osmotic adjustment substances

To resist the exogenic stress, soybean seedlings forwardly improved the osmotic adjustment. Under drought conditions, this phenomenon was more prominent, particularly in the presence of salinity stress simultaneously. However, it is not clear that, under drought conditions,

Table 2
The osmotic adjustment substances in soybean leaves under different treatments (mean $\pm$ SD, n = 5).

Treatment	Total osmolality (mmol kg^{-1})	Osmolarity of inorganic ions (mmol kg^{-1})	Osmolarity of organic osmolytes (mmol kg^{-1})	Ratio of osmotic adjustment from inorganic ions (%)	Content of inorganic osmolytes (mg g^{-1}DW)	Content of organic osmolytes (mg g^{-1}DW)
Ctrl	392.74 $\pm$ 19.70d	131.90 $\pm$ 6.27d	250.84 $\pm$ 13.71c	34.47 $\pm$ 0.46d	35.92 $\pm$ 1.91c	242.22 $\pm$ 6.66c
S	414.80 $\pm$ 14.75c	178.80 $\pm$ 7.32c	236.00 $\pm$ 9.56c	43.11 $\pm$ 0.97b	40.26 $\pm$ 3.15b	243.58 $\pm$ 5.29c
D	673.56 $\pm$ 23.67b	261.82 $\pm$ 10.65b	411.74 $\pm$ 14.37a	38.86 $\pm$ 0.60c	42.68 $\pm$ 2.66b	281.14 $\pm$ 8.35a
D + S	710.76 $\pm$ 23.11a	342.68 $\pm$ 13.81a	368.08 $\pm$ 13.94b	48.21 $\pm$ 1.10a	53.34 $\pm$ 2.46a	268.30 $\pm$ 6.01b

Ctrl: control; S: salt stress alone; D: drought stress alone; D + S: combined drought and salt stress. Ratio of osmotic adjustment from inorganic ions refers to the percentage of inorganic osmolarity in the total osmolarity of leaf sap. Different letters in the columns represent significant differences at P < 0.05.

whether the presence of moderate salinity could induce soybean seedlings to more effectively utilize inorganic osmolytes for osmotic adjustment? To access this question, the osmolarity and content of organic and inorganic osmolytes in soybean leaves under different treatments were determined (Table 2). The osmolarity of total osmolytes, organic osmolytes, and inorganic ions in the drought-treated soybean leaves (D, and D + S) were significantly higher than those in non-drought-treated leaves (Ctrl, and S). Furthermore, under drought conditions, the osmotic adjustment (Table 1), total osmolarity, and the osmolarity and content of inorganic osmolytes (Table 2) of the treatment D + S leaves were significantly higher than those of the treatment D leaves, but the osmolarity and content of organic osmolytes of D + S were significantly lower than the latter. Thus, the increased total osmolarity in the treatment D + S leaves needed to be supplemented by higher osmolarity of inorganic ions. This similar phenomenon also appeared under non-drought conditions: increased osmotic adjustment and total osmolarity in the treatment S leaves mainly resulted from the significantly increased osmolarity of inorganic ions, while the osmolarity of organic osmolytes between under S and Ctrl had no significant change (Tables 1 and 2). The increased accumulation of inorganic ions mainly was resulted from the passive absorption of inorganic ions (mainly Na$^+$) caused by the exoteric soil salinity (Chen et al., 2007). This hypothesis was validated by that the ratio and content of inorganic osmolytes in the salt-treated soybean leaves (S, and D + S) were significantly higher than those in the corresponding salt free-treated ones (Ctrl, and D, Table 2).

In view of the contents of main organic osmolytes, the contents of proline, betaine, and soluble sugars were all increased significantly under all stress treatments compared to those of control. However, under drought conditions, the presence of soil salinity (D + S treatment) caused a significant decrease in the contents of proline, and betaine, and a slight decrease in the contents of soluble sugars than drought alone (D treatment, Table 3). To supplement the highly increased total osmolarity under the D + S treatment (Table 2), the content of Na$^+$ increased significantly in the D + S treatment leaves compared to that in the D treatment leaves (Table 3). Therefore, it could be postulated that, under drought conditions, the presence of moderate soil salinity enabled soybean seedlings to lower their osmotic potential by accumulating more inorganic ions and reducing the synthesis of organic osmolytes. As the increased Na$^+$ contents under salinity conditions mainly relied on the passive absorption from the

external environments, thus, this action was more energy-efficient than active transferring and accumulating in vivo. In addition, combined with the less synthesis of more energy-consuming organic osmolytes, the presence of moderate soil salinity could save more photoassimilate and energies used for osmotic adjustment in soybean seedlings under drought conditions.

