

Effect of Sodium Chloride on Seed Germination and Seedling Growth of Yellow Fluted Pumpkin (*Telfairia occidentalis*) in the Niger Delta, Nigeria

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Abstract Experiments were carried out in the laboratory and screen-house to investigate the effects of sodium chloride on seed germination and seedling growth of yellow fluted pumpkin (*Telfairia occidentalis*). In both experiments, five levels of sodium chloride viz: 0 dS/m (control), 0.75, 1.5, 3.0, and 6.0 dS/m. The treatments were laid out in a completely randomized design (CRD) with five replications (in Experiment 1) and 3 replications in Experiment 2. Emergence percentage (EP) and Emergence speed index (ESI) were significantly higher (up to 80% and 0.87 respectively) at low concentrations of NaCl (0.75, 1.5, and 3.0 dS/m; particularly at 0.75 dS/m NaCl) than the control. In the control, EP and ESI were 13.3% and 0.14, respectively. Although NaCl at the concentrations studied improved the synchrony of germination, they caused a delay in mean time to emergence (MTE). At higher concentration of NaCl (6.0 dS/m), EP, ESI and MTE were negatively affected. The growth (evaluated using by vine length, number of leaves per plant, leaf relative water content, dry matter content of stem, root and leaves) of established seedlings was not significantly ($p < 0.05$) inhibited by irrigating soil with the range of NaCl concentrations tested. N, P, K Mg and Na contents increased compared to the control with K, Mg and Na being at least double that in the control. Uptake of K, Na and Mg increased with the application of NaCl while others declined. Chlorophyll content declined significantly with increasing concentration of NaCl, and it was negatively ($p < 0.05$) related to leaf phosphorus content alone ($y = 2974.14 \pm 31.71P$; $p = 0.03$). Thus, yellow fluted pumpkin appears to be tolerant to NaCl levels below 3.0 dS/m, and so, should be conserved as a food security crop.

Keywords Fluted pumpkin, Seed germination, Seedling growth, Sodium chloride food security

1. Introduction

Secondary salinization occurs in arable lands, and this is mainly caused by two factors amongst others: (1) the use of poor quality surface or saline underground water for irrigation particularly in regions where evaporation exceeds precipitation, and (2) ingress of sea water [1]. Secondary salinization accounts for the degradation of as large as a third of the worlds' agricultural land and this occur in all climates, with Africa and Asia being most affected [1].

With the current promotion of off-season crop production and the apparent consequences of climate change, this value is even likely to increase. Since man's existence depends on soil as primary source of resources for food, fuel and fiber production, efforts must be geared towards identifying and conserving crop species that are adapted to adverse conditions such as salinity as well as protect lands from further degradation.

When sodium chloride (NaCl) salt dissolves in water, they disintegrate into ions (tiny electrical particles) that alter the electrical conductivity (EC) of the water, and as the concentration of the ions increase, the electrical conductivity (in dS/cm^2 or dS/m) of the water increases [2]. Plant activities are completely halted by EC levels synonymous with that of seawater, i.e., 54 dS/m or 54,000 $\mu\text{S}/\text{cm}$. At salt stress concentrations far lower than seawater, soil water and nutrient acquisition by plants is hindered due to negative osmotic effects, direct cell damage, or failure of soil particles to aggregate [3]. These in turn lead to reduced yields, farmers' income and nutrition. Salt sensitive plants are shown to suffer from salt concentrations at all stages of development with the most sensitive process being germination [4], [5], [6].

Salt resistant plants on the other hand, develop adaptive mechanisms that enable them escape, exclude, ameliorate or tolerate the ions. Many crop plants are however known to have low tolerance to NaCl [3], [1]. In the Niger Delta region of Nigeria, the soils in some coastal areas are saline and seasonally water logged as a result tidal flow. Mangrove forests are also common.

Fluted pumpkin (*Telfairia occidentalis* Hook. F.) is one of

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the most commonly cultivated leafy vegetables in the Niger Delta region of Nigeria. It is a tropical vine grown for its edible leaves and seeds. It belongs to the family Cucurbitaceae [7]. The common names include; fluted gourd, fluted pumpkin, and "Ugu". In Nigeria, there are two major cultivars: 'ugu ala' with black/ dark red seeds and 'ugu elu' with brown/ yellowish seed [8].

Fluted pumpkin is cultivated in many West African countries, where it is used primarily in soups and herbal medicines [9]. Although the fruit is not edible, the seeds are high in protein and fat, and can, contribute to a well-balanced diet. The plant is a drought-tolerant; dioeciously perennial that is grown trellised [7].

