**Final Report: Salt Tolerance in Soybean-2021**

**Summary:**

Global climate change and anthropogenic activities cause severe deterioration of soil health by increasing the levels of salts. This situation is more critical in rainfed and coastal areas of the Delmarva (Delaware-Maryland-Virginia) Peninsula. Salt-water intrusion and well irrigation during the summer season impairs the growth and development of salt sensitive row crops (soybean and corn) with subsequent marginalization of the productive land. Recent data showed (Lambrecht and Todd, 2020) higher salt levels in the coastal area of the Delmarva Peninsula ranges from 1.3 to 4.5 ppt (parts per trillion). This range of salt concentration can inhibit the growth of row crops and perpetuate salt tolerant invasive plants. Soybean cannot tolerate more than 3 ppt of salt concentration. Seawater represents 35 ppt of salt concentration; this concentration may be higher in inlands ditches. Due to continuous rise in sea level, soil salinity will be a serious threat for the cultivation of soybean on the Delmarva Peninsula. Soybean is moderately tolerant to salinity with a threshold of 5 dS/m (approximately 50 mM NaCl). However, salt sensitive cultivars failed to produce seeds at 8 dS/m (approximately 80 mM NaCl; Liu et al., 2016). Salinity imparts negative effects on plant growth and development by manipulation of osmotic and ionic stresses. To prevent soil degradation from salinity, salt tolerant crops are required. In the current work, we screened three varieties (V1: Patent pending, V2: P46A16R, and V3: P48A94PR) of soybean for salt tolerance in potted experiments. Varietal differences were observed in salt tolerance. The variety V1 showed higher yield in salt stress regimes in comparison to V2 and V3. In addition, V1 showed higher leaf fresh weight, root fresh weight, leaf dry weight, root dry weight, nodule per plant, and seed yield per plant in comparison to V2 and V3 in salt stress regimes. Similarly, higher levels of proline were detected in V1 leaves. In addition, V1 showed an improved antioxidant defense system in terms of higher activities of superoxide dismutase (SOD) and peroxidase (POD) with concomitant decrease in hydrogen peroxide levels under salt stress. V1 can be a good candidate for salt affected soils. However, these results were the outcome of a greenhouse study and require testing in field conditions.

**Report:**

Global climate change, seawater intrusion in coastal areas, rainfed agriculture, and poor-quality irrigation water caused salt contamination with concomitant decline in soybean yields. Salt imparts negative effects on all the stages of soybean life cycle through osmotic and ionic effects. Osmotic effects cause lowering of osmotic potential in soil solution, thus restricts water availability in plant and associated metabolic processes. Similarly, ionic effects caused accumulation of toxic ion (Na+, Cl-) in plant cells and therefore, decreased photosynthesis, irreversibly inactivates enzymes, and finally plant cell senescence. In the current work, soybean seed germination potential and crop growth and development indices were evaluated on three (V1: Patent pending, V2: P46A16R, and V3: P48A94PR) commercially available cultivars from BASF and Pioneer companies in saline regimes. Information generated through this work helps to understand salt induced morphological, biochemical, and antioxidant defense mechanisms in soybean for further selection and breeding purposes. In addition, survival of salt tolerant variety ‘V1’ in salt regimes help soybean growers to cultivate this variety in salt affected areas.

