Germination, Growth, Chlorophyll Fluorescence and Ionic Balance in Linseed Seedlings Subjected to Saline and Alkaline Stresses

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Abstract: Both saline and alkaline stresses involve osmotic stress and ion injury; however, alkaline stress involves the stress due to a high pH. The aim of this study was to evaluate the physiological responses of linseed seeds and seedlings to saline and alkaline stresses and to elucidate the adaptive mechanisms involved. Stresses were generated by exposure to neutral saline solutions of saline stress and alkaline stress for 7 days. The relative growth rate (RGR) and water content (WC) of linseed seedlings were scarcely affected by salinity stress, but significantly reduced by alkaline stress. Photosynthetic activity and pigment indices were hardly changed under saline stress but were inhibited by alkaline stress. This implies that alkaline stress may be mediated by Na⁺ uptake and accumulation up to toxic levels, leading to a decrease in photosynthetic pigments and damage to the photosynthetic apparatus. Alkaline stress causes precipitation of phosphate and metal ions which causes a sharp decrease in ionic activity and in the concentrations of various other ions. The results indicated that carbohydrates and proline synthesis decreased osmotic potential, remedied the shortage of inorganic anions and maintained stability of the intracellular pH allowing the plant to cope with osmotic stress from a high Na⁺ vacuolar concentrations. However, the contribution of betaine to osmotic adjustment was small in linseed seedlings. With increasing solution concentrations, the rate of germination of linseeds was more severely inhibited under alkaline stress than under saline stress.

Key words: Alkaline stress, Linseed, Saline stress.

Over the last 300 years, since the beginning of the industrial revolution, our unbridled exploitation of the world’s natural resources has severely damaged its vegetation and has also resulted in worrying accumulations of industrial wastes and greenhouse gases. Together, these have upset many natural ecosystem balances and have created many global environmental and climatic problems, including rising sea and air temperatures, rising sea levels, increasing desertification, severe erosion and soil loss, soil salinization and damaging accumulations of soil nitrogen (Abrol et al., 1988; Richards, 1990; Rengasamy, 2002). With widespread use of low-grade water for irrigation, soil salinization has also come into especially sharp focus. Somewhat over 60% of the world’s arable land is now affected by salinity to some degree and this inhibits crop growth and lowers productivity and can even kill crops (Allakhverdiev et al., 2000; Munns, 2002).

In naturally saline/alkaline soils, the main salts responsible are NaCl, Na₂SO₄, NaHCO₃ and Na₂CO₃, so the predominant ions are Na⁺, Ca²⁺, Mg²⁺, K⁺, Cl⁻, SO₄²⁻, CO₃²⁻ and NO₃⁻ (Kawanabe and Zhu, 1991; Tanji, 2002). Based on the particular mix of salts present in the soil at any site, natural salt stress has been classified into neutral salt-stress, alkaline salt-stress and mixed salt-stress. Saline stress generally involves two stresses – osmotic stress and ion injury stress, whereas alkaline stress includes in addition high-pH stress (Yang et al., 2009). A high-pH root environment can significantly decrease the availability of...
certain mineral elements and can cause precipitation of certain ions in the soil such as Ca\(^{2+}\), Mg\(^{2+}\), and HPO\(_4\)\(^{2-}\). This can inhibit ion uptake and disrupt the ion homeostasis of plant cells (Xue and Liu, 2008). Compared with saline stress, little attention has been paid to alkaline stress.

Linseed (Linum usitatissimum L.) is a diploid, self-pollinated, annual crop, with modern cultivars being typically short, many branched and producing a large amount of seed (Deyholos, 2006). It is one of the earliest cultivated plants known and, since ancient times, it has been grown widely for its high-quality, cellulose-rich, bast fibers and also for its oil (Zohary and Hopf, 2000; Huis et al., 2010). The oil of linseed is an excellent nutritional supplement being rich in \(\alpha\)-linolenic acid and omega-3 fatty acid. The oil is also used in the production of industrial raw materials (Vaisey-Genser and Morris, 2003; Foster et al., 2009; McKenzie and Deyholos, 2011). In the textile industry, the fibers of linseed have nowadays been largely replaced by cotton or by synthetic fibers but linseed fibers are still used in high-quality linen materials and are also now being incorporated in the polymeric matrix of biocomposites to improve their mechanical properties (Bodros et al., 2007; Huis et al., 2010). In 2009, the top producers of linseed were Canada, India and China, with 45% of world production being in Canada (FAO, 2009).