Whilst the botany of fluted pumpkin is relatively clear, the germination and seedling growth response of its seeds and seedlings to NaCl is still poor. The work of [10], suggests that seedling growth of fluted pumpkin is highly sensitive to low concentrations of NaCl (0.005- 0.03%).

However, since the test cultivar is often not stated, it is unclear whether the finding is applicable to the yellow fluted pumpkin, which is the popular cultivar among farmers. In addition, the response of fluted pumpkin seeds and established fluted pumpkin seedlings to NaCl in irrigation cropping is unclear. The objective of this study was, to determine the effect of different concentrations of sodium chloride: 0 (control), 0.75, 1.5, 3.0, 6.0 dS/m, on germination and seedling growth of yellow fluted pumpkin.

2. Materials and Methods

The study consisted of Laboratory and Screen -house experiment conducted in the Department of Crop and Soil Science, Faculty of Agriculture, University of Port Harcourt. The Yellow fluted pumpkin pods were obtained from Rivers State Agricultural Development Agency (ADP), Rivers State, Nigeria.

2.1. Soil Analysis

Prior to experimentation, composite soil sample were taken from heaps of soil that was randomly collected within the study area. The samples were analyzed for particle size, total organic carbon, available phosphorus, total nitrogen, exchangeable bases (Ca, Mg, K and Na). Total exchangeable acidity ($H^+ + Al^{3+}$), and pH; using standard methods.

2.2. Preparation of Sodium Chloride Solution

The salt concentrations were chosen based on knowledge of the general response of plants to salt levels ranging from 0 to 7.5 dS/cm [2]. Based on [2], electrical conductivity of 0-0.75 dS/m has no effect on most plants. At 0.75-1.5ds/cm, sensitive crops are negatively affected. At 1.5 – 3.0 ds/cm many crops are adversely affected. At 3.0 – 7.5, most plants are adversely affected except tolerant plants. The test

concentrations were prepared using analytical grade sodium chloride.

2.3. Experiment 1. Effect of Sodium Chloride Concentration on Yellow Fluted Pumpkin Seed Germination (Laboratory)

Germination test was carried out on Whatman No.1 filter paper in 9 cm plastic bowls. Seeds were sterilized using sodium hypochlorite to avoid contamination by microorganisms during the germination process. The seeds were then rinsed with distilled water and soaked in different concentrations of sodium chloride solution (0, 1.0, 1.5, 3.0 and 5.0 dS/cm NaCl) for 6 hours. The seeds were then placed on moistened filter paper and observed daily for date of radicle, plumule emergence (germination).

The treatments were laid out in completely randomized design (CRD) replicated five times (9 seeds planted per treatment per replicate). The bowls were slightly covered to reduce evaporation and placed on the laboratory bench at 29.4°C. The seeds were observed daily for 12 d at the end of the experiment; data was collected on length of radicle and plumule (cm). These data were used to calculate emergence percentage (EP); emergence speed index (ESI) [11] and mean time of seedling emergence (MTE) in days [12].

2.4. Experiment 2: Effect of Sodium Chloride Concentration on Seedling Development (Pot Experiment)

The experiment was conducted in a Screen house in the Department of Crop and Soil Science. Ninety (90) ten (10) liter capacity plastic pots with perforations at bottom were filled with 10 kg of top soil. The pots were saturated with water and allowed to drain for 4 days to slightly below field capacity (FC). The plastic pots were arranged in a completely randomized design (CRD) in three replicates with six plastic pots per treatment per replicate. Four seeds of fluted pumpkin were planted into each plastic pot and subsequently each plastic pot was irrigated as required to FC until two days before treatment application.

The seedlings were first irrigated with 200 ml of the respective sodium chloride concentrations at 3 weeks after emergence (WAE) and again after 2 days to make a total of 400 ml of NaCl. The parameters collected include: vine length, number of leaves, relative water content following [13], and chlorophyll content [14]. At the end of the study, two plants per treatment per replicate were separated into their component parts and fresh and dry weights collected. Drying was performed at 80°C until constant weight was obtained. In addition, tissue samples were analyzed for nutrient content and nutrient uptake (g/plant) calculated. Data collected from experiments 1 and 2 were analyzed using analysis of variance (ANOVA). Means were separated using least significant difference (LSD) at 5% probability.

3. Results

3.1. Soil Characteristics

The physiochemical characteristics of the soil before planting shows that the soil was sandy loam, slightly acidic with a pH of 6.3 and fertility with high organic carbon content, total nitrogen, available P, exchangeable K, Mg, Ca and Na but low nitrogen content (Table 1).

