Comparative studies of chlorophyll concentration on different varieties of soybeans treated with different levels of salinity in salt mining site, in Yala Local Government Area, Cross River State, Nigeria.

**Abstract**

Salinity is a major abiotic stress limiting soybean productivity, particulary in salt-affected regions. A pot experiment was conducted in Okpoma, Yala Local Government Area of Cross River State, to compare the impact of increasing salinity levels (0, 4, 6 and 8 dS/m ) on chlorophyll concentration of different varieties of soybeans cultivated in salt mining site over a 12 week period. The experiment was laid in a Completely Randomized Design (CRD), with three replicates for each variety. Data collected was subjected to statistical analysis using Analysis of Variance (ANOVA) and means were compared using Duncan’s New Multiple Range Test (DNMRT). The results revealed a significant decline in chlorophyll content with rising salinity, with sensitive varieties such as TGX 1904-6F and TGX 1905-2F showing early and severe pigment degradation. In contrast, salt-tolerant varieties like TGX 1987-10F and TGX 1448-2E retained higher chlorophyll levels, suggesting presence of adaptive physiological mechanisms including osmotic adjustment, ion homeostasis, and antioxidant defense. These findings highlight chlorophyll content as a reliable physiological indicator for salt stress tolerance and support its inclusion in breeding programs aimed at improving soybean resilience in saline environments.

**Keywords: Soybeans, Concentration, Chlorophyll, Varieties.**

1.0 **Introduction**

Soybeans (*Glycine max* L.) is a vital leguminous crop globally recognized for its rich protein and oil content, contributing significantly to food security and economic development (Lia *et al*., 2018). As a leguminous crop, soybeans are also valued for their nitrogen fixing ability, which improves soil fertility and reduces the need for synthetic fertilizers (Hartwig & Amarger, 2014). However, soybean cultivation faces numerous abiotic stresses, among which soil salinity is a prominent limiting factor affecting crop growth, yield and quality (Munns & Tester, 2008).

Soil salinity is a growing concern worldwide, especially in arid and semi-arid regions, due to natural processes and human activities such as mining, irrigation practices, and degradation (Shahid *et al*., 2019). Salt-affected soils impose osmotic stress, ion toxicity, and nutritional imbalances on plants, leading to reduced photosynthetic efficiency and chlorophyll degradation (Parida & Das, 2005). Chlorophyll content serves as a reliable indicator of plant health and photosynthetic capacity under salinity stress (Khan *et al*., 2019).

Understanding the response of different soybean varieties to salinity, particularly in terms of chlorophyll concentration, can inform breeding programs and management strategies aimed at enhancing salt tolerance.

Yala Local Government Area in Cross River State, Nigeria, hosts salt mining activities that contribute to soil salinization in the region (Nsowu *et al*., 2020). Despite the economic importance of soybeans and the increasing salinity challenges, limited research has been conducted on the comparative effects of soil on different soybean varieties in this locale.

Investigating how varying levels of salinity influence chlorophyll concentration across different soybean varieties can shed light on their relative tolerance and potential for cultivation in salt-affected soils.

This study aims to compare the chlorophyll concentrations of selected soybean varieties subjected to different salinity levels at salt mining site in Yala Local Government Area, Cross River State, Nigeria. The findings will contribute to understanding varietal tolerance mechanisms and support the development of salt -resilient soybean cultivars suitable for saline environments.

**2.0 Materials and Method.**

**2.1Study Area**

The study area was Yala Local Government Area of Cross River State, Nigeria. It is located in the northern part of the State, its headquarters is in Okpoma. It is between 60 42’ N 80 36’E with altitude of 144m and annual rainfall estimated between the range of 2000mm and 3000mm. It has a temperature range between 180C to 400C with optimum temperature of 290C, and a total area of 1,739km2 with a population of 210,843 as the second most populated Local Government in the State. It has abundant salt deposits which are mined locally and can sustain any small to medium scale industry, their major economic activities are farming, mining, and trading. The people of Yala are historically and predominantly subsistence farmers and traders. They cultivate white yams, water yam, black yam and cassava as their main crops for home consumption and sell the surplus in the village market. They also plant other crops like Bambara nuts, groundnut, sesame, maize, pepper, vegetables and African yam bean (NPC, 2006).

