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Comparative analysis of responses to field salinity stress in contrasting soybean accessions highlights NaCl exclusion in leaves as a key mechanism for salinity stress tolerance.

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Abstract

Salinity is one of the main limitations to legume productivity in many regions of the world and it is estimated that 50% of all arable land will become affected by salinity by 2050. The impact of field salinity on soybean performance was assessed using two soybean accessions (KCW and HMC) cultivated in salt-treated soil and non-salt treated soil. We used a 30 cm deep field system layered with 0.4 kg/m^2 NaCl for the salt-treated experiment while the control field received no salt treatment. The results show that salinity reduces soybean growth and yield as evident from the reduction in the plant shoot length, stem diameter, number of branches, number of pods and seed weight. However, the reduction in these growth parameters was less pronounced in the HMC accession than the KCW accession. Furthermore, Na^+ content in leaves of the HMC accession was lower than that of the KCW accession. This proved that salinity has a damaging effect on soybean growth and yield and the relative tolerance of the HMC accession is attributed in part to its ability to restrict Na^+ transport to the leaves. While the study emphasizes salt exclusion as a potentially useful mechanism for salinity tolerance in soybean, it provides evidence that the HMC accession is a good genetic resource for breeding soybean varieties with improved salinity stress tolerance.

1. INTRODUCTION

As the world population continues to increase, the amount of arable land keeps decreasing (Essa, 2002). Soil salinity is one of the most common agricultural constraints rendering land non-arable in arid and semi-arid regions of the world (Amirjani, 2010). Due to increased soil salinity, which renders previously arable land unsuitable for crop production (Butcher et al., 2016), plant responses to salinity is one of the most widespread research topics in plant physiology. The excessive use of fertilizers and saline irrigation water are the major factors that increase soil salinity (Shahid et al., 2018). In light of the predicted 70-100% increase in food demand needed to feed the growing population by 2050, and with no modern alternative for broadening arable agricultural land, an increase in traditional crop breeding for salt tolerance serves as a vital step towards sustaining food security (Guan et al., 2014). Salinity becomes a burden when there is an excessive amount of soluble salts in the soil or water, mainly due to excessive Na^+ levels in the soil, mostly originating from NaCl (Grewal, 2010). This can limit water potential and induce ionic stress in plant tissue, thereby causing oxidative stress in plants (AbdElgawad et al., 2016). Many studies have reported that salinity reduces plant growth and productivity through changes in plants physiology, morphology and biochemistry (AbdElgawad et al., 2016; Chawla et al., 2013; Hernández, 2019). These changes are usually quantified by measuring plant shoot length, biomass, leaf anatomy, yield, stomatal density, photosynthetic capacity, phytohormones, osmolytes and antioxidants, among others. Soybean (*Glycine max*) is a glycophytic crop for which growth and yield can be adversely impacted by salinity. This crop species is the most produced grain legume worldwide, providing an excellent source of high quality protein (35%) and oil (21%) to human and animal diets (Wang et al., 2020). This economically important crop is also rich in vitamins B1, B2, B6 and contains good levels of micronutrients such as Fe, Cu, Mn, Ca, Mg, Co, K, and Zn (de Vargas et al., 2018). Soybean grain is mainly used as animal feed (Willis, 2003). Soybean oil, soybean cake, soya sauce, flavoured soymilk and yoghurt are all soybean final products for direct human consumption (Wang et al., 2020). Soybean is also used industrially in the production of biofuels, emulsifiers, cosmetics, soaps and pharmaceuticals (Hart, 2017). Soybean is classified as moderately tolerant to salinity, with a threshold of 2 to 5 $dS.m^{-1}$, beyond which its growth is remarkably reduced (Guan et al., 2014). Many studies on soybean salinity responses where molecular studies were conducted make use of pot-based experiments. However, this does not reflect true field responses because the physiological effect of soybean salinity in real scenarios may not be effectively scored and thus limits advancement towards the development of salinity tolerance in soybean. In this study, we used a field system to assess the salinity effect on two contrasting accessions of soybean.

2. MATERIALS AND METHODS

The study was conducted on a 240 m^2 field (at Lukholweni village, Matatiele, Alfred Nzo District, Eastern Cape province in South Africa, GPS coordinates 30°37’54.8”S 28°51’29.3”E), consisting of a 120 m^2 block for the salt stress treatment and 120 m^2 for the non-saline (control) experiment, as illustrated in Fig. 1. The experiment was initiated on 30 September 2020 and ended at soybean maturity on 30 January 2021. For the 120 m^2 salt block, the field was dug

