

Evaluation of salt tolerance of some varieties of *Capsicum* species during germination using morphometric features

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Abstract

Evaluation of salt tolerance on morphometric features of some pepper species in their germination stage was carried out using a completely randomized design with four replications. Data were analysed using analysis of variance (ANOVA) and compared with duncan multiple range test (DMRT). Morphometric features studied include; rate of germination, germination percentage, root length, shoot length, root ratio, shoot ratio and dry weight of plantlet. Sodium chloride was used for salinity treatments. Salinity stress with slow decline in germination of plants was observed in bell pepper, bird pepper and sweet pepper, thus the salinity was 12 ds/m⁻¹ and germination percentage was high in the three plants. Germination rate under salt stress linearly decreased in all plants. The germination rate compared to germination percentage in plants assessment showed greater sensitivity to salt stress. The results showed that the germination stage of bell pepper and bird pepper were tolerant to salinity, sweet pepper was moderately resistant to salinity and cayenne pepper was highly sensitive to salt. The results of data analyses showed salinity effect on studied varieties and interaction of pepper varieties to salinity on the studied characters were significant at 1% level. Also some characters as rate and percentage of germination, root and shoot length, root to shoot ratio and dry weight of plantlet with increasing salinity in four pepper varieties decreased.

Keywords: Germination, Salinity stress, *Capsicum, annum, frutescens*, Sweet pepper, Bird pepper, Bell pepper, Cayenne pepper, Plumule length, Radicle length.

1. Introduction

Salinity is one of the most important environmental factors that limits crop yield in arid and semi-arid regions of the world. Saline soil in most parts of the world is about 23.8 million hectares of total land area. Nowadays, almost half of the more than 18 million hectares of cultivated land in some regions are irrigated where salinity is one of the major and challenging problems. The development of salinity-tolerant cultivars is an economical and effective approach to cope with salinity [1]. Salinity is one of the key environmental factors that adversely influence crop productivity in several regions of the world [2]. [3]. Soil salinity is a major abiotic stress that severely affects crop production worldwide [4]. A considerable amount of land in the world is affected by salinity and more than 45 million hectares (M ha) of irrigated land which account to 20% of total land have been damaged by salt and 1.5 M ha are taken out of production each year due to high salinity levels in the soil [5]. High salt concentrations cause various events that negatively impact agricultural production, such as delays in plant growth and development, inhibition of enzymatic activities and a reduction in the photosynthetic rate [6]. It has been reported that excess Na⁺ and Cl⁻ in the soil solution caused osmotic stress, and plants accumulate osmotically active compounds to lower the osmotic potential [7]. Salts also interfere with plant growth through two major processes: initially, the growth slows due to osmotic stress, as the water uptake by root is impaired; subsequently, the salts accumulate in toxic concentration in old leaves and cause its death [5]. When initially exposed to high salt content, plant growth rapidly reduces due to osmotic (non-

specific) effects. Over longer periods (days to weeks), individual salts may accumulate to toxic levels, thereby inducing specific-ion toxicities [8].

Pepper is an important crop not only because of its economic importance but also for the nutritional value of its fruits being a major source of natural colours and antioxidant compounds [9]. The intake of these compounds in food is an important health protecting factor, they have been recognized as being beneficial for prevention of widespread human diseases, including cancer and cardiovascular diseases when taken daily in adequate amounts [10]. Over 25 percent of the world's population consumes peppers everyday [11]. In Nigeria two cultivated species (*Capsicum annum* and *Capsicum frutescens* L.) comprising of six varieties represent the genus. The following varieties are widely grown in Nigeria and commonly consumed in south west, that is *Capsicum frutescens*: these are cayenne red pepper, they are known as bird pepper (local name Ata wewe), cayenne pepper (locally known as Ata Sombo) and bird eye chilli pepper (known locally as Ata bawa) and *Capsicum annum*: Bell pepper (tatase) and sweet pepper (Ata rodo). These peppers are used either fresh or dried in preparation of traditional diets but they are commonly used fresh. Although pepper has been reportedly used as medicine in the management of arthritis pair, diabetic, neuropathy, post mastectomy pan among others, there is paucity of information about the antioxidant capacity of the pepper varieties used as major condiments in the traditionally prepared diet in the south west of Nigeria. Hence this study is aimed at evaluating the proximate, mineral composition, antioxidant activity and total

phenolic content of the four varieties that are commonly used as condiments- bird pepper (Atawewe) cayenne pepper (Ata sombo), bell pepper (Tatase) and sweet pepper (Ata rodo). It is believed that this will provide the much needed data in formulating diets and fill the missing gap in the food composition table of an indigenous food plant.