**2.2 Planting Material**

Ten different varieties of soybeans seeds *(*(*Glycine max* (L.) Merill)) which included TGX 1910-11F, TGX 1485-1D, TGX 1951-3F, TGX 1835-10E, TGX 1445-2E, TGX 1905-2F, TGX 1904-6F, TGX 1987-10F, TGX 1448-2E and TGX 1987-62F were obtained from the International Institute of Tropical Agriculture (IITA), Ibadan Nigeria, and used for the research.

The salt (NaCl) used for the experiment was procured from a commercial laboratory in Calabar, Cross River State Nigeria. Thirty polybags (30) of equal sizes were procured from the Ministry of Agriculture, Cross River State Nigeria, for the experiment.

**2.3 Soil sampling**

Soil samples were collected at different points in the experimental site after clearing of the experimental site, at a depth of 0 – 25cm using soil auger for pre – planting and post – harvest soil analysis. For the post – harvest soil analysis, samples were taken from all. The soil samples were bulked, air – dried and sieved through a 2mm mesh sieve before analyzing for physical and chemical properties. The soil particle sizes were determined by the hydrometer method (Bouyocos, 1962), textural class (USDA, 1960), phosphorus (Trough, 1930) cation exchangeable bases were also estimated (A.O.A.C., 2005).

0.1g of fresh soybeans leaves was collected at different salinity levels and placed in a test tube filled with 10ml of acetone and was incubated in a dark room for 24 hours at 40C to obtain a green extract. The green extract was collected into a cuvette for spectrophotometric measurement to measure the absorbance of the chlorophyll extract at 663nm for chlorophyll a and 645nm for chlorophyll b. The chlorophyll content was determined using the formular:

Total Chlorophyll content: Total Chl(mg/g) = (8.2x A663) + (20.2 xA645).

**Data analysis:** The data were analysed with SPSS version 20 software and all data collected were subjected to Analysis of Variance (ANOVA) according to Gomez and Gomez, (1984) and treatments were compared using Duncan Multiple Test Range (DMRT) 1995.

**3.0 Results**

The data in the Table below, demonstrated the effects of increasing salinity levels (0, 4, 6, and 8 dS/m) on the chlorophyll content of various soybean (*Glycine max*) varieties over twelve weeks (Week 4, 8, and 12). Chlorophyll content is a critical indicator of photosynthetic capacity and overall plant health, thus providing insight into the plant’s physiological response to salt stress.

At week 4, all varieties generally exhibit high chlorophyll content under control conditions (0 dS/m), with TGX 1448-2E and TGX 1987-10F showing the highest values (7.47 and 7.44, respectively). As salinity levels increase to 8 dS/m, a significant decline in chlorophyll content is evident across all genotypes, with the most pronounced reductions observed in sensitive varieties such as TGX 1904-6F and TGX 1905-2F, where chlorophyll content drops below 1.5. Conversely, more tolerant varieties like TGX 1987-10F and TGX 1448-2E maintain relatively higher chlorophyll levels even at higher salinity, indicating better preservation of photosynthetic pigment under stress.

By week 8, the decline in chlorophyll content becomes more evident. For example, TGX 1987-62F's chlorophyll levels decrease from approximately 6.13 at 0 dS/m to about 5.13 at 8 dS/m, while TGX 1904-6F exhibits a sharper reduction from around 7.43 to below 1.5 at the highest salt concentration. The data suggest that salt stress progressively impairs chlorophyll synthesis and stability, likely due to ionic toxicity and osmotic imbalance disrupting chloroplast function. These effects are more severe in susceptible varieties, which show the lowest chlorophyll levels at high salinity.

At week 12, the trend of declining chlorophyll content persists, with most varieties showing drastic reductions at 8 dS/m. For instance, TGX 1904-6F’s chlorophyll content drops to nearly zero, indicating severe impairment of photosynthesis and potential plant senescence. On the other hand, genotypes like TGX 1987-10F and TGX 1448-2E still retain measurable chlorophyll levels (4.1 and 4.0, respectively), implying that these varieties possess intrinsic mechanisms to mitigate salt-induced damage, such as antioxidant activity or efficient ion regulation.