The tolerance of linseed to biotic and abiotic stresses has been studied extensively but, while this species is known to be a crop well adapted to saline conditions (Bodros et al., 2007; Huis et al., 2010). In 2009, the top producers of linseed were Canada, India and China, with 45% of world production being in Canada (FAO, 2009).

The plants and growing conditions

Seeds of linseed (Linum usitatissimum L.) NingYa-15 were sown 20 seeds per pot in 17-cm diameter plastic pots each containing 2.5 kg of washed sand. Seedlings were watered daily with 0.5 strength Hoagland’s nutrient solution. All pots were placed outdoors but were sheltered from the rain. The day/night temperature range experienced during the experiment was 21.0 – 25.5°C / 18.5 – 21.0°C.

2. Stress treatments

Four weeks after seeding, 40 pots containing uniformly growing seedlings were selected and divided randomly into 8 sets, each with 5 pots. One set was used as the untreated control, a second set was used for determining the growth index at the beginning of treatment, and the 6 remaining sets were used for the stress treatments. Each pot was a single replicate, with 5 replicates per set. Pots were thoroughly watered daily with nutrient solution containing the appropriate stress solution at 1700 – 1800. Control plants were watered with nutrient solution. The duration of stress treatments was 7 d.

3. Simulated saline and alkaline conditions

The neutral salts, NaCl and Na\(_2\)SO\(_4\), were mixed at a 1 : 1 molar ratio for the saline stress (SS) at 30, 60 and 90 mM (5 pots per concentration). Similarly, the two alkaline-pH salts, NaHCO\(_3\) and Na\(_2\)CO\(_3\), were mixed at a 1 : 1 molar ratio and used for the alkaline stress (AS) at the same three Na\(^+\) concentrations. The electrical conductivity (EC), pH and osmotic potential of the stress solutions were measured using a conductivity meter (DDG-2080-S, Anhui, China), a pH meter (PSH-3C) and a water potential meter (Psypro Wescor Corporation, US).

4. Measurement of growth

After 7 days of treatment, all seedlings in a pot were removed from the sand, washed with ultra-pure water and the shoot was divided from the root. The fresh weight (FW) was recorded after removing surface water by blotting and the dry weights (DW) were determined after drying for 15 min in an oven at 80°C and then in a vacuum dryer at 40°C to constant weight. The relative growth rate (RGR) was defined as (ln DW after treatment – ln DW before treatment) / treatment duration (Kingsbury and Epstein, 1984). The water content (WC) of the seedlings was calculated as: 100 × (FW – DW) / FW (Yang et al., 2008).

5. Measurement of chlorophyll fluorescence and photosynthetic pigments

The maximal PS II quantum yield (Fv/Fm), the effective PS II quantum yield (\(\Phi_{\text{PSII}}\)), nonphotochemical quenching (NPQ) and the coefficient of nonphotochemical quenching (qN) were determined between 0900 and 1100 from fully-expanded leaves using an Imaging-PAM (Walz, Effeltrich, Germany) (Genty et al., 1989; van Kooten and Snell, 1990). The leaves were held in the dark for about 20 min before measurement. The intensities of the actinic and saturating light settings were 185 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) and 2500 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) PAR, respectively. Randomly selected 500-μg aliquots of fresh leaves were extracted in acetone and tested for absorbance at 440, 645 and 663 nm was measured three times to determine the contents of carotenoids (Car) and of chlorophylls (Chl) a and b. For the calculations we used the equations of Arnon (1949).
6. **Measurement of cations and anions**