Seeds were tested to find out the seed germination percent (SGP) in control conditions before experimentation and results confirmed > 90% seed germination as provided on the label by the company. Uniform sized and intact seeds were selected for germination and seedling leaf studies. Seeds were sterilized with 2.5% sodium hypochlorite for 10 minutes followed by extensive washing with distilled water and air drying. Sterilized seeds were transferred to a 14 cm diameter Petri dish containing two layers of filter paper (P5 grade, Fisher Scientific). Seeds were placed randomly in between the layers of filter paper and soaked with 11 ml of five different NaCl concentrations (0, 50, 80, 100, 150, and 200 mM). Petri dishes were kept at 27 ± 2°C for 5 days using a G24 environmental incubator (New Brunswick Scientific Co., Inc., NJ, USA). Seeds were considered germinated upon display of 1 mm long radicle. SGP was calculated by dividing the number of seeds germinated by the total number of seeds and multiplying by 100. For leaf studies, seeds were planted in pots [18 cm (diameter) x 16 cm (depth)] containing 306 g of sterilized vermiculite (Therm-O-Rock-East, Inc., PA, USA) and kept on plastic saucer to provide water and nutrients from the bottom of the pots. Salt treatments were provided by adding 1000 ml each of 0, 50, 80, and 100 mM NaCl solution before planting seeds. Plants were grown for 100 days and nutrients were provided using Hoagland solution on a weekly basis. Pots were kept in greenhouse in natural light condition [700-750 µmole m-2 s-1 photosynthetically active radiation (PAR)]. The PAR reached to 400 µmole m-2 s-1 (Spectrum Technologies, Inc., IL, USA) on a cloudy day. Greenhouse were operated at 27 ± 2°C temperature, 75% relative humidity, and 12 h photoperiod. Position of pots were changed randomly to avoid positional effect. Plants were removed by washing roots with deionized water. Leaf and root samples were collected at day 15, 41, and 100 days after sowing (DAS) and processed for enzymes [superoxide dismutase (SOD) and peroxidase (POD)], H2O2, and proline. Plant height, and fresh and dry weight of leaf and root was measured at different stages of soybean growth and development [V1 (First trifoliate leaf), R5 (Beginning seed), R8 (Physiological maturity)]. Nodule number, number of pods, number of seeds, and seed yield was also determined. Experiments were conducted in a randomized complete block design during June-Oct. 2021. Fifteen replicates were used for the estimation of all the biochemical parameters and morphological parameters. SGP was evaluated up to 200 mM NaCl while other biochemical parameters were evaluated up to 100 mM NaCl. Plants failed to produce seeds at 150 mM NaCl. The means per plant were determined and subjected to analysis of variance (SAS OnDemand for Academics) and separated using a protected least significant difference (LSD) at P < 0.05. The standard error (SE) of the mean was also calculated.

Seed germination percent declined with increase in NaCl concentration in all the varieties. However, the decline was higher in V2 and V3 in comparison to V1. Decline in SGP was observed at 150 mM in V1 and at 50 and 80 mM NaCl in V2 and V3, respectively. The decline was 8%, 23%, and 13% in V1, V2, and V3 at 150 mM NaCl concentration respectively in comparison to controls. V3 seeds showed germination at 200 mM NaCl, but did not survive. Plant height declined in all the varieties at 80 mM NaCl concentration at 41 DAS. However, plant height increased at 50 mM NaCl in all the tested cultivars, but the increase in height was more in V3 in comparison to V2 and V1. Elongation of stem caused lodging of stem in V3. The decline in plant height was 44%, 15%, and 34% in V1, V2, and V3 at 150 mM NaCl, respectively, in comparison to controls.

Root fresh weight declined in all the varieties at 50 mM NaCl except V1 at 100 DAS. V1 showed an increase in root fresh weight at 50 mM NaCl and then declined. The decline in root fresh weight was 36%, 70%, and 56% in V1, V2, and V3 at 100 mM NaCl, respectively, in comparison to controls. Similarly, root dry weight decreased with increase in NaCl concentration in all the tested varieties. The decline in root dry weight was 62%, 81%, and 81% in V1, V2, and V3 at 150 mM NaCl receptively in comparison to controls. The decline was more in V3 > V2>V1.

Leaf fresh weight declined in all the varieties at 50 mM NaCl, but remained higher in V1 in comparison to V2 and V3 at 41 DAS in all the tested NaCl concentrations. The decline in leaf fresh weight was 33%, 58%, and 36% in V1, V2, and V3 at 100 mM NaCl, respectively, in comparison to controls. Leaf dry weight declined with increase in NaCl concentrations except in V1 at 50 mM NaCl. The decline in leaf dry weight was 12%, 41%, and 23% in V1, V2, and V3 at 100 mM NaCl, respectively, in comparison to controls at 41 DAS.