2. Materials and methods

2.1 Evaluation of salt tolerance during germination figures

The study was carried out in the laboratory using a completely randomized design with four replications of petri dish with a diameter of 11 cm and 20 seeds. Sodium chloride was the salt used for the experiment. The petri-dish was placed in the oven at 65 °C for 24 hours before use and was further disinfected with sodium hypochlorite. Also, the surface where the experiment was to be done was sterilized with 2.5% sodium hypochlorite solution and rinsed with distilled water. The seeds were then transferred to sterile moist filter paper and were put in a petri dish for germination at 25 °C. In this experiment, number of seeds was reviewed on a daily basis and roots were counted as seeds germinate the root and shoot length was also measured. The dry weight (DW) was measured after the samples were dried at 70 °C for 48 h; these parameters were taken to calculate the relative growth rate (RGR) of the seedlings.

2.2 Statistical analysis

Data were statistically analyzed using analysis of variance (ANOVA). The data were compared using Duncan Multiple

Range Test. Analysis of variance was significant at $P < 0.05$. Correlation analyses were conducted to determine the relationships between the traits by software SPSS v.16.

$GP = 100 \cdot (NG/NT)$

GP= germination percentage

NG= number of germinated seeds

NT= total number of seeds

3. Results and Discussion

Results of analysis of variance revealed significant effects of salinity on seed germination, root growth, shoot dry weight and ratio of root and shoot of the seedling in all four pepper plants studied and their interactions were significant at 1% (Table 1). Germination percentage and germination ratio decreased with increasing salinity. The highest percentage of germination is sweet pepper with zero salinity and cayenne pepper was lowest with salinity of 12 ds/m^{-1} (Figure 1). Average seeds germination of studied plants at different salinity levels is shown in Figure 1. In this study, the salinity decreased all components of the plant germination in the following hierarchy; sweet pepper, cayenne pepper, bird pepper and bell pepper. Several studies have shown that the percentage of seed germination decreases with increasing salinity. [12] stated that with increasing salinity, plant height, shoot and root dry weight and root length was reduced. Generally with increasing salinity germination decreased in all four plants.

Table 1: Analysis of variance for germination characteristics of pepper plants

| S.O.V | df | Radicle length | Plumule length | Seedling dry weight | Radicle to plumule ratio | Percentage Germination | Rate of Germination |
|----------------------|----|----------------|----------------|---------------------|--------------------------|------------------------|---------------------|
| Salt (A) | 3 | 5.75** | 3.11** | 0.0002** | 0.05** | 301.80** | 170.99** |
| Capsicum species (B) | 3 | 2.84** | 1.45** | 0.001** | 1.57** | 246.28** | 322.88** |
| A*B | 9 | 0.176** | 0.045** | 0.00008** | 0.04** | 16.91** | 4.87** |
| (Error) | 48 | 0.022 | 0.017 | 0.000008** | 0.008 | 23.14 | 2.62 |
| CV(%) | | 6.55 | 5.91 | 5.54 | 8.15 | 5.27 | 9.40 |

* and **: significant at 5 and 1% level of probability respectively

In Table 1 above, analysis of variance showed a significant effect of different levels of osmotic stress caused by salinity on root and shoot of all four pepper plants. Analysis of variance showed that the effect of salinity on germination of plant is also significant. In general it was observed that with increasing salinity, germination rate was reduced. Also the plants'

reaction to salinity was different, as cayenne pepper and sweet pepper had more decreased rate of germination while the rate of germination in bell pepper and bird pepper were less affected by salinity, this implies that the two plants were more resistant and less sensitive to salinity.

Table 2: Mean comparison of germination characteristics of four pepper plants in control

| Treatment | Radicle length | Plumule length | Seedling dry weight | Radicle to plumule ratio | Percentage Germination | Rate of Germination |
|----------------|----------------|----------------|---------------------|--------------------------|------------------------|---------------------|
| Sweet pepper | 2.81a | 2.47a | 0.84c | 0.02a | 95.83a | 23.41a |
| Cayenne pepper | 2.36b | 2.31b | 1.33b | 0.02b | 91.66a | 17.21b |
| Bird pepper | 2.12c | 1.93c | 1.44a | 0.017c | 91.24a | 17.21b |
| Bell pepper | 1.81d | 1.77d | 0.85d | 0.014d | 86.24b | 13.87c |

Similar letters in each column show non-significant differences according to Duncan's Multiple Range Test.