**Table 1: Number of Branches as affected by different salt concentration rates**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **VAERIETIES** | **Week 4** | | | | **Week 8** | | | | **Week 12** | | | |
| **Concentration (dS/m)** | | | | **Concentration (dS/m)** | | | | **Concentration (dS/m)** | | | |
| **0** | **4** | **6** | **8** | **0** | **4** | **6** | **8** | **0** | **4** | **6** | **8** |
| **TGX 1987-62F** | 4.4333±0.30a | 4.1333±0.15a | 4.1333±0.15a | 4.0500±0.11a | 10.2833±0.49a | 9.3000±0.52a | 9.0333±0.06a | 5.0900±0.08b | 11.6667±0.58a | 10.2167±0.37a | 9.4667±0.35b | 5.0333±0.06b |
| **TGX 1448-2E** | 3.5000±0.00a | 4.0500±0.42a | 3.5000±0.43a | 3.8667±0.11a | 7.3000±0.26a | 6.9667±0.06a | 6.5500±0.39a | 6.8000±0.10a | 8.4500±0.39a | 8.0000±0.10a | 7.1667±0.29ab | 4.5667±0.20b |
| **TGX 1987-10F** | 4.0000±0.00a | 4.2667±0.15a | 5.0500±0.09a | 5.1867±0.32a | 8.0000±0.00a | 7.1000±0.10a | 7.1333±0.15a | 6.1000±0.17a | 13.0833±0.38a | 8.5833±0.52ab | 6.1667±0.20b | 5.0500±0.09b |
| **TGX 1904-6F** | 4.1933±0.17a | 4.190±00.6a | 3.2667±0.30a | 3.3167±0.08a | 9.30± 0.51a | 7.0500±0.09a | 6.3167±0.05a | 5.5833±1.01b | 11.8167±0.76a | 9.2833±0.04a | 7.5967±0.45ab | 6.2500±0.43ab |
| **TGX 1905-2F** | 4.6200±0.10a | 4.2667±0.15a | 4.1167±0.03a | 3.3333±0.15a | 10.0833± 0.14a | 7.5000±0.10a | 6.2000±0.17a | 3.3000±0.26b | 13.6500±0.38a | 7.1833±0.08ab | 6.1500±0.25ab | 0.0000±0.00c |
| **TGX 1445-2E** | 4.1333± 0.56a | 4.2667±0.25a | 3.7167±0.12a | 3.1833±0.17a | 10.8000±0.44a | 7.4000±0.10a | 6.2667±0.15ab | 3.1667±0.20b | 12.6833 ±0.59a | 7.5500±0.13ab | 6.2500±0.22b | 0.0000±0.00c |
| **TGX 1835-10E** | 4.6167± 0.20a | 4.0333±0.58a | 4.0833±0.14a | 3.6833±0.17a | 9.4333± 0.38a | 8.2667±0.38a | 8.1167±0.10a | 7.2667±0.20a | 10.6167±0.54a | 8.4333±0.40ab | 8.2200±0.02ab | 0.0000±0.00c |
| **TGX 1951-3F** | 4.3167± 0.12a | 4.1000±0.10a | 4.0500±0.09a | 3.0000±0.00a | 9.3000±0.26a | 8.2833±0.37a | 8.1500±0.05a | 5.2833±0.49b | 11.9500±0.18a | 8.4500±0.13ab | 8.1000±0.10ab | 5.1967±0.10b |
| **TGX 1485-ID** | 4.6667±0.58a | 4.1833±0.08a | 4.3000±0.10a | 3.2500±0.35b | 10.6533 ±0.30a | 7.4667±0.15a | 6.2667±0.20ab | 3.4833±0.22b | 13.5167±0.55a | 7.6000±0.10ab | 6.4000± 0.10b | 0.0000±0.00c |
| **TGX 1910-11F** | 4.3080±0.37a | 4.4333±0.06a | 4.2500±0.21a | 3.4333±0.02a | 9.9667±0.15a | 7.2833±0.08ab | 6.1333±0.15b | 3.6333±0.11c | 12.4167±0.52a | 7.4000±0.10ab | 6.4000±0.10b | 0.0000±0.0c |

