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-1) on chlorophyll concentration of different varieties of soybeans cultivated in salt mining site over at 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 (McNeil, 2010). 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**.

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.

We hypothesize that salt-tolerant soybean varieties will retain higher chlorophyll concentrations under salinity stress compared to salt-sensitive varieties.

**2.0 Materials and Method.**

**2.1 Study 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).

**2.4 Experimental Design and Replication:** The experiment was laid out in a Completely Randomized Design (CRD) with three replicates per soybean variety. Each replicate consisted of a pot containing three soybeans’ plants, making a total of nine plants per variety per treatment. Three fully expanded leaves per pot (n= 3leaves per pot per replicate) were sampled and measured to obtain mean values for each replicate.

Salinity levels of 4, 6 and 8dS/m were selected to present mild, moderate and severe salt stress conditions based on literature of (Sofy, 2020). EC levels reported in the salt-affected soils of Yala Local Government, Cross River State (Nsowu *et al*., 2020).

**2.5. Treatment application**: Salinity was imposed by irrigating the pots with Nacl solutions prepared at different concentrations equivalent to electrical conductivity (EC) values of 4 dS/m and 8 dS/m-1. Solutions were applied thrice a week, using 200 mL per pot, staring at 7 days after sowing. Soil EC was monitored weekly using a portable EC meter (Hanna H198331 Soil Test Direct) to ensure treatment uniformity.

**2.6. Estimation of chlorophyll content:**

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:

The concentrations of Chlorophyll a, chlorophyll b and total chlorophyll were calculated using the following equation (Arnon, 1949):

Chlorophyll a (mg/gm tissue): [12.7(A663) -2.69 (A645)] \*V/100\*W

Chlorophyll b (mg/gm tissue): [22.9(A645) -4.68 (A663)] \* V/1000\*W

Total Chlorophyll content: (a+b) (mg/gm tissue): [20.21 (A645) + 8.02(A663)] \*V/1000\*W

A= Absorbance of specific wavelength; V= Final volume of chlorophyll extract in 80% Acetone; W= Fresh weight of tissue extract.

**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 New Multiple Range Test (DNMRT) 1955 at 5% probability level performed with SPSS version 20.

**3.0 Results**

The data in the Table below, demonstrated the effects of increasing salinity levels (0, 4, 6, and 8 dS/m-1) 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-1), with TGX 1448-2E and TGX 1987-10F showing the highest values (7.47 and 7.44, respectively) which were significantly different. As salinity levels increase to 8 dS/m-1, a significant decline in chlorophyll content is evident across all genotypes (F (6, 42, =48.73, P < 0.05) 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.

At 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-1to about 5.13 at 8 dS/m-1to, while TGX 1904-6F exhibits a sharper reduction from around 7.43 to below 1.5 at the highest salt concentration. The analysis of variance revealed a significant effect of salinity on chlorophyll content (F (3, 48) = 3.89, P < 0.05). 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-1. For instance, TGX 1904-6F’s chlorophyll content drops to nearly zero, indicating severe impairment of photosynthesis and potential plant senescence.

