Effect of sodium chloride salinity on germination and growth of pumpkin seedlings in Maseno (Kenya)

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Abstract*: Salinity is one of the most serious abiotic factors that limit agricultural productivity with an adverse effect on germination and growth parameters throughout the world. Sodium ions (Na^+) and chlorides (Cl^-) ions have been identified to turn agricultural land into unproductive lands unable to support the growth of plants for food and nutrient supplements which results in food insecurity. *C. pepo* L. plant is a fruit vegetable, a source of food, and nutrients, a medicinal and short seasoned crop that has not only been neglected but also is underutilized. The sodium Chloride salinity tolerance of *C. pepo* is not well understood; therefore this study was initiated to determine the effect of Sodium Chloride salinity on the germination and growth of *C. pepo* seedlings. Seed materials were purchased from the East Africa Seed Company (Kitale). They were carried in sealed labeled envelopes in the botany laboratory. The germination test was done using ten seeds under treatment of NaCl (Control 0, 35mM, 70mM, 105mM, and 140mM) using a completely randomized design. A growth experiment was set up in the greenhouse using varied salinity levels of NaCl (Control 0mM, 35mM, 70mM, 105mM, and 140mM). Growth parameters were collected after three days intervals for a period of one month. Chlorophyll a, b, and total chlorophyll were estimated. Analysis of variance (ANOVA) test ($p \leq 0.05$) showed that germination was inversely affected by salinity treatment with complete inhibition at higher concentrations. Salinity was significantly different as concentrations increased. Since *C. pepo* L. could withstand salinity levels of upto70 mM with germination of up to 47%, farmers within saline areas or those who use irrigation water should use water that has a salinity concentration that is not beyond 70mM.

***Keywords*: Salinity, *Cucurbita pepo* L., Sodium Chloride, Germination, Sodium ions, Chloride ions**

I. INTRODUCTION

Salinity refers to the occurrence of high concentrations of dissolved major inorganic ions in solution which include Na^{2+}, Cl^-, $SO_4^{2-}$and Mg^2[1]. Salinity is one of the most serious factors that limit the agricultural productivity of crops with an adverse effect on germination, plant vigor, and crop yields [2] [3].

Salinization is a common problem in Kenya and occurs as a result of increased Sodium ions (Na^+), Potassium ions (K^+), Calcium ions (Ca^{2+}), Magnesium ions (Mg^{2+}), and Chloride ions (Cl^-) [4]. Soils predominated by Na^+ can become acidic and tend to have a very poor structure which limits water infiltration or drainage over a long period of time.

High salinity causes water stress, ion toxicity, osmotic stress, mineral deficiency, membrane disorganization, reduction of cell division, and cell expansion [5]. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. Long-term exposure to salinity leads to ionic stresses that result in senescing of adult leaves, therefore, reducing the photosynthetic area available to support growth. Excessive presence of Na^+ and Cl^- ions cause swelling and toxicity symptoms such as leaf necrosis and chlorosis which occur in leaves due to high Na^+ that affect protein synthesis [6].

Since salinity affects crops differently, knowledge of levels of salinity concentration in relation to agricultural crops needs to be understood well. At low concentrations, highly sensitive plants will be affected while tolerant plants will not be affected at all. Increasing salinity concentration lowers yields to zero. Other workers have shown that most plants will not be able to grow in high salt concentrations and their growth is severely inhibited or even killed by NaCl when salinity levels range between 100-200mM [5].

The pumpkin plant (*Cucurbitapepo* L) is an indigenous fruit-vegetable that is known as "Liondo" in Luhya, and "Ilenge" in Kamba and scientifically belongs to the Cucurbitaceae family. This family is one of the largest families with edible plant species. It has consumable leaves, vines, and fruits as per the local communities in Kenya.