Table 3: Mean comparison of germination characteristics of pepper plants in salinity stress

| Treatment | Radicle length | Plumule length | Seedling dry weight | Radicle to plumule ratio | Percentage Germination | Rate of Germination |
|----------------|----------------|----------------|---------------------|--------------------------|------------------------|---------------------|
| Sweet pepper | 3.01a | 2.62a | 1.18a | 0.019a | 95.83a | 21.17a |
| Cayenne pepper | 2.49b | 2.24b | 1.14ab | 0.017b | 93.74a | 17.96b |
| Bird pepper | 1.94c | 1.81c | 1.10ab | 0.014c | 89.16b | 16.38b |
| Bell pepper | 1.66d | 1.63d | 1.04b | 0.012d | 86.24b | 13.36c |

Similar letters in each column show non-significant differences according to Duncan's Multiple Range Test.

Table 4: Correlations between germination characteristics in four pepper plants

| Trait | Radicle length | Plumule length | Seedling dry weight | Radicle to plumule ratio | Percentage Germination | Rate of Germination |
|------------------------|----------------|----------------|---------------------|--------------------------|------------------------|---------------------|
| Root length | [| | | | | |
| Shoot length | 0.48** | [| | | | |
| Root to shoot ratio | 0.18** | 0.57** | [| | | |
| Seedling dry weight | 0.65** | 0.32** | -0.68** | [| | |
| Percentage germination | 0.27** | 0.64** | 0.38** | -0.22ns | [| |
| Germination rate | 0.47** | 0.75** | 0.22ns | 0.12** | 0.31** | [|

Ns, * and **: Non-significant and significant at 5% and 1% level of probability respectively.

In Table 4 above there was a relatively consistent and positive correlation between root length and shoot length $r=0.48^{**}$ as well as a positive correlation between root length and root shoot ratio $r=0.65^{**}$. Correlation analysis of the positive relationship between root and shoot length can confirm that, the root's water and useful minerals uptake can increase dry matter accumulation in both root and shoot and elongate them.

Correlation analysis of the positive and significant relationship between shoots and root length and germination rate was observed. A positive significant correlation was found between shoot length and seedling dry weight. Non positive significant correlation between the percentage of germination and seedling dry weight, root length was observed