The contents of cations (Na⁺, K⁺, Ca²⁺, Mg²⁺, Fe²⁺ and Zn²⁺) were determined using an atomic absorption spectrophotometer (TAS-990, Purkinje General, Beijing). The contents of the anions (NO₃⁻, Cl⁻, SO₄²⁻ and H₂PO₄⁻) were determined by ion exchange chromatography (DX-300 ion chromatographic system; AS4A-SC ion-exchange column, CD M-II electrical conductivity detector; DIONEX, Sunnyvale, USA) with a mobile phase comprising 1.7 / 1.8 mM Na₂CO₃ / NaHCO₃ (Yang et al., 2007).

7. **Measurement of proline, betaine and carbohydrates**

Proline was extracted with 3% sulfosalicylic acid for 30 min at 70°C and measured by the ninhydrin reaction (Zhu et al., 1983). Betaine was extracted with 80% methanol for 20 min at 70°C and measured as described by Grieve and Grattan (1983). Samples were dried and cut into small pieces and the anthracene ketone method was applied to 50 g samples from each treatment. Briefly, these were dipped into 3 cm³ of 80% ethanol held in a water bath at 80°C for about 40 min, centrifuged at 3000 × g for 15 min and the supernatant was collected. Polycondensation with anthrone resulted in a blue compound (Zhou et al., 2002). The carbohydrate contents of the leaves were determined using a UV-754 spectrophotometer at 620 nm. Fructose and sucrose were determined according to Wrigley (1994) and Li et al. (2003). Each measurement was repeated 3 times.

8. **Measurement of germination**

One hundred linseed seeds were germinated on filter paper in germination boxes. The dry seeds were submerged in 100 mL of the SS and AS solutions described in Table 1.

### Table 1. The electrical conductivity (EC), pH and osmotic potential of SS and AS treatment solutions.

| Treatment | Na⁺ (mM) | EC (mS cm⁻¹) | pH | Osmotic potential (MPa) |
|-----------|----------|--------------|-----|------------------------|
| Control   | 0        | 1309         | 7.02 | –0.05                 |
| Saline    | 30       | 4300         | 6.97 | –0.23                 |
| Stress    | 60       | 7210         | 6.94 | –0.42                 |
| (SS)      | 90       | 10880        | 6.93 | –0.62                 |
| Alkaline  | 30       | 3990         | 9.76 | –0.20                 |
| Stress    | 60       | 7070         | 9.84 | –0.39                 |
| (AS)      | 90       | 9250         | 9.88 | –0.58                 |

Fig. 1. Effects of SS and AS on the relative growth rate (RGR) (A and B), and on water content (WC) (C and D) of shoot (A and C) and root (B and D). The values are the means of 5 replicates. Means followed by different letters in the same graph are significantly different at $P < 0.05$, according to the least significant difference (LSD) test.
above (with distilled water as the control). The boxes were maintained at 20°C in the dark for 10 d, 5 replicates of each salinity stress treatment were prepared. Germinated seeds were scored daily, based on the emergence of the radicle.

9. Statistical analyses

Statistical analyses included one-way analysis of variance (ANOVA) and SPSS (Version 13.0, SPSS, Chicago, IL, USA) and the least significant difference (LSD) methods used to detect differences ($P < 0.05$) in physiological parameters under saline and alkaline stresses. The means and standard errors (SE) of 5 replicates were obtained for each measurement.

Results

1. Electrical conductivity (EC), pH and osmotic potential of stress treatment solutions

As shown in Table 1, with increased salinity, the EC increased, while the osmotic potential of stress treatment solutions decreased. The magnitudes of these changes in the saline solutions were greater than those in the alkaline solutions. The SS and AS solutions had a pH of 6.93 – 6.97 and 9.76 – 9.98, respectively.