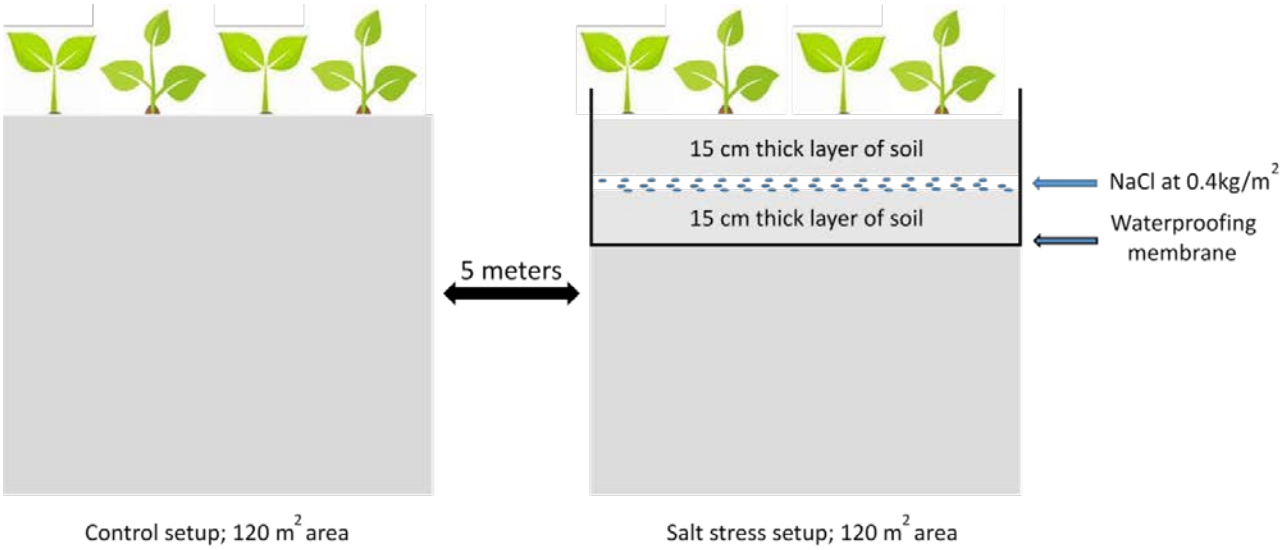

Fig. 1. The salt stress field setup. A field measuring 120 m^2 was used for each of the salt and non-salt stress study. The distance between the control setup and the salt-stressed setup was 5 meters. The salt-stress setup was 30 cm deep and was covered with a waterproofing membrane to restrict salt penetration to other parts of the field. The membrane was covered with soil layer 15 cm thick, following which NaCl (0.4 kg/m^2, thus 48 kg for the 120 m^2 area) was evenly distributed on the soil. The salt-treated soil was then layered with a soil layer 15 cm thick on which the seeds were sowed. The control filed was also 120 m^2 and set up similarly to the salt block but without any addition of salt.

up to a depth of 30 cm and the dug up soil was set aside such that a plastic waterproof membrane was laid at the 30 cm deep base of the dug up field. Following the plastic membrane laying, the dug up soil was returned on top of the plastic membrane to make an even layer of soil that is 15 cm thick. This was followed by applying salt (NaCl) on this soil layer at a rate of 0.4 kg/m^2. A 'no salt' control block was set up in a similar way as the salt block at a space of 5 meters away from the salt block, except that no salt was applied to the control block. Seeds of two soybean accessions (KCW and HMC) were sowed 6 cm deep with a spacing of 10 cm between seeds and 40 cm between rows such that each accession occupied a block of 40 m^2 repeated three times for each treatment, with 100 plants per block. The soil was irrigated daily with water at a rate of 2 L/m^2. After 123 days of sowing, the plants were harvested and yield parameters such as the shoot length, stem diameter (at the cotyledonary node), number of branches, number of pods and seed weight were recorded. The level of salinity in both salt-stressed and non-saline field was measured in soil sampled at a depth of 5 cm, using the Extech EC410 ExStik Conductivity/TDS/Salinity Kit (Extech instruments, New Hampshire, USA). Na^+ content in leaves was measured as described in Liu et al. (2016) using a Thermo ICap 6200 ICP-AES emission spectrometer. Statistical analysis was done using a one-way analysis of variance (ANOVA) and was tested for significance using GraphPad Prism 6.01 software (GraphPad Software. San Diego, CA) via the application of the Tukey-Kramer test at a 5% level of significance.

3. RESULTS AND DISCUSSION

Soil salinity increased in the soil approximately 4 times higher in the salinity block than the control block (Fig. 2A.). Soybean growth and yield were significantly impaired by salinity as demonstrated by reduced shoot length, stem diameter and number of branches (Fig. 2B-D.).

