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RESEARCH ARTICLE

DETERMINATION OF THE INFLUENCE OF TEMPERATURE AND SALINITY ON THE GERMINATION OF *ABELMOSCHUS ESCULENTUS* SEEDS

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ABSTRACT

The environmental stress such as salinity and temperature are among the major obstacles for field crops around the world. The objective of this study was to test the effects of different salinity levels and temperature on the seed germination and early seedling growth of *Abelmoschus esculentus* (Okra). Each set of triplicate petridishes were filled with moist sterile cotton wool and ten Okra seeds were placed and allowed to germinate in different water baths set at different temperatures in dark, warm condition, for seven days. Similar set up was made with different concentrations of NaCl. Parameters measured from these two set ups were, the percentage of germination, germination index, general normal seedling percentage, mean time to germination and the length of germ tube. The decline in the characteristic features of germination in response to higher temperature and higher NaCl concentration are a consequence of Okra establishing its life system in less saline and moderate warm environment. The highest germination characteristics were observed at the moderately warm temperatures and lower salinity conditions of germination of Okra seeds and the lowest at the higher saline and the outside the range of moderate temperatures. Optimum germination responses were expressed by Okra at 35°C temperature and 50mM salinity.

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INTRODUCTION

Salinity is the accumulation of salt in soil and water. Higher level of salt concentration can adversely affect the plant growth, soil structure, water quality and infrastructure. Salinization of soil is one of the major factors limiting the production of crop particularly in tropical regions of the world (Ahmed, 2009). Salt stress is known to perturb a multitude of physiological processes (Noreen and Ashraf, 2008). It exerts its undesirable effects through osmotic inhibition and ionic toxicity (Munns *et al.*, 2006). Increased salinity level beyond the tolerance level caused a significant reduction in germination percentage, rate of germination, individual lengths of the root and shoots and fresh weights of the root (Jamil *et al.*, 2006).

Temperature plays a major role in seed germination and the distribution of plant species in a territory (Guan *et al.* 2009). Cellular metabolic activity mechanism and the seedling growth rate are directly affected by the soil temperature. Some seeds show optimum germination rate in cool temperature but others seeds show optimum germination in between warm and cool temperatures. Different seeds require different temperature to break the dormancy. Cellular metabolism of the germinating seeds mainly depends on the amount of water uptake by seed.

Abelmoschus esculentus (Ladies finger / Okra) is one of the nature's gift vegetable for general human consumption. It is valued for its edible green seed pods. The plant is cultivated widely in tropical, subtropical and warm temperate regions around the globe. Seeds of Okra are very easily susceptible to the adverse environmental conditions. The contents inside the seeds can undergo different biochemical reactions and this will lead to the damage of the seed, during the period of storage (Kausar *et al.*, 2009).

Seed germination is a critical stage in the life of plants and salinity tolerance during germination is crucial for the establishment of plants that grow in saline soils. Seed germination depends on both internal and external factors. The most important external factors are temperature water, oxygen and sometimes light or darkness. Plants require different components at different rate for the successful germination of seeds (Rizzadri *et al.*, 2009). As Okra is cultivated in a wide range of climatic and geographic locations of the Island of Sri Lanka as a vegetable, which is surrounded by the sea water, the seed germination and yield differs from place to place. Since Sri Lanka is located in the tropical region, the climatic conditions here facilitates the cultivation of diverse plant varieties like okra which are of greater economical importance. There have been no studies conducted to compare the differences in the germination potential / index and seedling

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growth rates of Okra growing in different saline and temperature zones of Sri Lanka. Therefore, this study was aimed to test the effects of different salinity levels and temperature on the seed germination and early growth of *Abelmoschus esculentus*.

MATERIALS AND METHODS

Source of seeds

The seeds of *Abelmoschus esculentus* (Lady's finger) were collected from Thirunelvely agricultural farm school of Northern province Sri Lanka.

Effect of temperature

A set of three sterile petridishes were taken and they were filled with sterile cotton wool moist with sterile water. In each petridish, ten seeds of *Abelmoschus esculentus* were placed separately. After moisten by 20ml of sterile water, the petridish was kept at a water bath set at 20°C, under dark warm condition that is favourable for natural seed germination, for 7 days. The same experiment was repeated to 25, 30, 35 and 40°C temperatures. To avoid the seeds getting dried, the cotton wools were moisten by 5ml of sterile distilled water every day. The seeds were carefully observed daily and the percentage of germination, germination index, general normal seedling percentage and mean time to germination and the length of germ tube were measured. At the end of the germination period, germination percentage, germination index and mean time to germination were calculated.