Means with the same alphabet under the same week and concentration are not significantly different at (P < 0. 05)

Overall, the results highlight that salt stress induces a significant decline in chlorophyll content, which correlates with the reduction in photosynthetic efficiency and plant vigor. The differential responses among soybean varieties suggest genetic variability in salt tolerance. Varieties like TGX 1987-10F and TGX 1448-2E demonstrate better resilience, maintaining higher chlorophyll levels under saline conditions. This trait is crucial for sustaining photosynthesis, growth, and yield in salt-affected environments. The progressive decrease in chlorophyll content over time and increasing salt levels underscores the importance of selecting and breeding salt-tolerant soybean cultivars to enhance productivity in saline soils.

Salinity markedly impairs chlorophyll content in soybean plants, with tolerant varieties exhibiting less reduction. These findings provide valuable insights for breeding programs aimed at improving salt tolerance, emphasizing the need to focus on traits associated with chlorophyll retention under salinity stress to ensure crop productivity in challenging environments.

4. 0 **Discussion**

The progressive reduction in chlorophyll content across across soybean varieties exposed to increasing salinity levels (0, 4, 6 and 8 dS/m) observed in this study confirms that salinity stress negatively impacts photosynthetic pigments synthesis and stability. Chlorophyll is fundamental to light harvesting during photosynthesis, and its reduction is a key physiological indicator of plants stress and health deterioration (Parihar *et al*., 2015; Munns & Gilliham; 2015). At week 4, the relatively high chlorophyll levels across all varieties at control suggest optimal physiological conditions. However, the subsequent decline in chlorophyll content with increasing salinity levels reflects the onset of ionic toxicity and osmotic stress, which impair chloroplast development and function (Zor *et al* 2019). Susceptible varieties such as TGX 1904-6F and TGX 1905-2F showed more rapid and severe reductions, indicating their lower capacity to cope with Na+ and Cl- accumulation, as reported by Yadav *et al.* (2021).

At week 8, the worsening impact of salinity was evident, aligning with the work of Khan et al. (2021), who reported significant chlorophyll degradation in soybean under saline irrigation. The sensitive genotype TGX 1904-6F’s sharp reduction from 7.43 to <1.5 highlights its vulnerability, whereas TGX 1987-10F and TGX 1448-2E retained more chlorophyll due to enhanced antioxidant systems and ion compartmentalization.

At week 12, near-zero chlorophyll levels in the more sensitive varieties point toward irreversible damage to the photosynthetic apparatus, likely leading to premature senescence. In contrast, the tolerant varieties maintaining chlorophyll levels above 4.0 demonstrate adaptive traits like better osmotic adjustment and reactive oxygen species (ROS) detoxification (Hasanuzzaman *et al*., 2017, Qin *et al*., 2020).

This differential varietal response underlines the genetic diversity in salt stress tolerance among soybean genotypes. The observed resilience in some varieties suggests the presence of protective physiological mechanisms such as efficient Na+ exclusion, vacoular sequestration, or increased synthesis of osmo-protectants (Zhou *et al*., 2021). The ability to retain chlorophyll content even at high EC levels makes them ideal candidates for breeding programs targeting salinity-affected regions.

Moreover, consistent with recent studies, chlorophyll retention under salinity is highly correlated with sustained photosynthetic activity and yield stability (Nadeem *et al.,* 2020). Therefore, chlorophyll content not only serves as a stress biomarker but also a selection criterion for screening salt tolerant soybean lines (Nguyen *et al*.,2022).

**Conclusion**   
Salinity stress led to significant reduction in chlorophyll content in soybean, particularly in sensitive varieties. However, some varieties exhibited resilience, maintained higher pigment levels under stress, suggesting their suitability for cultivation in salt-affected areas. This study reinforces the importance of incorporating chlorophyll stability into selection indices in soybean breeding for salt tolerance.

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