Number of nodules per plant declined with an increase in NaCl concentration at 15 DAS. However, the decline was less in V1 and V3 in comparison to V2. The decline in number of nodules was 23%, 72%, and 23% in V1, V2, and V3 at 80 mM NaCl, respectively, in comparison to controls at 15 DAS. The number of nodules per plant declined at 50 mM NaCl onward at 41 DAS. The decline was 52%, 81%, and 67% in V1, V2, and V3 at 80 mM NaCl, respectively, in comparison to controls. The number of nodules per plant remained higher in V1 in comparison to V2 and V3 at all the levels of NaCl.

The hydrogen peroxide (H2O2) concentration declined in V1 up to 100 mM NaCl and then increased at 15 DAS. However, H2O2 levels increased in V2 at all the levels of NaCl while in V3 at 80 mM NaCl onward. The decline in H2O2 was 6.7% at 100 mM NaCl in V1 and the increase in V2 and V3 was 43% and 19% respectively in comparison to controls at 100 mM NaCl. The superoxide dismutase (SOD) activity increased in the leaves of V1 up to 100 mM NaCl and then declined at 15 DAS. However, SOD increased up to 80 mM NaCl in V2 and V3 and then declined. SOD activity remained higher in V1 in comparison to V2 and V3 at all the levels of NaCl concentrations. POD activity increased up to 100 mM NaCl in the leaves of both V1 and V3 at 15 DAS. However, POD activity declined in V2 at 80 mM NaCl onward. POD activity remained higher in V1 in comparison to V2 and V3 at all the levels of NaCl concentrations. Proline accumulation occurred in the leaves of all the varieties at 15 DAS with successive increase in NaCl concentration. The higher proline accumulation was observed in the leaves of V1 followed by V3 and V2.

The number of pods per plant declined in all the varieties with increase in NaCl concentrations. The decline was 57%, 80%, and 84% in V1, V2, and V3 at 80 mM NaCl, respectively, in comparison to controls. Similarly, the number of seeds per plant declined in all the varieties at all the levels of NaCl concentrations. The decline was lower in V1 in comparison to V2 and V3 at all the concentrations of NaCl. The decline in number of seeds per plant was 55%, 97%, and 82% in V1, V2, and V3 at 80 mM NaCl, respectively, in comparison to controls. Seed yield per plant declined with successive increase in NaCl concentrations in all the varieties. However, the seed yield was higher in V1 in comparison to V2 and V3. The decline in seed yield was 54%, 81%, and 83% in V1, V2, and V3 at 80 mM NaCl, respectively, in comparison to controls.

The variety V1 showed improved yield in comparison to V2 and V3 due to better-regulated maintenance of water status in leaves and roots. Similarly, lower levels of H2O2 and higher activities of antioxidant enzymes SOD and POD also contributes to V1 survival in salt stress. Higher levels of proline in V1 were evident, which probably maintained osmotic adjustment. All these parameters were compromised or had lower levels in V2 and V3 in comparison to V1, thus causing failure of crop and decline in yield.

**References:**

**1:** 1: Lambrecht, B and T. Gracie. 2020. Coastal farmers in Maryland and across Mid-Atlantic being driven off their land as salt poisons the soil. The Baltimore Sun. <https://www.baltimoresun.com/news/environment/bs-md-coastal-farmers-salt-poisons-soil-20201215-jdzgwdv72nd6zbtnr74majvpui-story.html>.

**2:** Ying L., Y. Lili., Q. Yue., C. Jingjing., H. Huilong., L. Zhangxiong., C. Ruzhen., G. Matthew., Q. Lijuan, and G. Rongxia. 2016. GmSALT3 which confers improved soybean salt tolerance in the field, increase leaf Cl- exclusion prior to Na+ exclusion but does not improve early vigor under salinity. Front Plant Sci. 7 (1-14).