Chitosan, a bio-stimulant confer salt stress tolerance in sorghum (*Sorghum bicolor* L. Moench)

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Introduction: The agricultural sector has been facing enormous challenges ranging from the increased demand to produce more food to cater for the growing population (Calicioglu *et al*., 2019). Crop production is severely affected by environmental factors. Salinity is a major abiotic stress that has contributed to more than 50% loss of important crops including rice, wheat and maize (Shrivastava and Kumar*,* 2015*)*. The use of fertilizers and pesticides played a vital role in improving crop yield and quality throughout the seasons. However, in the last decade, there has been a rising concern about their negative effects on human health and the environment (Davydov *et al*., 2018). It is therefore necessary to develop novel technologies that will assist the agricultural sector to produce more crops in an environmentally friendly manner and to overcome the negative effects of stress on crops. One of the promising targets is the application of signaling compounds including bio-stimulants. Chitosan is a natural compound obtained from the deacetylation of chitin. Chitin is a component of the cell wall of fungi and crustaceous, making its use safe and cheap. Chitosan is beginning to receive noticeable attention in agriculture due to its attractive features, which includes biodegradability, nontoxicity, and biocompatibility for application in different areas (Chopra and Ruhi, 2016). However, the mechanism of chitosan mediated stress tolerance remains elusive. This study reports the role of chitosan in conferring salt stress in sorghum towards increasing crop production.

Methodology*:* Surface sterilized seeds were soaked overnight in autoclaved distilled water (ddH_2O), followed by airdrying under the laminar flow. Seeds were sown in medium containing different NaCl (0, 100, 200, and 300 mM NaCl) and Chitosan (0, 0.25, 0.5 mg/ml) concentrations. Samples were incubated in the tissue culture for 7 days in the dark, while germination rate was measured daily. Germination parameters included Germination Percentage (GP), Germination Index (GI), Mean Germination Time (MGT), and Total Germination (TG), while Fresh and Dry weights were measured on the 7^{th} day. Seedlings germinated in ddH_2O only were further transferred into potting soil and then subjected to different treatments of NaCl (0 and 300 mM NaCl) and chitosan applied every second day for 7 days. Seedlings were harvested, followed by assaying physiological (growth assays), biochemical (osmolytes and oxidative stress markers), and anatomical responses (Mulaudzi *et al*., 2020).

Results: Salt stress reduced germination and growth of sorghum as evident by low GP, GI, MGT, TG and fresh weights. Salt-reduced growth was improved by the application of chitosan, whereas chitosan showed no significant effect on non-salt treated plants (control). In response to high salt stress, sorghum plants accumulated 319.84% proline and 45% soluble sugars. Furthermore, oxidative damage was evident by the over production of H_2O_2 (~40%) under salt stress, which was sufficient to degrade polyunsaturated lipids as shown by over 60% increase in malondialdehyde content. Superoxide dismutase and ascorbate peroxidase activities increased by 66.67% and 180% in salt stressed plants as compared to the control respectively. Interestingly, all chitosan concentrations significantly reduced the antioxidant enzyme activities in salt-treated plants. The negative effects of salt stress were reversed by the application of chitosan, which resulted in a significant decrease of both osmolytes and oxidative stress markers by more than 50% in all salt-treated plants.

Discussion and conclusion: Results showed that chitosan serves as an excellent bio-stimulant by improving sorghum germination, growth, and conferred a high degree of salt tolerance under NaCl treatment. Preliminary findings suggested that chitosan-induced stress tolerance in sorghum is related to the regulation of osmolytes and the antioxidant system. Future work will analyze the molecular response of chitosan by profiling the transcripts of selected genes. And in a long run, these results will lead to the use of chitosan in a larger scale to improve agricultural productivity and sustain food security globally.

Keywords: Germination, Chitosan, bio-stimulants, NaCl, Osmolytes, Oxidative stress, Salt stress, *Sorghum bicolor*

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