Effect of Salinity

A set of three sterile petridishes were taken and they were filled with sterile cotton wool moist with 50mM. In each petridish, ten seeds of *Abelmoschus esculentus* were placed separately. Then the petridishes were moistening by 40ml of 50mM sodium chloride solutions. All petridishes were placed in water baths set at 35°C, and left in dark for 7 days for natural seed germination. The same experiment was repeated with 100, 150, 200, 250 and 300mM concentration of sodium chloride solutions. 25, 30, 35 and 40°C the growth measurements of germ tube changes were recorded. The seeds were carefully observed daily and the number of seeds germinated, the length of germ tubes and the rate of germination were measured.

Germination Mean Time.

$$GMT = \frac{(nT)}{n}$$

n- Number of seeds newly germinated at a time T
T- Hour from the beginning of the germination test.
n- Final germination.

Germination Index

$$\text{Germination Index} = \frac{[\text{Number of germinated seed}] + \dots + [\text{Number of germinated seed}]}{[\text{Days of first count}] + \dots + [\text{Days of first count}]}$$

Statistical analysis

Statistical analyses were performed using R 2.15.3 statistical software at $\alpha = 0.05$ confidence level. The data sets were checked for the parametric assumptions of normality (Shapiro-Wilk and Kolmogorov-Smirnov tests) and homogeneity of variances (Bartlett's test). Box plots were used for identifying outliers from the data set that were removed before the statistical analysis. When necessary to meet the assumptions of normality and homogeneity of variance, the data were transformed, either by log transformation or square root transformation. The data were analyzed using ANOVA. Tukey's multiple comparison test was used to determine significant differences at $p < 0.05$ (R Development Core Team, 2011).

RESULTS AND DISCUSSION

Abelmoschus esculentus has the capacity of growing in a wide range of temperatures. The germination percentage of Okra, after five days of germination was very low, at 20°C (Figure 1). Germination percentage increased with the temperatures up to 35°C, and beyond this temperature, it started to decline. Highest germination percentage was observed at 35°C and this is considered as optimum. The germination percentage at 20°C, was significantly lower than that of 35°C ($p=0.05$) after 5 days of germination. The length of the germ tube also showed similar trend as the germination percentage (Figure 2). It was significantly lower at 20°C, than that of 35°C.

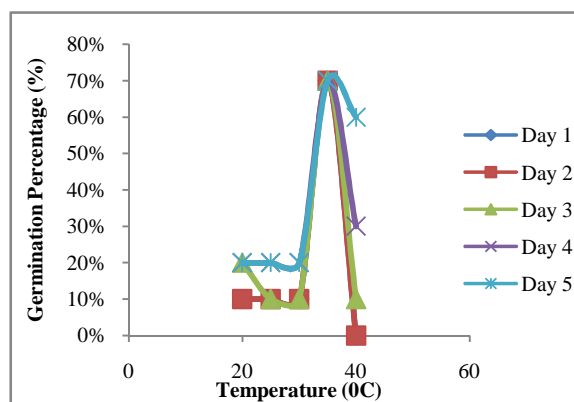


Figure 1 Percentage of germination of *Abelmoschus esculentus* at different temperatures.

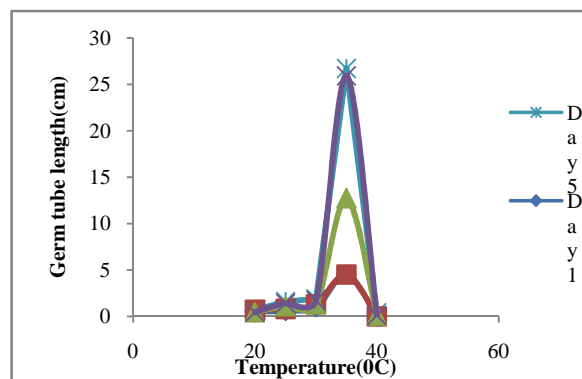


Figure 2 Length of germ tube of *Abelmoschus esculentus* at different temperatures.

Mean germination time (MGT) of Okra after seven days of germination period, was stable at low temperatures. At 35°C, mean germination time of Okra was significantly higher than the other temperatures. When the temperature was increased to 40°C, again, the MGT declined (Figure 3). Germination index of Okra was also showed the similar trend, after 7 days of germination (Figure 4). Seeds of Okra showed differences in responses of seed germination under different temperature treatment (Figure 5).