3.3. The leaf construction cost

The presence of soil salinity can reduce the synthesis of organic osmolytes and increase the accumulation of inorganic salts, which may lead to changes in photoassimilate utilization and energy strategy. Leaf construction cost and other related parameters were determined in this experiment (Table 4). Under a natural water supply, although the ash content of soybean seedlings subjected to salt stress alone (S) was higher than that in the seedlings subjected to the control (Ctrl), the ash-free caloric value (AFCV) and construction cost per unit mass (CC$_{mass}$) of the leaves showed no significant difference between the S, and Ctrl treatments (Table 4). However, the SLA was significantly lower in the treatment S than in the treatment Ctrl, the leaf construction cost per unit area (CC$_{area}$) in S was, therefore, significantly higher than that of Ctrl. This indicated that under a natural water supply, the presence of moderate soil salinity reduced soybean leaf area but had little effect on photoassimilate and energy utilization.

Under drought stress, although the different of the AFCV between D and D + S was not significant, the ash content and SLA of D + S were significantly higher than those of D, while the leaf construction cost per unit mass (CC$_{mass}$) and per unit area (CC$_{area}$) were significantly lower in the former than those in the latter (Table 4). These results could be inferred that, under drought stress, the presence of moderate soil salinity could lessen the consumption of photoassimilates and energy.

3.4. The biomass accumulation

To resist the exogenic single salinity stress, soybean seedling used more passively absorbed inorganic ions for osmotic adjustment. This action was surely the more energy-efficient way for osmotic adjustment compared to using organic osmolyte accumulation, but it did required extra energy consumption for osmotic adjustment compared to the control. The shoot biomass of the treatment S was significantly lower than that of the Ctrl seedlings (Fig. 1), suggesting that the soil salinity

Table 3
The contents of main organic osmolytes (proline, and betaine), inorganic salt ions (Na$^+$, and K$^+$), and soluble sugars in soybean leaves under different treatments (mean $\pm$ SD, n = 5).

Treatment	Proline (μmol g^{-1}DW)	Betaine (μmol g^{-1}DW)	Soluble sugars (mg g^{-1}DW)	Na$^+$ (mg g^{-1}DW)	K$^+$ (mg g^{-1}DW)
Ctrl	15.68 $\pm$ 0.46d	8.65 $\pm$ 0.25d	21.68 $\pm$ 1.67c	0.59 $\pm$ 0.08c	23.4 $\pm$ 1.43a
S	34.13 $\pm$ 1.02c	15.41 $\pm$ 0.70c	36.33 $\pm$ 2.08b	4.57 $\pm$ 0.33b	23.43 $\pm$ 0.67a
D	68.64 $\pm$ 1.19a	27.10 $\pm$ 1.46a	52.29 $\pm$ 1.27a	0.97 $\pm$ 0.14c	23.11 $\pm$ 1.23a
D + S	58.23 $\pm$ 2.02b	22.92 $\pm$ 0.90b	52.03 $\pm$ 1.18a	6.46 $\pm$ 0.46a	23.51 $\pm$ 1.59a

Ctrl: control; S: salt stress alone; D: drought stress alone; D + S: combined drought and salt stress. Different letters in the columns represent significant differences at P < 0.05.

Table 4
The leaf construction cost and other related parameters in soybean seedlings under different treatments (mean $\pm$ SD, n = 5).

Treatment	Ash content (%)	AFCV (kJ·g^{-1}DW)	SLA (m^2·kg^{-1}DW)	CC$_{mass}$ (g glucose·g^{-1})	CC$_{area}$ (g glucose·m^{-2})
Ctrl	8.82 $\pm$ 0.29c	18.93 $\pm$ 0.10c	27.22 $\pm$ 0.75a	1.338 $\pm$ 0.005bc	49.189 $\pm$ 1.400d
S	9.27 $\pm$ 0.20b	19.06 $\pm$ 0.06bc	24.04 $\pm$ 0.77b	1.341 $\pm$ 0.006b	55.818 $\pm$ 1.772c
D	9.33 $\pm$ 0.14b	19.21 $\pm$ 0.09a	21.54 $\pm$ 0.81d	1.352 $\pm$ 0.007a	62.875 $\pm$ 2.271a
D + S	10.50 $\pm$ 0.27a	19.16 $\pm$ 0.11 ab	22.70 $\pm$ 0.62c	1.330 $\pm$ 0.011c	58.632 $\pm$ 1.492b