2. Growth

At a lower Na’ concentration, RGR and WC were not significantly affected by SS and AS; however, at a higher Na’ concentration (> 90 mM) the RGR and WC of linseed were lowered by SS and AS; AS having a greater effect than SS (Fig. 1A – D; $P < 0.05$).

3. Chlorophyll fluorescence

SS and AS had no significant impact on $Fv/Fm$, and there was no difference between the two responses (Fig. 2A; $P < 0.05$). The value of $\Phi_{PSII}$ decreased with increasing salinity under AS, but not under SS (Fig. 2B; $P < 0.05$). The effects of salinity on $NPQ$ and $qN$ values showed similar change trends, these values increased under AS, but did not change significantly under SS (Fig. 2C, D; $P < 0.05$).

4. Photosynthetic pigments

Salinity stress significantly increased the $Chl\ a$, $Chl\ b$ and $Car$ contents of leaves in linseed seedlings (Fig. 3; $P < 0.05$). The $Chl\ a/b$ ratio under SS was higher than that in the control, except for 90 mM of SS. In contrast, AS led to a marked decline in photosynthetic pigments parameters (Fig. 3; $P < 0.05$). The extent of the changes in photosynthetic pigment content and $Chl\ a/b$ under AS were markedly greater than those observed under SS (Fig. 3; $P < 0.05$).

5. Cations

In the shoot, the $K^+$ content decreased slightly with increasing stress, but in the root it decreased sharply (Fig. 4A; $P < 0.05$). The Na’ content of the shoot increased with increasing stress, more greatly under AS than under SS (Fig. 4B; $P < 0.05$). In the roots, the Na’ content increased with increasing salinity of AS solution up to 30
unchanged, whereas it tended to decrease in the roots (Fig. 5A1, 2; \( P < 0.05 \)). The \( \text{NO}_3^- \) contents of shoot and root under SS were lower than those in the control. The changes in the root under AS were significantly greater than those under SS (Fig. 5B1, 2; \( P < 0.05 \)). With increasing SS, the \( \text{H}_2\text{PO}_4^- \) content increased, while with increasing salinity in AS it decreased. The changes under AS were greater than those under SS in the shoot and root (Fig. 5C1, 2; \( P < 0.05 \)). In the shoots, the \( \text{SO}_4^{2-} \) content increased with increasing salinity (Fig. 5D1; \( P < 0.05 \)). In the roots the \( \text{SO}_4^{2-} \) content decreased with increasing AS, but, it increased with increasing salinity in SS (Fig. 5D2; \( P < 0.05 \)).

**6. Anions**

Under SS, the \( \text{Cl}^- \) content increased with increasing salinity in the shoot and the root (Fig. 5). With increasing AS, the \( \text{Cl}^- \) content of the shoot remained relatively unchanged, whereas it tended to decrease in the roots (Fig. 5A1, 2; \( P < 0.05 \)). The \( \text{NO}_3^- \) contents of shoot and root under SS were lower than those in the control. The changes in the root under AS were significantly greater than those under SS (Fig. 5B1, 2; \( P < 0.05 \)). With increasing SS, the \( \text{H}_2\text{PO}_4^- \) content increased, while with increasing salinity in AS it decreased. The changes under AS were greater than those under SS in the shoot and root (Fig. 5C1, 2; \( P < 0.05 \)). In the shoots, the \( \text{SO}_4^{2-} \) content increased with increasing salinity (Fig. 5D1; \( P < 0.05 \)). In the roots the \( \text{SO}_4^{2-} \) content decreased with increasing AS, but, it increased with increasing salinity in SS (Fig. 5D2; \( P < 0.05 \)).