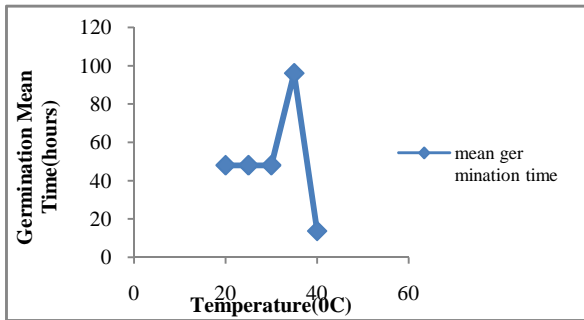


Figure 3 Mean germination time of *Abelmoschus esculentus* after 7 days at different temperatures.

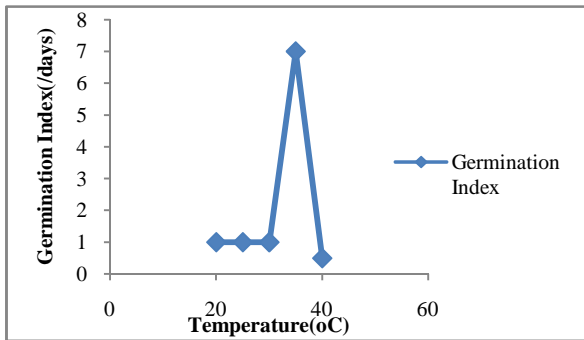


Figure 4 Germination index of *Abelmoschus esculentus* at different temperatures.



Figure 5 Seeds of *Abelmoschus esculentus* showing difference responses at different temperatures after 5 days. A - 20°C, B- 25°C, C- 30°C, D- 35°C and E - 40°C

When low temperature is accompanied by relatively high air temperature that promotes high transpirational demand, low soil temperature tolerance is frequently correlated with drought resistance thus it promotes seed germination (Janowiak *et al.*, 2002). Low temperature decreases root water flux (Lee *et al.*, 2012) by reducing root water absorption area due to root growth inhibition (Wan *et al.* 1999,2001), increasing resistance to apoplastic root water flow due to increased water viscosity, and decreasing cell-to-cell water transport through the effects on aquaporins (Lee *et al.*, 2012). Aquaporins play a key role in plant water homeostasis in response to various environmental stresses (Wan *et al.* 2001). When the temperature is high, rate of evapo-transpiration will become higher and this might be a reason for the inhibition of seed germination, at higher temperatures.

At lower salinity (50 mM of NaCl) the percentage of germination of Okra was significantly higher than the other salinity concentrations (100, 150, 200 and 250 mM, at $p=0.05\%$) after 5 days of germination under dark condition. With the increasing salinity, the percentage of germination decreased (Figure 6). At very high salinity concentrations, germination of *Abelmoschus esculentus* was very much inhibited. Beyond 200mM NaCl, increase in the concentration of NaCl did not induce germination. The variation in the length of the germ tube also showed similar trend as the germination percentage (Figure 7). Okra had very limited tolerance to salinity and Okra plants cannot grow in a wide range of salinity variations.

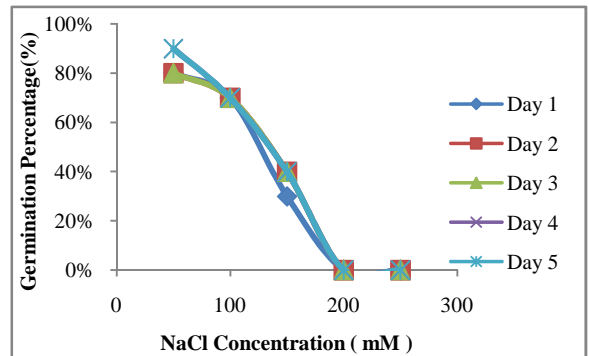


Figure 6 Percentage of germination of *Abelmoschus esculentus* at different NaCl concentrations.

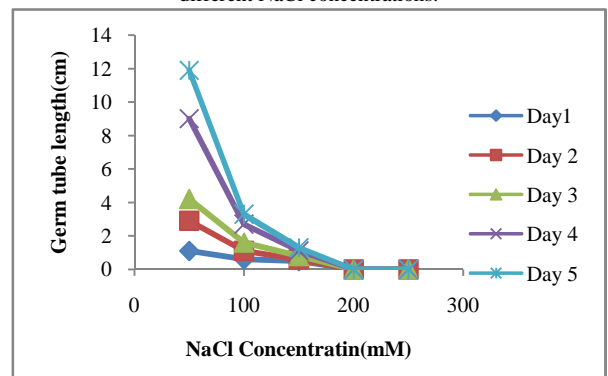


Figure 7 Length of germ tube of *Abelmoschus esculentus* at different NaCl concentrations.