The pumpkin plant is a notorious plant with a high level of nutrients. According to previous work [7], pumpkin leaves have 43.8% protein content that is comparable to soybean. They contain more nutrients per unit of land area than staples such as rice. Consumption of this pumpkin plant is therefore important as it contains components of a healthy diet, i.e. fruit vegetables which could help prevent a wide range of diseases human diseases [8] [9], further more it has been reported that indigenous vegetables on average contain levels of Ca^{2+} and vitamins that would provide 100% of the daily requirement and up to 40% of the protein [9]. Given that pumpkin leaves are an indigenous vegetable in Kenya, it is not excluded. Indigenous vegetables are therefore a valuable source of nutrition in rural areas where they contribute substantially to proteins, minerals, and vitamin intake [10].

Although effects of salinity stress have been studied on horticultural crops such as pepper, Melons [11], and the

African nightshade [12]. Few studies have reported effects of salinity on some pumpkin varieties such as *Obez*, *RS841*, *and Ferro* F_1 which are used as rootstocks in grafting, but not on *C. pepo* grown from seeds [13]. Therefore, this study was set up to investigate the effect of NaCl salinity on germination and growth of seedlings of *Pumpkin*.

II. MATERIALS AND METHODS

2.1 Collection of seed materials

Cucurbita pepo seeds (Pumpkin seeds) were purchased from the Kitale Branch for East African Company. The seeds were carried in sealed labeled envelopes to Maseno University Botany Laboratory where they were kept beforethe start of research.

2.2 Germination test

Ten seeds were subjected to a germination test on petri dishes lined with whatman number one filter paper under treatments of NaCl (0, 35mM, 70mM, 105mM, and 140mM) with three replications at room temperature. Germination percentages were calculated using the formula:

Germination percentage= (Number of germinated seeds/Total number of planted seeds) ×100 according to the procedure by Liu, et al. [14].

2.3 Greenhouse experimental setup

These seeds were grown in plastic pots of a volume of 4.5 liters under polythene coveredgreenhouse (Day Temperatures 25°C-40°C and Night Temperatures 20°C-30°C, 14/10h photoperiod and humidity 70-90%) located at the University Research farm in Maseno. Maseno is situated in western Kenya, its geographical coordinates are 0° 10' 0" South, 34° 36' 0" East, and the altitude is 1,503 meters or 4,934 feet above sea level (KNBS, report, 2013). Maseno receives both short and long rains averaging 1750mm per annum with a mean temperature of 28.7°C. Pots were first cleaned by washing to remove any soil material or any material that could cause contamination. Maseno soils are classified as acrisol deep reddish brown clay and well drained with a pH range of 4.5- 5.4 [15]. Soils were solarized for 48 hrs.in the greenhouse for sterilization. After which each pot was three-quarterfilled with topsoil and eight seeds were planted at a depth of 3cm below the soil surface.

Tap water was used for irrigation and continued after germination which took 16 days and no more germination occurred. Each pot had at least four plants germinated (Plate 1). NaCl salinity treatment levels of NaCl (0mM, 35mM, 70mM, 105mM, and 140mM) were initiated three weeks after plants had germinated and this was to reduce osmotic shock which might have killed the seedlings.

Salinity treatments were irrigated after every three days withthree replications in a completely randomized layout. Growth parameters were collected throughout the experiment.

2.4 Determination of shoot height

Shoot height was measured after three days using a meter rule. Measurements were taken from the base of the plant to the last foliage leaf according to the procedure earlier described [16].

2.5 Determination of leaf area

Leaf samples werefrom one plant per treatment and leaf area was estimated according to the procedure earlier described [17].

The formula for estimating leaf area

$$L = 0.5 (L1 \times W1)$$

Where; L1- was leaf length andW1 - was the maximum width for each leaf per plant.

2.6 Determination of stem diameter

The pumpkin stem diameter was measured each time other growth parameters were collected using a vernier caliper. Measurements were taken 1 cm above the soil surface according to the procedure earlier described [18]

2.7 Determination of root length

Root length was determined at the end of the experiment. The pots containing the plants were immersed in a bucket containing full water and left for ten minutes to allow soils to get wet and ensure no root material was left behind during the uprooting of the targeted plant. The plant was uprooted and the main root was measured from the base of the stem to the tip according to the procedure earlier described [19].