**7. Organic solutes**

With increasing salinity in SS, the total carbohydrates (TTC) content increased as compared with the control in shoots and roots. AS appeared not to affect TTC content,
Fig. 4. Effects of SS and AS on the contents of K⁺ (A₁), Na⁺ (B₁), Ca²⁺ (C₁), Mg²⁺ (D₁), Fe²⁺ (E₁) and Zn²⁺ (F₁) in the shoots of linseed seedlings, and of K⁺ (A₂), Na⁺ (B₂), Ca²⁺ (C₂), Mg²⁺ (D₂), Fe²⁺ (E₂) and Zn²⁺ (F₂) in the roots. The values are the means of 5 replicates. Means followed by different letters in the same stress type are significantly different at \( P < 0.05 \), according to the least significant difference (LSD) test.
though it increased TTC at 30 mM Na\(^+\) concentration in shoots. In roots, however, TTC content decreased slightly with increasing Na\(^+\) concentration (Fig. 6A1, 2; \(P < 0.05\)). In shoots the impacts of SS on fructose and sucrose contents were similar to that on TTC, but in roots their contents decreased with increasing SS. Fructose and sucrose contents of shoots and roots decreased under AS were greater than those under SS (Fig. 6B1, 2, C1, 2; \(P < 0.05\)). The proline contents of shoots increased with increasing salinity and alkalinity, although the increases under AS were significantly greater than under SS (Fig. 6D1; \(P < 0.05\)). In the root, the proline content increased with
Fig. 6. Effects of SS and AS on the contents of total carbohydrates (TTC) (A₁), fructose (B₁), Sucrose (C₁), proline (D₁) and betaine (E₁) in linseed seedling shoots, and of the total carbohydrates (TTC) (A₂), fructose (B₂), Sucrose (C₂), proline (D₂) and betaine (E₂) in the roots. The values are the means of 5 replicates. Means followed by different letters in the same stress type are significantly different at $P<0.05$, according to the least significant difference (LSD) test.
increasing salinity in SS but decreased with increasing salinity in AS (Fig. 6D; $P < 0.05$). The betaine contents were not significantly changed under two stresses in shoot, but it decreased significantly at 90 mM Na$^{+}$ concentration under SS. The betaine contents decreased with increasing salinity in SS and AS in the root (Fig. 6E; $P < 0.05$).

8. Germination

The change in germination rate of linseed seeds under the two stress conditions was similar; germination tended to decrease with increasing salinity but the decrease was greater under AS than under SS (Fig. 7; $P < 0.05$).

Discussion

1. Growth

RGR and WC reflect the condition of the plant and are thus considered useful indexes of the degree of stress a plant is under at any time and its tolerance to various stresses (Lutts et al., 2004; Yang et al., 2007). In general, salinity inhibits growth and can lead to plant death (Munns and Tester, 2008). However, our results showed that RGR and WC decreased with increasing salinity, the extent of the decrease under AS was clearly greater than that under SS. This indicated that SS and AS are distinct types of stress and that the injurious effects of AS on plants are severer than those of SS. The injurious effect of SS is commonly thought to arise from a low water potential and ion toxicity, whereas AS involves high-pH stress in addition to these two stress factors (Yang et al., 2000). The high pH lead to the lack of protons, and the destruction or inhibition of transmembrane electrochemical-potential gradients in cells. The response of linseed growth to AS differed from that of wheat (Triticum aestivum) and rice (Oryza sativa L.). As low as 30 mM Na$^{+}$ concentration under AS significantly inhibited wheat and rice growth; however, only severe AS (Na$^{+}$ > 90 mM) inhibited the growth of linseed, indicating that linseed was stronger alkali-tolerant than other crops (Guo et al., 2011; Wang et al., 2011).