Table 1. Soil physiochemical properties before planting pumpkin seeds

Soil properties	Value
Sand (%)	90
Silt (%)	6.0
Clay (%)	4.0
pH (H ₂ O)	6.3
Total organic carbon (%)	2.93
Total nitrogen (%)	0.17
Available P (mg/kg)	38.50
Ca	0.87
Mg	1.65
Na	0.95
K	1.98
TEA	2.81
ECEC	8.26

3.2. Effect of Sodium Chloride Concentration on Germination of Yellow Fluted Pumpkin Seeds

3.2.1. Percentage Emergence (EP)

Percentage emergence increased by 66.7% when seeds absorbed NaCl solution at 0.75 dS/m compared to the control (0 dS/m) (Table 2). Although, higher concentrations of NaCl (1.5, and 3.0 dS/m) also significantly increased percent germination compared to the control, the increase was only by 53 and 47% respectively. At the highest NaCl concentration (6.0 dS/m NaCl, however, percentage germination significantly declined compared to that at all other concentrations of NaCl. However, it did not significantly differ from that in the control. Thus, 0.75 dS/m NaCl significantly ($P < 0.05$) had the highest emergence percentage which was statistically similar to that at 1.50 and 3.0 dS/m NaCl while the control had the lowest germination percentage and it did not vary significantly from that at 6.0 dS/ NaCl.

3.2.2. Emergence Speed Index (ESI)

The emergence speed index of the NaCl treated fluted pumpkin seeds followed the same trend as percentage emergence (Table 2). The highest number of germinating seeds per day (0.87) was observed at 0.75 dS/m and 3.0 dS/cm, which were not significantly different from that at 1.5 dS/m. Emergence speed index was lowest in the control and at 6.0 dS/m NaCl. Thus, the best synchrony of germination was observed at low NaCl concentration than without NaCl or at 6.0 dS/m NaCl.

Table 2. Effects of NaCl on germination characteristics of fluted pumpkin

NaCl (dS/m ⁻¹)	Sqrt. EP	ESI	MTE	Radicle length at 10 DAT
0	2.11 (13.3)	0.14	1.67	0.83
0.75	8.90 (80.0)	0.87	4.91	2.30
1.5	8.07 (66.7)	0.73	4.67	1.64
3.0	7.67 (60.0)	0.87	4.08	2.07
6.0	4.47 (20.0)	0.18	5.67	1.27
LSD (P=0.05)	3.510	0.216	2.535	2.17

Sqrt. EP = square root transformed of emergence percentage, ESI = Emergence speed index, MTE = Mean time of seedling emergence, RL = Radicle length. Values in parenthesis are actual EP.

3.2.3. Mean Time to Emergence (MTE)

In contrast to EP and ESI responses of fluted pumpkin seeds to NaCl, mean time to seedling emergence was significantly delayed by NaCl treatments (Table 2). Time to emergence was most delayed (5.67 d) at the highest NaCl level (6.0 dS/m), which was statistically the same as the other levels of sodium chloride except the control. Hence, germination was earliest by at least 2 d in the control than NaCl treatments.

3.2.4. NaCl Effect on Radicle Length

The longest radicle (2.30 cm) was observed on seeds given 0.75 dS/cm NaCl solution while the shortest radicle length was observed in the control. However, these differences were not statistically significant at $P < 0.05$. Radicle length was found to be strongly ($r^2 = 0.702$) and positively correlated with emergence speed index (ESI) but not mean time of seedling emergence (MTE) ($r^2 = 0.050$). The relationship between radicle length and ESI was explained by the equation: $RL = 0.522 + 1.975 \text{ ESI}$ ($P = 0.05$). Thus, mean radicle length depends more on a combination of number of germinating seeds and date germination (ESI) than date of germination alone.

3.3. Effect of Sodium Chloride on Yellow Fluted Pumpkin Seedling Growth and Development

3.3.1. Vine length

Vine length of the yellow fluted pumpkin seedlings did not differ ($P > 0.05$) significantly across treatments at each sampling date (4, 5 and 6 WAE). At 4 WAE, vine length ranged from 31.56 to 36.67 cm. At 5 and 6 WAE, it ranged from 41.03 to 70.22 cm and 78.39 to 98.67 cm respectively. At all sampling dates, the shortest vines were observed amongst vines in 6 dS/m NaCl treatment.

3.3.2. Number of Leaves Per Plant

Number of leaves per plant did not differ significantly among the various concentration of sodium chloride throughout the study. At 4, 5 and 6 WAE, the number of leaves ranged from 9 to 10, 9 to 16, and 13 to 17 respectively with 1.5 dS/ m NaCl having the greatest number of leaves

while 3.0 and 6.0 dS /m NaCl had the fewest number of leaves per plant.