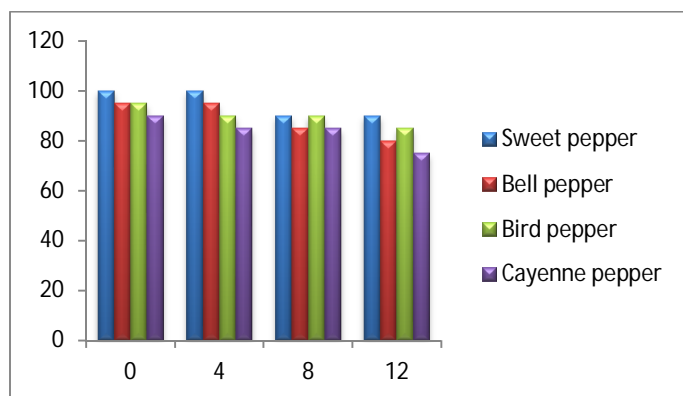


Fig 1: Effects of salinity on germination percentage of four pepper plants

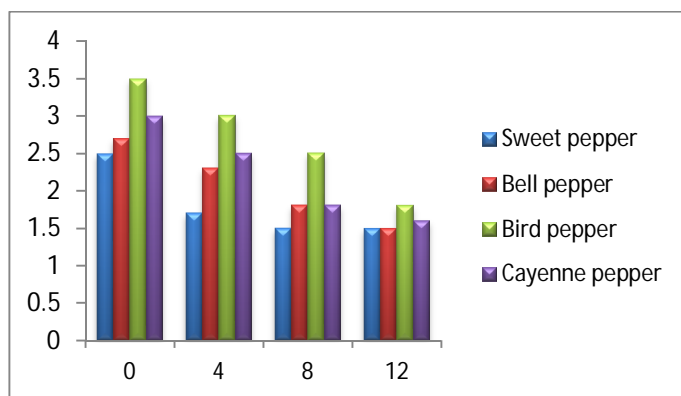


Fig 2: Effects of salinity on radicle length of plantlet in four pepper plants

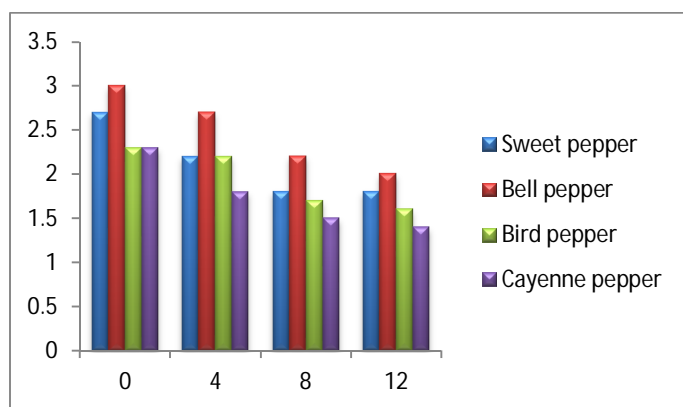


Fig 3: Effects of salinity on plumule length of plantlet in four pepper plants

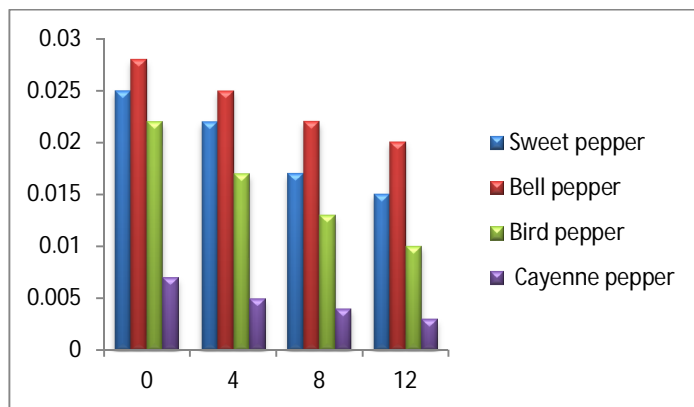


Fig 4: Effects of salinity on dry weight of plantlet in four pepper plants

Salinity is a growth reducing factor for most of the plant species. The major inhibitory effect of salinity on plant growth and development had been attributed to osmotic inhibition of water availability, toxic effects of salt ions responsible for salinization and nutritional imbalance caused by such ions, but the specific effects of soil salinity largely differ depending upon the predominant ions, their concentrations and the physiological stage of growth at which the plants were subjected to salinity^[13]. Salinity results in poor plant stand due to the decrease in the germination rate of seeds and seedling survival for most of the agricultural crops^[14]. Research on different plants showed that salinity reduced germination and this might be due to low osmotic potential and low ionic concentrations of the control^[15].

Increased salinity had harmful effects on root and shoot (Figure 2 and 3). Also in all pepper plants studied, increase in salinity levels brought about decrease in root length, shoot length and root/shoot ratio. In all experimented plants, root and shoot length in the salinity of 12 ds/m⁻¹ decreased compared to the control (Figure 2 and 3).^[8] reported that salinity decreases speed of germination and seedling growth. He also said that the salt stress effect on root and shoot of most crops is a way to evaluate salt tolerance. So far, there have been reports on crop germination and the impact of increased salinity on shoot length, root length and seedling dry weight which was significantly reduced compared to the control sample^[16, 17]. Studies have shown that plants that are tolerant to saline and drought conditions are forced to build some compounds such as proline and glycine for osmotic regulation and since making these products requires energy, thus, conditions such as growth and seedling dry weight are reduced^[18]. The results of the mean comparison showed that increasing salinity decreased all evaluated features.

There was a drop in the length of root and shoot with increase in salinity in bird pepper and bell pepper while in sweet pepper and cayenne pepper there was slow reduction at salinity 8 ds/m⁻¹ (Tables 2 and 3). One reason for the shoot decline during the plant osmotic stress was the slower degradation of the endosperm and thus reducing the transmission of substances or seed storage tissues of the fetus^[19]. Suggestions by^[20, 21] was that salinity can reduce the length of root or stem, and ultimately reduce the length of the plant while root shoot ratio increased (Table 2). The germination of plants in saline conditions and controls showed no significant difference (Tables 2 and 3). Mean comparison results showed the trend of seedlings dry weight under salt stress in studied plants. Salinity decreased dry weight of all evaluated pepper plants (Table 2).^[22] reported that high concentration of salt in sorghum reduced seed germination and seedling weight. They observed in ten plants that with increasing salinity levels, seedling dry weight decreased linearly. Similar results based on the dry weight of seedlings were observed in other plants^[23, 24].

4. Conclusion

The result of the study showed that increase in salinity levels decreased germination indices in sweet pepper, cayenne pepper, bell pepper and bird pepper. Also from the study, bell pepper and bird pepper were observed to be resistant, sweet pepper was moderately tolerant to salt and cayenne pepper was more sensitive. Increase in salinity, reduced the percentage of germination of the *Capsicum species*. From the study, it can be concluded that there was a significant difference in all

characteristics between plants while increase in salinity significantly reduced the rate of germination, seedling growth (root and shoot) and seedling dry weight. Therefore, germination, seedlings growth and their development is directly dependent on the plant's tolerance to salinity.