2. Chlorophyll a fluorescence and pigment content

The impacts of SS and AS on photosynthetic activity led us to investigate the mechanisms involved in more detail by examining chlorophyll a fluorescence as an index of stress (Ralph and Burchett, 1998; Rau et al., 2007; Naumann et al., 2008) since this could provide insight into the nature of the stress-induced damage to the photosynthetic apparatus (Maxwell and Johnson, 2000). In the present study, the linseed seedling photosynthetic apparatus was not damaged by SS and AS consistent with previous reports, such as that on Citrus by Silva et al. (2010) Suaeda salsa by Lu et al. (2002) and Triticum aestivum by Guóth et al. (2008). Under AS the values of $\Phi_{NPQ}$ showed decreases, it may reflect the reduction of PSII electron flow, which is resulted from either the inhibition of activities of photosynthetic enzymes or the stomata closing by the massive Na$^{+}$ influxes and high pH. In contrast, $NPQ$ and $qN$ increased in AS, indicating that Na$^{+}$ influx and the high pH may enhance dissipation of excess light energy, thus protecting the photosynthetic tissue and mitigating the effects of AS on photosynthesis. With increasing salinity in AS, the contents of pigment decreased, this may be because AS causes precipitation of Mg and leads to inhibition of chlorophyll synthesis by enhancing the activity of the chlorophyll degrading enzyme chlorophyllase (Reddy and Vora, 1986; Shi and Zhao, 1997). Alternatively, the pigment reduction may be a strategy for protection and/or acclimation; the reduction of energy waste, carbon skeletons and nutrients in chlorophyll synthesis may favor other physiological processes (Chaves et al., 2009; Silva et al., 2010).

3. Ion balance

The main toxic ion in saline soil is Na$^{+}$; plants accumulate high concentrations of Na$^{+}$ in their vacuoles to decrease cell water potential (Munns and Tester, 2008). In the studies with other plants, such as Triticum aestivum (Guo et al., 2009), Setaria viridis (Guo et al., 2011) and Aneurolepidium chinense (Shi and Wang, 2005), Na$^{+}$ content increased with increasing AS. In this study, however, no significant difference was observed between the effects of SS and AS on the Na$^{+}$ content of shoots at a low stress intensity (< 30 mM) in linseed, implying that the adaptive mechanism of linseed shoots to the AS (high pH) may differ from that of other plants (Table 2). Our results showed that the K$^{+}$ content of shoots under SS was similar to that under AS in linseed, and this differed from that in Triticum aestivum (Guo et al., 2009) and Choris virgata (Yáng et al., 2010); in these two species, the K$^{+}$ content of shoots under AS was lower than under SS (Table 2).
Many plant species have a Na⁺ exclusion mechanism that depends on a Na⁺/H⁺ antiporter, which exchanges cytoplasmic Na⁺ with external H⁺ (Munns and Tester, 2008). Those data suggest that there is little effect of osmotic stress on ionic balance perhaps because SS regulates the activity of H⁺-ATPase and H⁺-pyrophosphate synthase in both the cell and vacuole membranes. Under high AS, the exchange activity of the Na⁺/H⁺ antiporter in the roots plasmamembrane decline; and possibly reduce exclusion of Na⁺ into the rhizosphere and enhance accumulation of Na⁺ in shoots even to a toxic level.

Availability of free Ca²⁺ is necessary to maintain membrane stability, participating in signaling and this is needed for synthesis of cell walls. Meanwhile, Mg²⁺ is an important component of chlorophyll, and Fe²⁺ and Zn²⁺ play important roles in various physiological and biochemical processes (Knight et al., 1997; Mori, 1999; Parida and Das, 2005). The responses of free Ca²⁺, Mg²⁺, Fe²⁺ and Zn²⁺ to both SS and AS were similar, their accumulation in shoots was enhanced but it was inhibited in roots by increasing saline stress. In shoots Ca²⁺, Mg²⁺ and Fe²⁺ increased under stress but their contributions to osmotic adjustment were small because their ratios to the total cations present were very low (Table 2, 3). Under SS and AS, the absorption of Ca²⁺ and Mg²⁺ was inhibited, especially under AS (Table 2, 3). This may be a cultivar-specific response or simply a specific adaptive response. In roots, the Fe²⁺ increased dramatically at 30 mM Na⁺ and then decreased sharply with increasing Na⁺ concentration under both stresses (Fig. 3E; P < 0.05). Fe²⁺ competes with K⁺, Mg²⁺ and Zn²⁺ for uptake into roots because they have similar hydrated ionic radii. The contributions of Fe²⁺ and Zn²⁺ to stress adjustment are small because their ratio to the total ions present is very low (Table 2, 3).