3.3.3. Dry Matter Content

Analysis of dry matter content was carried out at 5 WAE. At this date, there were no significant difference in root, stem and leaf dry weights across the various concentrations of sodium chloride. Root dry weight ranged from 0.07 to 0.302 g with the control (0 dS/m NaCl) having the highest root dry weight (0.302 g). Stem dry weight ranged from 0.453 to 0.670 g with the control having the highest stem dry weight than other treatments (NaCl treatments). Leaf dry weight ranged from 0.763 g – 1.303 g. Thus, NaCl only slightly reduced root, stem and leaf dry matter content. Also, leaves accumulated the greatest dry matter followed by the stem and then the root.

3.3.4. Mineral Nutrient Concentration and Uptake

The mineral nutrient concentration (N, P, K, Mg, Na and CL ions) of fluted pumpkin tissue at 5 WAE suggests that N and K contents were clearly higher in the tissues than Mg, P and Cl⁻ (Fig. 1). Also, the figure shows that N, P, K, Mg and Na contents increased with the application of NaCl. Phosphorus content was 0.0175% in the control while values at 0.75, 1.5, 3.0 and 6.0 dS/m NaCl were 0.0187, 0.0177, 0.0186 and 0.0187% respectively. Potassium, Mg and Na contents in tissues under 1.5, 3, and 6 dS/m NaCl were at least double those in the controls. In contrast, chloride content declined with treatment in NaCl.

Uptake of Nitrogen, P and Cl⁻ were slightly lower with NaCl application than the control while K, Na and Mg and uptake increased with the application of NaCl (Fig. 1). Phosphorus uptake in the control was 0.00021 g/pot while values at 0.75, 1.5, 3.0 and 6.0 dS/m NaCl were 0.00014,

0.00011, 0.00016 and 0.00015 g/pot respectively.

3.3.5. Leaf Chlorophyll Content at 5 WAE

There was significant difference in mean chlorophyll content across the various concentrations of sodium chloride at 5 WAE, with leaf chlorophyll being the highest in the control (1975 µg/mg FW) and about 66% less under 6.0 dS/m NaCl treatment (Table 3). Generally, therefore, chlorophyll content declined with increasing concentration of NaCl.

Regression of chlorophyll content with nutrient content or uptake showed that chlorophyll content was strongly and negatively (p=0.05) related to phosphorus content alone. The relationship was explained by the equation: chlorophyll= 2974.14 + - 31.71P (P<0.05).

Table 3. Effect of different concentrations of NaCl on chlorophyll content (µg/mg FW) of yellow fluted pumpkin at 5 WAE

NaCl conc. (dS/m)	Chlorophyll content (µg/mg FW)
0	1975
0.75	1475
1.5	1792
3.0	1436
6.0	1312
LSD (p=0.05)	496.4

3.3.6. Leaf Relative Water Content at 5 WAE

The relative water content ranged from 61.5 to 71.5% with the control (0 dS/m NaCl) having the highest (71.5%) relative water content and 6.0 dS/m NaCl the lowest (61.5%). However, the differences in mean relative leaf water content across the treatments were not significantly different at p<0.05.