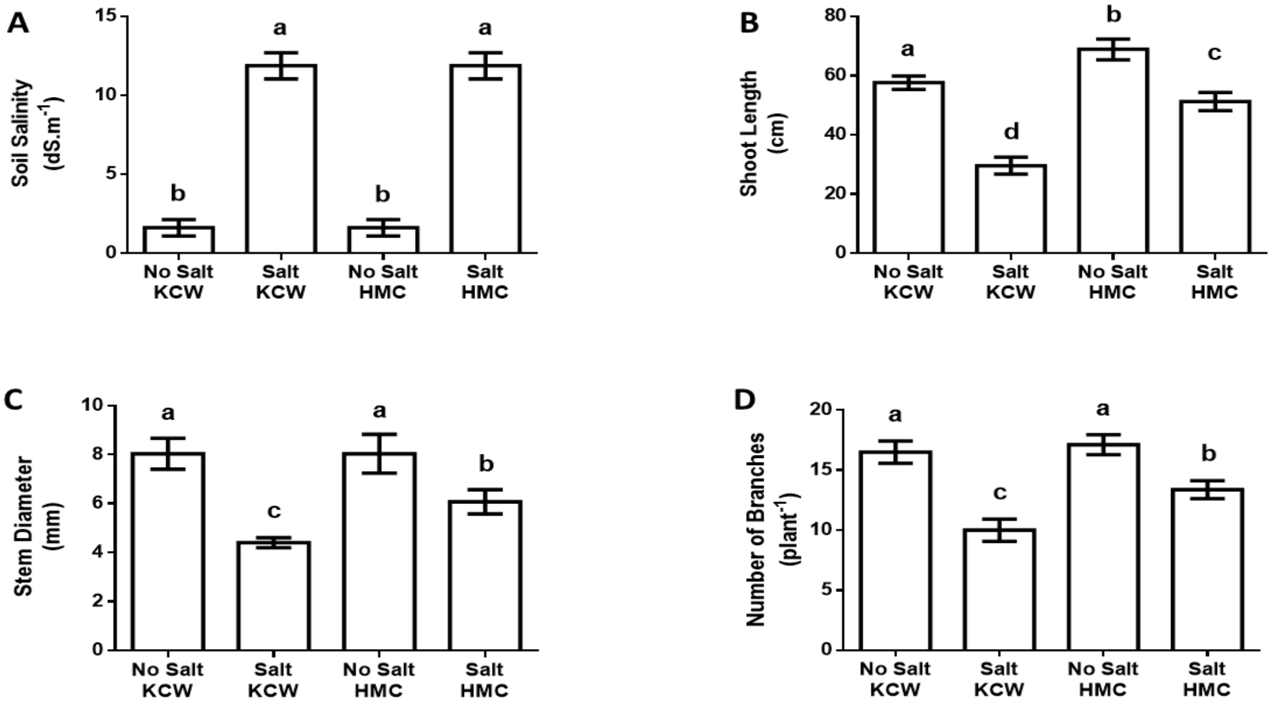

Fig. 2. **Effect of salt stress on soybean growth**. Changes in soil salinity (A), shoot length (B), stem diameter (C) and number of branches (D) as a result of application of salt on the soil. Data are means ± SD of five soil samples (taken from the four cornes and the centre of each of control and salt stress blocks) and twelve plants randomly selected from each treatment for each of the two accessions. Bars with different letters indicate statistically different means ($P < 0.05$).