Ion imbalance in plants is caused mainly by the influx of superfluous Na⁺. To maintain ionic balance, plants usually accumulate inorganic anions or synthesize organic anions (Blumwald, 2000; Yang et al., 2007, 2008). The accumulation of anions will decrease cell water potential but if excessive levels appear, they will inhibit photosynthesis and reduce growth. In the present study, under SS, the linned seedlings showed a proportionate increase in the uptake of Cl⁻, H₂PO₄⁻ and SO₄²⁻, whereas the levels of NO₃⁻ declined (Table 2, 3). There were interesting responses of anions to AS in the roots. The gradual decline of Cl⁻, NO₃⁻, H₂PO₄⁻ and SO₄²⁻ may be related to a lack of external

### Table 2. Molarity of different solutes expressed as a percentage of total solute molarity in linned seedlings shoots.

| Na⁺ (mM) | K⁺ [%] | Na⁺ [%] | Ca²⁺ [%] | Mg²⁺ [%] | Fe²⁺ [%] | Zn²⁺ [%] | Cl⁻ [%] | NO₃⁻ [%] | H₂PO₄⁻ [%] | SO₄²⁻ [%] | Proline [%] | Betaine [%] | TTC [%] |
|----------|--------|--------|---------|---------|---------|---------|--------|--------|------------|-----------|------------|------------|---------|
| Control  | 0      | 20.70  | 3.69    | 8.94    | 7.21    | 0.00    | 0.01   | 6.35   | 23.76      | 14.74     | 6.43       | 6.81       | 0.17    | 1.19    |
|          | 30     | 17.18  | 12.64   | 6.89    | 6.30    | 0.00    | 0.01   | 11.14  | 14.27      | 11.20     | 8.20       | 11.02      | 0.14    | 1.03    |
| Saline stress | 60   | 13.30  | 17.14   | 5.69    | 5.07    | 0.00    | 0.01   | 12.48  | 12.68      | 11.06     | 8.66       | 12.96      | 0.11    | 0.84    |
|          | 90     | 8.19   | 18.14   | 6.09    | 5.03    | 0.00    | 0.01   | 15.77  | 10.19      | 9.42      | 9.85       | 16.10      | 0.08    | 0.82    |
| Avg.     | 12.89  | 15.97  | 6.22    | 5.47    | 0.00    | 0.01   | 13.13  | 12.38     | 10.56      | 8.91       | 13.36      | 0.11    | 0.90    |
| Alkaline stress | 30 | 17.66  | 13.10   | 7.86    | 6.54    | 0.00    | 0.01   | 5.81   | 13.09      | 10.82     | 6.30       | 16.18      | 0.14    | 1.10    |
|          | 60     | 12.17  | 39.49   | 5.19    | 4.42    | 0.00    | 0.01   | 4.07   | 11.00      | 6.23      | 5.03       | 12.29      | 0.09    | 0.48    |
|          | 90     | 7.83   | 38.96   | 4.81    | 3.89    | 0.00    | 0.01   | 3.72   | 8.18       | 5.06      | 5.31       | 21.78      | 0.06    | 0.99    |
| Avg.     | 12.55  | 30.78  | 5.95    | 4.95    | 0.00    | 0.01   | 4.53   | 10.96     | 7.37       | 5.55       | 16.75      | 0.10    | 0.65    |