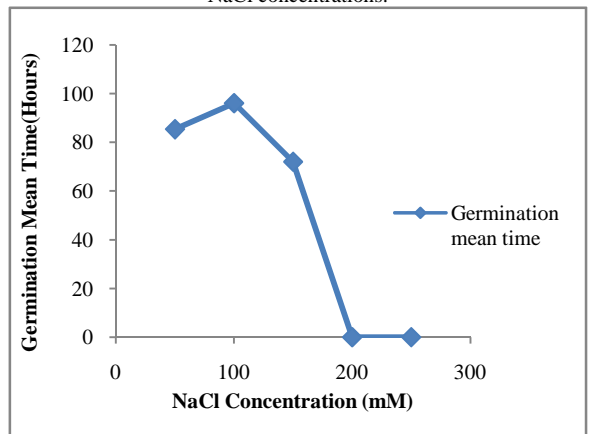


Figure 8 Mean germination time of *Abelmoschus esculentus* at different NaCl concentrations.

Mean germination time (MGT) of Okra after seven days of germination period, was stable at lower concentrations of NaCl (50, 100 and 150mM). Beyond 150mM of NaCl, mean germination time of Okra was significantly lower than the other

NaCl concentrations. When the NaCl concentration was further increased to 200mM of NaCl, the MGT declined and no germination was reported beyond this concentration of NaCl (Figure 8). Germination index of Okra was also showed the similar trend, after 7 days (Figure 9). Seeds of Okra showed differences, in responses of seed germination under different salinity treatments (Figure 10).

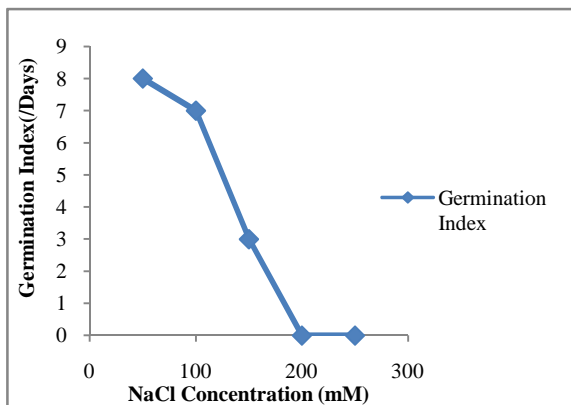


Figure 9 Germination Index of *Abelmoschus esculentus* at different NaCl concentrations.

The effect of the temperature and salinity treatments on the parameters such as percentage of germination, germination index, general normal seedling percentage and mean time to germination, under conditions provided for germination of *Abelmoschus esculentus* (Okra) were significant ($P < 0.05$). The presence of saline conditions in the substrate, when temperatures are high and there is high evapo-transpiration, can induce temporarily unfavourable environmental conditions for the germination and the normal development of the seedlings. However, this tendency is not the normal pattern for all plants (Ungar, 1977, Khan and Ungar 1997 and Khan and Gulzar, 2003). It is known that the effect of salts on seed germination and plant growth leads to physiological drought. Salt reduces the water potential of the substrate solution, which inhibits the supply of water by plants. They in the salty media receiving large amounts of salt in root cells, and thus reduces the water potential, so it increases the absorption of water under physiological drought conditions (Bajji *et al.*, 2002).



Figure 10: Seeds of *Abelmoschus esculentus* showing difference responses at different NaCl concentrations. A – 50 mM, B- 100 mM, C- 150 mM, D- 200 mM and E - 250 mM

Growth of plant roots generally declines with decreasing root temperature in the 5–30°C range, and reduced whole-plant growth and photosynthetic rate may be a result of reduced water and nutrient uptake (Bowen, 1991). In a plant life cycle, seeds have the highest resistance to extreme environmental stresses, whereas seedlings are most susceptible to the environmental changes (Gutterman, 1993). Therefore, successful establishment of a plant population is dependent on the adaptive aspects of seed germination and of early seedling growth in the particular environment (Duan *et al.*, 2007).

Decreasing in the percentage of germination might be due to the enzymatic reactions that take place under the treatment conditions of seeds (Geressu and Gezahagne, 2008). The interaction of temperature and salinity plays an important role in germinative behaviour and in the growth of seedlings in natural habitats (Ungar 1995).

CONCLUSION

The decline in the characteristic features of germination in response to higher temperature and higher NaCl concentration are a consequence of *Abelmoschus esculentus* establishing its life system in less saline and moderate warm environment. The highest germination characteristics were observed at the moderately warm temperatures and lower salinity conditions of germination of *Abelmoschus esculentus* and the lowest at the higher saline and the outside the range of moderate temperatures. Optimum germination responses were expressed by *Abelmoschus esculentus* at 35°C temperature and 50mM salinity.

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