2.8 Determination of fresh and dry weights of shoots and roots.

The fresh and dry weights of shoots and roots were determined at the end of the experiment in the laboratory. The plants were carefully uprooted in the morning hours (08:20hrs.) when evaporation was low, carried in well-labeled polythene bags each containing water so that the plants never lost any moisture during transportation from the greenhouse to the laboratory. At the laboratory, shoots were separated from roots packed in well-labeled envelopes and weighed immediately using an electronic balance. The fresh shoot and root weight was obtained by subtracting the envelope material weight which was then subjected to oven drying at 60°C for 48 hours after which they were reweighed to obtain dry weights [20].

2.9 Determination of Chlorophyll a, b, and total chlorophyll content

Chlorophyll content was determined by taking a sample of fourth fully expanded leaves from the shoot apex of all treatments. 0.3 grams from Pumpkin leaves were cut into small piecesand soaked overnight in 5ml of 80% v/v acetone according to the procedure earlier discussed [19]. Readings were taken at wavelengths 664nm and 647nm using a UV-visible spectrophotometer. The total chlorophyll content was then calculated [21] using the equation below;

Total chlorophyll= 7.93 *A664* +19.53 *A647* (mg g^{-1}fresh weight).

Where;

A664 is the absorbance at 664 nm

A 647 is the absorbance at 647 nm.

III. RESULTS

3.1 Effect of NaCl salinity on germination and growth parameters of pumpkin

NaCl salinity generally had a negative effect on germination and most growth parameters of pumpkin

3.2 Effect of NaCl salinity on germination,

When the germination was inversely related to salinity percentages of pumpkin seeds, it showed that a high level of salinity significantly inhibited the germination of pumpkin seeds. The germination percentage significantly decreased as the concentration of salinity increased. In the control experiment (0mM), the germination percentage was above 76.67 percent and significantly different from treatments 35mM, 70mM, 105mM, and 140mM. Treatments 35mM and 70mM were not significantly different from each other, i.e. 40.67 and 40.0 but these two treatments were significantly different from treatments 105mM and 140mM which means of 10.0c and 0.0c.Table 1.

3.3 Effect of NaCl salinity on shoot height of pumpkin

NaCl salinity significantly affected shoot height at higher concentrations. Control 0mM was significantly different from Treatment 105mM and 140mM on shoot length. The control showed the highest height treatment 0mM was not significantly different from treatment 35mM and 70mM which had shoot height means of 27.867mm, 25.917mm, and 24.604mm while treatment 35mM and 70mM were not significantly different from treatment 105mM and 140mM on shoot height with a mean height of 25,917mm, 24.604mm, 22.07mm, and 22.05mm respectively.

3.4 Effect of NaCl salinity on stem diameter of pumpkin

NaCl significantly affected stem diameter. Treatment 0mM, was significantly different from treatment 35mM, 70mM, 105mM and 140mM with stem diameter means of 1.300a,0.924b,0.849b,0.661b and 0.658b respectively at $p \leq 0.05$ (Table1) .Treatment 35mM, 70mM, 105mM, and 140mM were not significantly different from each other at $p \leq 0.05$. Stem diameter was strongly negatively correlated to moisture content with a value of -0.309.This is due to increased ionic concentration which checked water absorption that indirectly affected cell division of the stem. The introduction of salinity affected the stem diameter but irrespective of the concentration, the effect felt was not significantly different at $p \leq 0.05$.

3.5 Effect of NaCl salinity on leaf area of pumpkin

NaCl had no significant difference on the leaf area of the pumpkin plant. Treatments 0mM, 35mM, 70mM, 105mM and140mM were not significantly different although there was a general observation of salinity symptoms in salinity treatments.

3.6 Effect of NaCl salinity on root length of pumpkin.

NaCl salinity significantly affected root length. Control treatment was significantly different from NaCl treatments of 70mM,105mM, and 140mM.Treatment 0Mm and 35mM were not significantly different from each other(Table 1).