5. References

1. Arzani A. Improving salinity tolerance in crop plants: A biotechnological view: In-vitro Cell. Dev. Biol. Plant. 2008; 44:373–383.
2. Saleem A, Ashraf M, Akram NA. Salt (NaCl) induced modulation in some key physio-biochemical attributes in okra (*Abelmoschus esculentus* L.): J. Agron Crop Sci. 2011; 197: 202-213.
3. Shahbaz M, Ashraf M. Improving salinity tolerance in cereals. Crit. Rev. Plant Sci. 2013; 32:237-24.
4. Kumar V, Shriram V, Jawali N, Shitole MG. Differential response of indica rice genotypes to NaCl stress in relation to physiological and biochemical parameters: Arch. Agron. Soil Sci. 2007; 53:581–592.
5. Munns R, Tester M. Mechanisms of salinity tolerance: Annu. Rev. Plant Biol. 2008; 59:651–681.
6. Lee MH, Cho EJ, Wi SG, Bae H. Divergences in morphological changes and antioxidant responses in salt-tolerant and salt-sensitive rice seedlings after salt stress: Plant Physiol. Biochem. 2013; 70:325-335.
7. Ahmad P, Sharma S. Salt stress and phyto-biochemical responses of plants: Plant Soil Environ. 2008; 54:89-99.
8. Munns R. Comparative physiology of salt and water stress: Plant cell Environ. 2002; 25:239-250.
9. Howard LR, Smith RT, Wagner AB, Villalon B, Burns EE. Provitamin A and ascorbic acid content of fresh pepper cultivars (*Capsicum annuum*) and processed jalapenos: Journal of Food Science 1994; 59:362-365.
10. Brainley PM. Isolycopene beneficial to human health? Phytochemistry. 2000; 54:233-236.
11. Namiki M. Antioxidant/antimutagens in food: Food Science and Nutrition. 1990; 29:273-300.
12. Gain P, Mannon MA, Pal PS, Hossien MM, Parvi S. Effect of salinity on some yield attribution of rice: Pak J Biol Sci. 2004; 7:760-762.
13. Greenway H, Munns A. Mechanism of salt tolerance in non-halophytes: Ann. Rev. Plant Physiol. 1980; 31:149-190.
14. Karim MA, Utsunomiya U, Shigenaga S. Effect of sodium chloride on germination and growth of hexaploid triticale at early seedling stage: Japan J. Crop Sci. 1992; 61:279-284.
15. Munns R, Schachtman DP, Condon AG. The significance of a two-phase growth response to salinity in wheat and barley: Aust. J. Plant Physiol. 1995; 22:561-569.
16. Kaya MD, Okcu G, Atak M, Cikili Y, Kolsarici O. Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.): Europ. J. Agron. 2006; 24:291-295.
17. Alebrahim MT, Janmohammadi M, Sharifzade F, Tokasi S. Evaluation of salinity and drought stress effects on germination and early growth of maize (*Zea mays* L.): Electronic Journal of Crop Production. 2009; 1(2):35-43.
18. Serraj R, Sinclair TR. Osmolyte accumulation: can it really help increase crop yield under drought conditions: Plant Cell Environ. 2002; 25:333-341.

19. Soltani A, Gholipour M, Zeinali E. Seed reserve utilization and seedling growth of wheat as affected by drought and salinity: *Environ. Exp. Bot.* 2006; 55:195-200.
20. Akbari G, Modarres Sanavy SAM, Yousefzadeh S. Effect of auxin and salt stress (NaCl) on seed germination of wheat cultivars (*Triticum aestivum* L.): *Pakistan Journal of Biological Sciences.* 2007; 10(15):2557-2561.
21. Hosseini H, Rezvani MP. Effect of water and salinity stress in seed germination on Psylliom (*Plantago ovata*): *Iranian journal of Field Crop Res.* 2006; 4(1):15-22.
22. Almodarres A, Hadi MR, Dosti B. Effect of salt stress on germination percentage and seedling growth in sweet sorghum cultivars. *International Journal of Biological Sciences.* 2007; 7(8):1492-1495.
23. Safarnejad A, Hamidi H. Effect Fennel morphological Fennel (*Foeniculum vulgare* Mill.) Under salt stress: *Iranian Journal of Genetics and improvement of pastures and forests.* 2008; 16:125-140.
24. Mahdavi B, Modares Sanavi SAM, Balochi HR. The effect of sodium chloride on the germination and seedling growth figures Grass pea (*Lathyrus sativus* L.): *Iranian Journal of Biology.* 2007; 20:363-374.