Ctrl: control; S: salt stress alone; D: drought stress alone; D + S: combined drought and salt stress. AFCV: ash-free calorific value; SLA: specific leaf area; CC$_{mass}$: leaf construction cost per unit mass; CC$_{area}$: leaf construction cost per unit area. Different letters in the columns represent significant differences at P < 0.05.
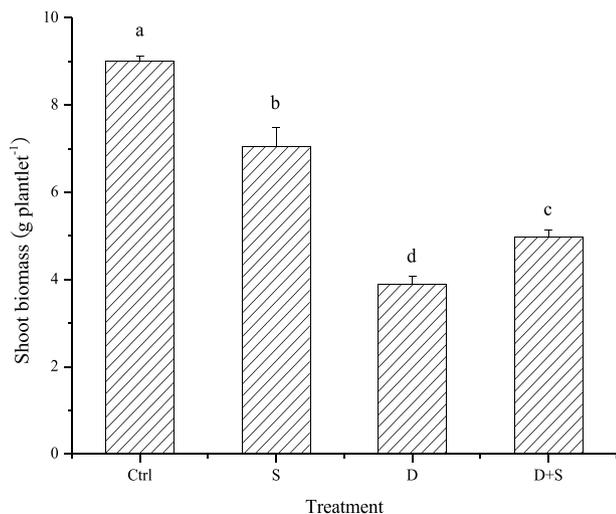

Fig. 1. Shoot biomass of soybean seedlings under different treatments. Ctrl: control; S: salt stress alone; D: drought stress alone; D + S: combined drought and salt stress. Different letters above bars indicate significant differences at P < 0.05.

investigated in this experiment could affect the biomass accumulation of the S treatment seedlings. Similarly, soybean seedlings exposed to single drought stress needed to invoke additional energies to synthesize osmolytes for osmotic adjustment, which also affected and hindered biomass accumulation (Fig. 1). However, the shoot biomass of the treatment D + S was significantly higher than that of the treatment D seedlings (Fig. 1), suggesting that under drought conditions, moderate soil salinity could enable soybean seedlings to use more inorganic ions for osmotic adjustment, and accordingly reduce the consumption of photoassimilate and energy. Thus, more saved energies could be used for biomass accumulation under the D + S treatment. This indicated that both drought and salinity could inhibit soybean growth and reduce its biomass accumulation, however, the presence of moderate soil salinity may alleviate the inhibitory effect of drought stress on soybean growth.

4. Discussion

Osmotic adjustment is considered as one of the key processes in plant adaptation to drought and salinity. It can reduce the osmotic potential of plant tissues through the accumulation of low-molecular weight organic solutes and inorganic ions, and therefore minimize water loss and maintain water uptake (Parida and Das, 2005; Gagneul et al., 2007; Blum, 2016). Both organic solutes and inorganic ions play crucial roles in osmotic adjustment, and their type, content and relative contribution vary with environmental factors, plant species, and so on (Chen and Jiang, 2010). Due to the energy consumption required for ion transport across membranes and the synthesis of organic osmolytes, some researchers believe that, the energy consumption required for synthesizing organic osmolytes is much greater than that required for accumulating inorganic ions (Yeo, 1983; Schröppel-Meier and Kaiser,

1988; Munns, 2002; Parida and Das, 2005; Zeng et al., 2015). Thus, in order to mediate osmotic adjustment, the transfer and accumulation of inorganic ions are more energy economical for plants compared with the synthesis of organic osmolytes (Tyerman et al., 2019). Especially, under osmotic stress caused by drought and salinity, some plant species were induced to passively absorb more inorganic ions from exoteric environments for osmotic adjustment, that consumed few energies. Despite an active removal of Na$^+$ from the cytosol into the vacuole through enhancing relevant transporters (Rea and Sanders, 1987; Tyerman et al., 2019) and Na$^+$ sequestration in vacuole are also a process requiring energy consumption for plants (Shabala et al., 2019), we can still obtain many evidences that moderate soil salinity is beneficial to plant growth, especially under drought conditions (Ikeda et al., 2007; Schulze et al., 2012; Ma et al., 2014; Kang et al., 2015; Zeng et al., 2015). In terms of this conclusion, the energies required for Na$^+$ transfer to vacuole and vacuolar sequestration must be no more than that saved by the synthesis of organic osmotics. Thus, we boldly deduced that for some kinds of plant species, a certain degree of soil salinity could improve osmotic adjustment, plant growth, stress tolerance, and even crop production, resulting from using a more energy-efficient method to accumulate osmolytes for osmotic adjustment (more inorganic ions and less organic osmolytes) and thereby having relatively more residual photoassimilate and energies for plant growth and other stress adaptive responses.