Table 1 Showing the effect of NaCl salinity on germination, shoot height, stem diameter, leaf area, shoot length, and root length of *C. pepo*.

Category	Germination (%)	Shoot height (cm)	Stem diameter (cm or mm)	Leaf area (unit?)	Root Length (unit?)
0Mm	76.667a	27.867a	1.300a	38.867a	26.500a
35mM	46.667b	25.917ab	0.924b	33.119a	20.333ab
70mM	40.000b	24.604ab	0.849b	30.668a	13.833b
105mM	10.000c	22.071b	0.661b	29.891a	11.800b
140mM	0.000c	22.054b	0.658b	28.699a	11.667b
Standard error	4.472	1.537	0.142	3.933	3.419

Means in the same column with the same letters do not differ significantly at $p \leq 0.05$ using LSD

3.7 Effect of NaCl salinity on shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, soil wet weight, soil dry weight, and soil moisture content.

The shoot fresh weight, dry weight, root fresh, dry weights, and soil moisture percentage were not significantly different. from control 0mM.

Soil wet weight was found not to be significantly different at$p \leq 0.05$. The control was not significantly different from soil wet weights for treatments 35mM, 70mM, 105mM, and 140mM respectively.

Soil dry weights were significantly different at $p \leq 0.05$. The salinity effect on treatments increased as salt concentration increased. Soil dry weight obtained from control was significantly different from soil dry weight obtained from treatments 105mM and 140mM but soil dry weight obtained from treatment 35mM and 70mM was not significantly different from soil dry weight obtained in treatment 105mM and 140mM (Table 2).

Table2 Showing effect of NaCl salinity on fresh shoot weight, dry weight, root fresh weight, dry weight soil wet weight, dry weight and moisture content

concentration	Fresh shoot weight	Dry weight shoot	Root fresh weight	Dry weight root	Soil wet weight	Dry weight soil	% Moisture Content
0mM	24.400a	3.753a	4.300a	0.367a	11.533a	10.100a	25.337a
35mM	23.987a	3.350a	1.880a	0.303a	10.900a	8.433ab	25.113a
70mM	19.210a	2.840a	1.607a	0.297a	10.767a	8.133ab	24.253a
105mM	13.050a	2.817a	1.337a	0.283a	10.300a	7.700b	22.867a
140mM	12.187a	2.433a	1.247a	0.233a	9.967a	7.433b	12.343a
Standard error	3.848	1.372	1.716	0.124	0.664	0.731	5.105

Means in the same column with the same letters do not differ significantly at $p \leq 0.05$ using LSD

3.8 Effect of NaCl salinity on Chlorophyll a, b, and total chlorophyll content.

Chlorophyll a, b, and total chlorophyll content of pumpkin under NaCl salinity was found to be significantly different from control (Table 3).

Generally, there were salinity symptoms that indicated low chlorophyll content in leaves. This included light-colored leaves with others yellowing as well as leaf tip burning.

Table 3 showing the mean performances of treatments for chlorophyll parameters of pumpkin

Concentrations	Chlorophyll a 647	Chlorophyll b 664	Total chlorophyll
0mM	23.020a	14.527a	37.547a
35mM	19.377b	8.887b	28.263b
70mM	17.187b	8.690b	25.870b
105mM	17.180b	8.497b	25.683b
140mM	17.103b	8.330b	25.433b
Standard error	1.542	1.150	2.417

Means in the same column with the same letters do not differ significantly at $p \leq 0.05$

Figure 1: showing the effects of 105mM salinity on C. pepo leaves.

Figure 2: Showing the effect of 140mM NaCl salinity on Pumpkin leaves

IV. DISCUSSION

NaCl salinity inversely inhibited the germination percentage of pumpkin seeds. The reduction in germination percentage was about 100% at the highest concentration of 140mM.Germination inhibition could be due to the osmotic potential difference between the seeds and the saline water used which significantly affected the *Pumpkin* embryo seeds. For any successful germination to occur enough water should be available to initiate processes such as oxidation, and cell division as well as to keep the cells alive.