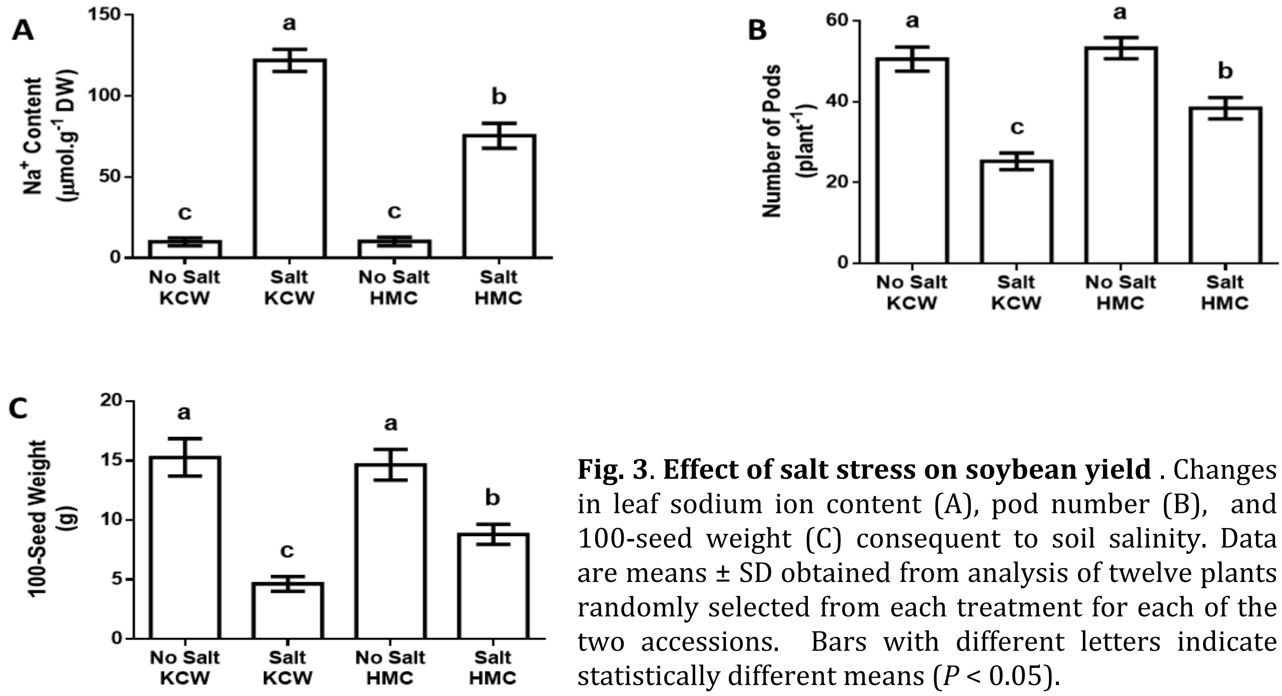

Fig. 3. **Effect of salt stress on soybean yield** . Changes in leaf sodium ion content (A), pod number (B), and 100-seed weight (C) consequent to soil salinity. Data are means ± SD obtained from analysis of twelve plants randomly selected from each treatment for each of the two accessions. Bars with different letters indicate statistically different means ($P < 0.05$).

These changes in the soybean growth parameters are in agreement with the study from Amirjani (2010) and Ghassemi-Golezani et al. (2009). The reduction in shoot length, stem diameter and number of branches should be due to increased Na^+ in shoots, inducing an ionic toxicity effect on the plants (Roy et al., 2019). It could also be exercebated by a reduction in water potential caused by the elevated salt levels in the soil, which hinders plant water uptake to ultimately lead to reduced growth (Yan et al., 2020, Arif et al., 2020). Luo et al. (2019) observed that reduction in shoot length and biomass under salt stress was due to the excessive accumulation of Na^+ and Cl^- in the chloroplasts, which affects the electron transport activities of photosynthesis, thereby inhibiting PSll activity. Kiełkowska (2017) showed that reduction in plant height under salt stress may be as a result of changes in plant-water relationships, which attenuates meristem activity and cell elongation. Some studies have established that salinity stress reduces leaf area, stem diameter, biomass, and shoot length (Beinsan et al., 2009, Basal, 2010, Taïbi et al., 2016). This ultimately leads to low yields in crops. In both accessions, Na^+ was accumulated in leaves in response to the salinity treatment. However, the Na^+ accumulation was higher in KCW than in HMC (Fig. 3A.). This suggest that HMC is better equipped to either restrict uptake of Na^+ by the roots or restrict Na^+ transport onto the leaves. The association between salinity tolerance and salt exclusion has been demonstrated in soybean, where it is associated with the activity of a protein encoded by the *GmSALT3* gene, which is proposed to be a cation/H^+ exchanger (Do et al., 2016; Guan et al., 2014; Qi et al., 2014). Further work to identify more genes associated with salinity stress tolerance in soybean can be pursued through comparative proteome-wide and genome-wide analyses between highly salt stress tolerant and sensitive soybean accessions. Such pursuit will aid in enhancing soybean tolerance to salinity and subvert the impact of soil salinization on food security. The KCW accession suffered a more pronounced reduction in shoot length, stem diameter, number of branches, number of pods and 100-seed weight than HMC, which implies that KCW has a higher degree of sensitivity to salt stress than HMC (Fig. 2B-D, Fig. 3B-C).

The above growth and yield parameters have been used as common biomarkers to screen for tolerant and sensitive cultivars under salt stress in maize (Cha-Um and Kirdmanee, 2009), sorghum (Yang et al., 2020) and wheat (Yang et al., 2014). Therefore, we infer that HMC is more salt stress tolerant than KWC, and this is in part due to the ability of HMC to limit Na^+ accummulation in leaves. Future studies assessing other biochemical markers such as reactive oxygen species accummulation, oxidative stress tolerance and antioxidant responses of these soybean accessions under salt stress contribute to further elucidation of other mechanisms mediating salinity stress tolerance.

4. CONCLUSION

This study shows that decreased soybean yield under salt stress is manifested as a decrease in shoot length, stem diameter, number of branches, pod number and seed weight. The study suggests that such negative impacts can be mitigated by improving the ability of soybean to limit salt accumulation in leaves.

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