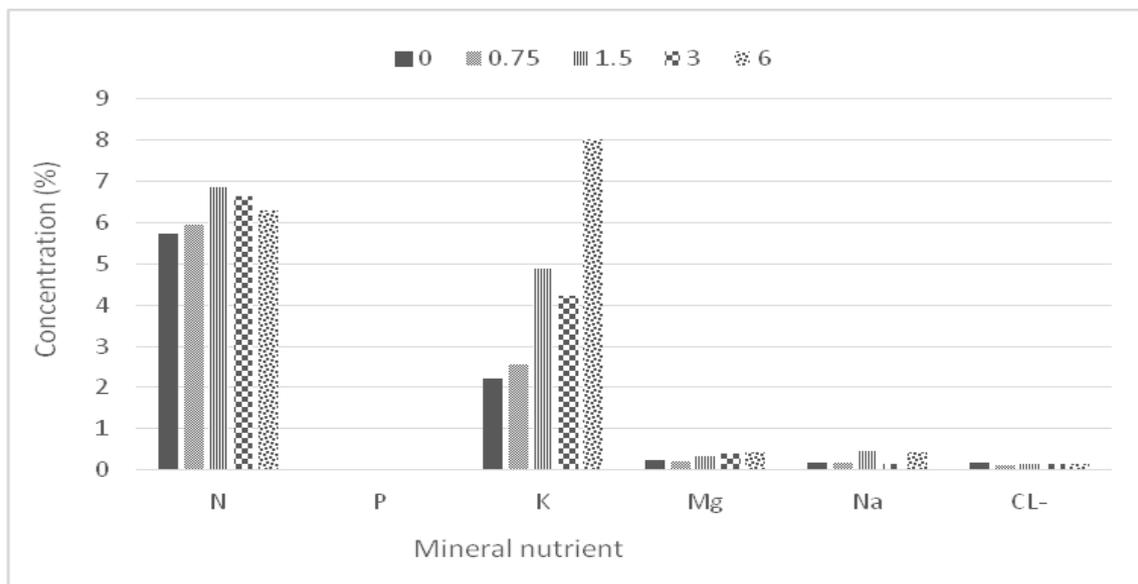


Figure 1. Fluted pumpkin tissue mineral nutrient concentration (%) at 5 WAE under five NaCl concentrations

4. Discussion

Sodium and chloride are essential in minute quantities for metabolic activities of salt loving plants. In this study, sodium chloride solution at concentrations between 0.75 to 3 dS/m significantly improved seed germination of yellow fluted pumpkin judging from their effect on percentage emergence, emergence speed index and mean time to seedling emergence compared with the control. In contrast, sodium chloride concentration above 3.0 dS/m (*i.e.* 6.0 dS/m) negatively affected germination of yellow fluted pumpkin. Similar delays in time to germination have been reported in snake melon (*Cucumis melo* var. *flexuosus*), *Lactuca sativa*, pepper etc. [15], [16], [6] but not in yellow fluted pumpkin. The observed delay in mean time to germination as NaCl concentration increased may be related to inhibitions associated with slow imbibition of water; since imbibition is strongly correlated to the available water content and the resistance of water flow [3] or the time taken to adjust internal seed mechanisms to the low extracellular osmotic potential.

Although germination was delayed under the sodium chloride salt treatments, the number of germinating seeds per day (ESI) was higher in the salt treatments than the control. Hence, NaCl solutions less than 3.0 dS/m increased the rate and synchrony of germination in yellow fluted pumpkin. The good germination indices at the different levels of sodium chloride suggests a combination of the effects of low osmotic pressure in the seeds, which supports the absorption of water via osmosis for germination, and increased cellular respiration (salt respiration). As reported in [17], germination and seedling development was encouraged in barley under concentrations up to 500 mM NaCl (4.55 dS/cm). Therefore, based on [2] the yellow fluted pumpkin seeds studied here may be classified as 'tolerant' to sodium chloride with respect to germination. Also, according to [18] germination of seeds under salt stress could be used as a criterion for identifying species that are salt tolerant.

When sodium chloride solution was applied after seedling establishment, leaf chlorophyll; the green pigment important during photosynthesis, content at 5 WAP was significantly ($p < 0.05$) reduced compared to the control and this was found to be negatively related to leaf phosphorus. This observed reduction did not however, translate to significant reductions in leaf, stem and root dry matter contents and leaf dimensions. Also, in spite of the presence of increased tissue Na content and uptake, vine length, number of leaves, fresh and dry weight and relative water content of NaCl treated plants did not significantly vary from the control. These findings contradict those of [11] perhaps due to differences in cultivars of fluted pumpkin, and/or the fact they transplanted seedlings into soils containing NaCl. Transplanting of seedlings, and into soil containing NaCl, may together delay seedling establishment. The response of yellow fluted pumpkin to the NaCl concentrations tested in this study could be attributed to the inherent genetic capacity of the fluted pumpkin plant to tolerate salinity and/or to the

presence of osmotic adjustment. The observed increase in potassium content may be connected with this osmotic adjustment. [19] noted that the ability of plants to tolerate and flourish under sodium chloride stress condition is of major significance in agriculture; it indicates a genetic potential for salt tolerance.

5. Conclusions

The results of this study showed that the control had the lowest of germination judging from its low percentage emergence, emergence speed index and mean time of seedling emergence and radicle length when compared to sodium chloride at different levels. Chlorophyll content was higher in the control and 1.5 dS/cm NaCl than in the higher NaCl treatments. At higher concentrations of sodium chloride, the uptake of K, Na, and Mg, ions slightly increased slightly while N, P and Cl reduced slightly at various levels of concentration of NaCl when compared to the control. However, seedling growth parameters were not significantly affected by NaCl. Therefore, yellow fluted pumpkin might be tolerant to NaCl concentrations below 3.0 dS/m. Continued cultivation of yellow fluted pumpkin in Niger Delta region of Nigeria is advised for food as well as for conservation as a food security crop in the event of climate change associated increases in sodium chloride level.

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