As the tolerance of salinity and drought stress varies markedly among plant type. For the drought and salt tolerance degree of soybean seedlings, our previous laboratory study suggested that the 50–100 mM NaCl treatment (considered as low to moderate salinity stress) could affect growth and water status, but alleviate the detrimental effects on growth and water status under drought conditions. The 150–200 mM NaCl treatment (considered as high salinity stress) could all cause serious damage under between single salinity and combined drought and salinity stress. Therefore, in this study, the 100 mM NaCl, equivalent to the moderate salinity stress for soybean seedlings, were used as the salt treatment concentration. When treated by 100 mM NaCl, the soybean seedlings had showed significant changes in biomass accumulation, water potential, and osmotic potential, but the situation could be obviously improved when overlapped with drought stress. Importantly, all of the above results are based on plants subjected to mild salt stress. Undoubtedly, severe salt stress or salt stress combined with drought will cause more serious damage to plants.

In view of the experimental results, all 3 treatments (D, S, and D + S) could all induce the osmotic potential of soybean leaves to decrease. The similar stress responses had been verified in some previous studies (Chen and Jiang, 2010). Under a natural water supply and drought conditions, the presence of soil salinity could significantly cause the osmolarity, ratio and content of inorganic ions to increase. When the drought stress lasted 30 days, the treatment D soybean leaves exhibited permanent wilting, and the cell turgor pressure became negative, indicating that the impact of drought stress had exceeded the capacity of osmotic adjustment. However, under the same drought stress, the presence of soil salinity made the treatment D + S soybean leaves only mild wilting, and the cell turgor pressure and water content in these leaves were significantly higher than those in the treatment D leaves. These findings indicate that the presence of moderate soil

salinity enables soybean seedlings to absorb and accumulate more inorganic ions to participate in osmotic adjustment, which is beneficial for absorbing and holding water, keeping water balance, and maintaining plant metabolism and growth in soybean seedlings under drought stress.

Calorific value refers to the combusted heat per unit dry mass (Lin and Cao, 2008). AFCV (ash-free calorific value) has been considered to be a reliable estimate of the synthetic cost of leaves (Mcdermitt and Loomis, 1981). Construction cost (CC) is a quantifiable measure of energy demand for biomass production or growth that represents the energy investment cost and utilization strategies of plants (Villar and Merino, 2001). A low construction cost is hypothesized to give plants a growth advantage by allowing them to utilize energy efficiently (Song et al., 2007). The significantly higher AFCV, CC_{mass} (leaf construction cost per unit mass) and CC_{aera} (leaf construction cost per unit area) of soybean leaves were found under each of 3 treatments (D, S, and D + S) compared with the control (Ctrl). This finding illustrated that soybean seedlings needed to consume energy to perform adaptive reactions, such as osmotic adjustment and antioxidant protection, thereby resulting in an increase in the biomass construction cost. However, under drought stress, the presence of moderate soil salinity could cause the ash content in the treatment D + S soybean leaves increase and the CC_{mass} and CC_{area} significantly decrease. This indicated that the presence of moderate soil salinity, together with drought, enabled soybean seedlings to accumulate more Na^+ and other ions, and most of the ions are compartmentalized into vacuoles and used as osmotic adjustment substances, which can enhance the water absorption and retention, and reduce the photoassimilate and energy consumption for the synthesis of organic osmolytes. When the living environments change, plants will adopt their corresponding ecological strategies to adapt to this change. Under drought with soil salinity, more inorganic ions mediating osmotic adjustment should be an optimized strategy for plants to cope with and adapt to the coexistence of dry and saline environments in terms of material and energy utilization.

5. Conclusions

This study clearly showed that the presence of moderate soil salinity can cause soybean seedlings to passively absorb and accumulate Na^+ and other inorganic ions. Compared with the soybean seedlings treated with drought alone, the salt- and drought-treated seedlings can accumulate more inorganic ions to mediate osmotic adjustment, which can enhance the water absorption and retention of plants and reduce the photoassimilates and energy consumption for the synthesis of organic osmolytes. This allowed more photoassimilates and energy to be used for plant growth and other adaptive responses, thereby increasing the accumulation of biomass and enhancing drought resistance. In summary, under drought conditions, the presence of moderate soil salinity enabled soybean seedlings to improve osmotic adjustment, reduced assimilation and energy consumption and alleviated the adverse effects of drought stress.

Author contributions

Xinfu Bai and Yanlin Sun designed research; Xinfu Bai, Liqiang Dai and Hongmin Sun performed research; Xinfu Bai wrote the manuscript; Yanlin Sun edited the manuscript. All authors discussed the results and commented on the manuscript. We acknowledge AJE for linguistic editing during the preparation of this manuscript.

Conflicts of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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