Though saline water with 35Mm 70mM will affect germination of *Pumpkin*, the extent of the effect was not excessively felt while in 105mM and 140mM which were the highest concentrations, germination of *Pumpkin* was adversely affected to an extent that germination was negligible [22]. This may have been as a result of increased toxicity of Na+ ions in the soil [22]. These findings on seed germination under saline conditions agree with the previous work on salinity stress on the *Cucumis melo* of the Cucurbitaceae family in which germination was negatively affected by salinity stress [22].

This germination percentage inhibition confirms other previous work done on vegetables. Other workers [23] while working on salt-stressed *Oryza sativa L*. observed that salinity stress inhibited germination. In this study, shoot height of Pumpkin were affected by salinity based on NaCl concentrations, where increasing salinity may have increased soil osmotic potential, that could have lowered the ability of water absorption by the plants thereby affecting shoot height. Water is a necessity during cell division in all plants, without it cell division is compromised leading to reduced or no growth in the shoot heights. When water deficit develops it retards plant height. In this study it was notable that the higher the concentration the more the plant suffers from height retardation. This data confirms previous work on salinity, where work on different pumpkin varieties obtained from rootstocks for grafting showed similar responses [24].

This was likely due to the short period of about one month of exposure to salinity. This resulted in minimal salinity effects to an extent that salinity concentrations would not have built up enough negative effect to produce a significant difference at $p \leq 0.05$. Pumpkin might have devised a mechanism such as

a physiological mechanism through which salinity stress was avoided.

According to previous studies [25] on the effect of different levels of salinity on rice plants, plants showed two physiological traits that could possibly enable them to tolerate salinity. This includes the compensatory growth following adjustments to salinity and they have the ability to increase both leaf area ratio and net assimilation rate to achieve increased growth. This information on salinity not being significantly different on leaf area disagrees with previous work [23] on salt stress *Oryza sativa* L. on which salinity affected salt-sensitive rice germplasm as the concentrations increased.

Stem diameter was affected by salinity in this study. The effect of salinity on stem despite the NaCl concentration was not significantly different apart from the control treatment. There was a similar effect felt at 35mM, 70mM, 105mM, and 140mM treatments of NaCl. Salinity lowers cell division and might be ion toxicity in cells. Na^+ ions accumulation in cells affects cell division in the stem which directly retards stem increase. While the highest concentration had an adverse effect on the stem such that it had an average of 22.06mm, findings agree with other workers [26] on salt-stressed sorghum that showed salinity reduced stem diameter.

Since this study has confirmed that increasing salinity retarded cell elongation thereby resulting in a reduction in length, these findings are in agreement with [27] and [24] on pumpkin. In this study, Plant biomass was not significantly different upon salt treatments possibly due to the effect of salinity that affects most physiological parameters of the plant reducing cell multiplication as well as its effect on plants weight, a finding that agrees with previous works [27] working on NaCl salinity in which it was found that salinity affected most physiological processes as well as anatomical processes that later resulted to reduction in plant biomass [27]. This study has shown that soil moisture content was not significantly different in all five treatments, and that even though water was available it could not be taken up by the plants because the salt concentration.

V. CONCLUSION

The results of this study have clearly indicated that NaCl salinity affects Pumpkin based on the concentration. It is now clear that salinity affects growth parameters and may result in adverse effects on germination at high concentration between 105mM and 140mM.

Therefore successful germination of Pumpkin seeds depends on the irrigation water with high concentrations of salts not above 70mM for the growth of Pumpkin.

Generally, it should be noted that the effects of salinity are dependent on plant species, type of salt, salt concentration, environmental conditions, and most importantly, the duration of exposure to salinity.

VI. RECOMMENDATIONS

1. This study has demonstrated that water with salinity content beyond 70mM should not be used for the growth of Pumpkin plants.
2. The germination test has clearly shown that for any successful germination to occur, the salt content of the water used is very important to Pumpkin plants and having shown maximum inhibition at the highest concentrations of 105mM and 140mM.
3. There is a need to determine the relationship between salt concentration and osmotic potential on the physiology and growth of pumkin

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