### Table 3. Molarity of different solutes expressed as a percentage of total solute molarity in linned seedlings roots.

| Na⁺ (mM) | K⁺ [%] | Na⁺ [%] | Ca²⁺ [%] | Mg²⁺ [%] | Fe²⁺ [%] | Zn²⁺ [%] | Cl⁻ [%] | NO₃⁻ [%] | H₂PO₄⁻ [%] | SO₄²⁻ [%] | Proline [%] | Betaine [%] | TTC [%] |
|----------|--------|--------|---------|---------|---------|---------|--------|--------|------------|-----------|------------|------------|---------|
| Control  | 0      | 12.7   | 4.27    | 2.52    | 5.41    | 0.00    | 0.00   | 7.15   | 60.40      | 3.23      | 1.14       | 2.33       | 0.08    | 0.77    |
|          | 30     | 10.82  | 15.31   | 2.54    | 5.48    | 0.00    | 0.00   | 8.30   | 47.32      | 4.66      | 1.66       | 3.14       | 0.10    | 0.67    |
| Saline stress | 60 | 8.33   | 18.90   | 1.77    | 4.09    | 0.00    | 0.00   | 9.62   | 44.51      | 5.55      | 2.03       | 4.44       | 0.07    | 0.68    |
|          | 90     | 7.56   | 23.74   | 0.88    | 3.63    | 0.00    | 0.00   | 14.83  | 36.17      | 4.82      | 2.81       | 4.96       | 0.07    | 0.54    |
| Avg.     | 8.90   | 19.32  | 1.73    | 4.40    | 0.00    | 0.00   | 10.92  | 42.67     | 5.01       | 2.17       | 4.18       | 0.08    | 0.63    |
| Alkaline stress | 30 | 11.90  | 22.86   | 3.48    | 5.97    | 0.00    | 0.00   | 2.79   | 45.30      | 1.66      | 1.23       | 4.11       | 0.09    | 0.59    |
|          | 60     | 4.29   | 52.64   | 10.42   | 8.67    | 0.00    | 0.00   | 5.26   | 8.64       | 2.49      | 2.70       | 3.72       | 0.28    | 0.80    |
|          | 90     | 2.74   | 60.90   | 8.55    | 7.09    | 0.00    | 0.00   | 5.03   | 6.09       | 1.85      | 2.96       | 3.23       | 0.46    | 1.10    |
| Avg.     | 6.31   | 45.47  | 7.48    | 7.24    | 0.00    | 0.00   | 4.36   | 20.01     | 2.00       | 2.33       | 3.69       | 0.27    | 0.83    |
protons due to the high pH (Table 2, 3). The excess charge is likely to disrupt many enzymatic reactions depending on pH, redox balance and on the charge state of substrates and their intermediates. This may have knock-on effects on plant development, growth and physiology, including photosynthesis and these should be investigated further.

4. Organic solutes
The water-soluble carbohydrates are mainly fructose and sucrose, and these are important osmosis regulating substances in plants, and so are relevant to stress tolerance (Pan, 2001). The results showed that the effect of AS on water-soluble carbohydrates was stronger than that of SS, indicating that the severe negative impact of AS on carbohydrate synthesis and storage may reflect the toxic levels of Na\(^+\) accumulation in the cells in a high-pH environment. The detection of high levels of pH in the root environment, suggests that carbohydrate accumulation is not a simple passive response to osmotic stress but, rather, is the result of active metabolic regulation. Proline and betaine are known to play important roles in osmotic adjustment (Stewart and Lee, 1974; Rhodes and Hanson, 1993). We found that proline accumulated in shoots of linseed seedlings in response to SS and AS (Fig. 6). This likely helped to balance the osmotic potential in the vacuoles and to protect various enzymes from the accumulation of Na\(^+\). The stresses did not significantly affect the accumulation of betaine and its contributions to osmotic solutes were small because its proportion to total organic matter was very low (Table 2, 3).

5. Germination
Our results indicate that moderate SS does not inhibit seed germination in linseed but it reduces the number of germinating seeds at concentrations exceeding 60 mM. The inhibition of germination by AS was greater than by SS at the same salinity (Fig. 7). Germination was not inhibited by high pH at low salinity but was inhibited at higher salinity. This implies that a degree of pH adjustment takes place in seeds that allows germination at low salinities. It also suggests that a high-pH environment and resulting ion imbalance has a direct effect on the absorption of minerals by the seed whereas excessive salinity merely delays the germination process. This suggests that linseed seeds can eventually germinate in saline soil. This is a complex response that needs to be studied further.

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