**Table 1. Chlorophyll as affected by different salt concentration rates**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| **VAERIETIES** | **Week 4** | | | | **Week 8** | | | | **Week 12** | | | |
| **Concentration (**dS/m-1**)** | | | | **Concentration (**dS/m-1**)** | | | | **Concentration (**dS/m-1**)** | | | |
| **0** | **4** | **6** | **8** | **0** | **4** | **6** | **8** | **0** | **4** | **6** | **8** |
| **TGX 1987-62F** | 6.1367±0.15a | 6.0000±0.00a | 5.1333±ab | 5.0000±0.00ab | 6.4000±0.61a | 3.3033±0.44ab | 3.0667±0.12ab | 2.3667±0.40b | 3.0367±0.06a | 2.0000±0.00b | 1.6000±0.26bc | 0.1667±0.12c |
| **TGX 1448-2E** | 7.4700±0.15a | 6.5033±0.10a | 5.1333±0.23a | 4.1100±0.12b | 3.0367±0.06a | 2.0433±0.08ab | 1.6967±0.04b | 1.2733±0.14b | 1.1333±0.06a | 0.7233±0.03a | 0.5067±0.02a | 0.2133±0.02ab |
| **TGX 1987-10F** | 7.4367±0.11a | 5.1000±0.010ab | 4.1000±0.10ab | 3.1033±0.10ab | 2.3700±0.12a | 2.0333±0.06a | 1.2667±0.20ab | 1.0667±0.06ab | 0.5000±0.01a | 0.3600±0.06a | 0.2333±0.02a | 0.1333±0.06ab |
| **TGX 1904-6F** | 7.4367±0.38a | 7.1100±0.19a | 6.0767±0.07a | 6.0667±0.12a | 5.2433±0.25a | 4.0767±0.07a | 1.2667±0.20b | 2.4233±0.14b | 2.0333±0.06a | 1.1667±0.15a | 0.2333±0.02ab | 0.6133±0.01b |
| **TGX 1905-2F** | 5.1033±0.18a | 4.0667±0.12a | 3.1667±0.12a | 3.0333±0.06a | 3.0667±0.12a | 2.0333±0.06ab | 1.2900±0.08b | 1.0000±0.00b | 1.0000±0.00a | 0.0000±0.00b | 0.0000±0.00b | 0.0000±0.00b |
| **TGX 1445-2E** | 4.1333±0.23a | 4.0333±0.06a | 2.7533±0.13ab | 1.7400±0.05b | 3.0333±0.06a | 2.0333±0.06b | 1.7033±0.10b | 0.7000±0.01b | 1.1667±0.15a | 0.0000±0.00b | 0.0000±0.00b | 0.0000±0.00b |
| **TGX 1835-10E** | 4.4000±0.05a | 4.0000±0.00a | 2.1000 ±0.10b | 1.5367±0.15b | 2.2000±0.17a | 2.1333±0.15a | 1.4000±0.10a | 0.6200±0.01b | 1.0333±0.06a | 0.0000±0.00b | 0.0000±0.00b | 0.0000±0.00b |
| **TGX 1951-3F** | 5.3367±0.04a | 5.0000±0.00a | 4.2000±0.26a | 3.1133±0.12a | 3.7533±0.13a | 3.0000±0.00a | 2.1433±0.16ab | 1.0000±0.00b | 2.1667±0.15a | 1.2667±0.12a | 1.2333±0.12a | 0.4133±0.01b |
| **TGX 1485-ID** | 5.2133±0.19a | 3.2667±0.21a | 2.4333±0.06ab | 2.0333±0.06ab | 3.1333±0.15a | 2.0333±0.06a | 1.5000±0.26ab | 0.8000±0.10b | 1.0333±0.06a | 0.0000±0.00b | 0.0000±0.00b | 0.0000±0.00b |
| **TGX 1910-11F** | 5.1333±0.15a | 3.1000±0.10ab | 2.2333±0.40b | 2.1667±0.06b | 3.1667±0.29a | 1.1000±0.10ab | 1.2333±0.12ab | 0.7133±0.02b | 1.2500±0.21a | 0.0000±0.00b | 0.0000±0.00b | 0.0000±0.00b |

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

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. The Overall result showed that, salt stress induced a significant decline in chlorophyll content, which correlates with the reduction in photosynthetic efficiency and plant vigor. The Anova revealed a highly significant effect of salinity on chlorophyll content (F (3, 48) = 34.72, P< 0.05), and a significant genotype x salinity interaction (F (12, 48) =5.21, P< 0.05). 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 (Swar *et al*., 2021).

4. 0 **Discussion**

The progressive reduction in chlorophyll content across soybean varieties exposed to increasing salinity levels (0, 4, 6 and 8 dS/m-1observed 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- accumulationas 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*., 2013).

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, vacuolar sequestration, or increased synthesis of osmo-protectants (Zhou *et al*., 2021). The decline in chlorophyll content under salinity stress observed in this study aligns with established physiological responses in soybean and other glycophytes. High salinity is known to disrupt chlorophyll biosynthesis and accelerate degradation due to oxidative stress and ion toxicity. Notably, tolerant varieties such as TGX 1987-10F and TGX 1448-2E maintained relatively higher chlorophyll levels at 8 dS/m-1, suggesting underlying mechanisms of salt tolerance. These may include efficient Na⁺ and Cl⁻ exclusion from leaf tissues, accumulation of compatible osmolytes such as proline to stabilize cellular structures, and enhanced activity of antioxidant enzymes (e.g., superoxide dismutase, catalase), which mitigate reactive oxygen species (ROS) damage to chloroplasts. Although these mechanisms were not directly measured in the current study, they have been reported in previous work as key components of salinity tolerance in soybean. For instance, IITA-based studies in Nigeria (Oyiga*et al*., 2018) and related research in Senegal and India have similarly shown that salt-tolerant genotypes maintain chlorophyll content through biochemical and ionic homeostasis. Compared to those studies, the degree of chlorophyll loss in sensitive genotypes like TGX 1904-6F in our trial is particularly severe, indicating possible varietal vulnerability in extreme saline environments such as the salt mining zones of Yala, Cross River. This highlights the need for breeding programs to prioritize genotypes combining both physiological tolerance traits and agronomic performance. Therefore, chlorophyll content not only serves as a stress biomarker but also a selection criterion for screening salt tolerant soybean lines cultivation.

**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 in salt-affected areas. This study reinforces the importance of incorporating chlorophyll stability into selection indices in soybean breeding for salt tolerance. The superior chlorophyll retention ability in TGX 1987-10F and TGX 1448-2E under salinity stress, future studies should evaluate their performances under field conditions and consider them as potential parent lines for developing salt-tolerant cultivars. Incorporating chlorophyll stability into selection indices could accelerate breeding progress. Moreover, molecular analysis to uncover the underlying genes controlling this trait would offer valuable insight into genetic architecture of salinity tolerance in soybean.

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