The Impact Of Salt Stress On The Growth Physiological Attributes Of Selected Okra (*Abelmoschus Esculentus* L.) Cultivars In The Sandy Regosols

Jeyapraba. J, *Mahendran. S, and Suganiya. S

Department of Agricultural Biology,

Faculty of Agriculture, Eastern University, Sri Lanka.

* thevamahen@yahoo.com

ABSTRACT

Salinization of underground water resource is a major problem that contributes in fixing the agricultural productivity. The salt stressed plants have shown stunted growth pattern with minimal lifespan of leaves and net productivity. By looking at these features, an experiment was conducted to assess the salt stress outcomes of selected okra cultivars on shoot and root length, shoot and root weight, number of leaves and flowers and chlorophylls 'a' and 'b' contents. The okra cultivars 'Haritha', 'EUOK 2' and 'MI 5' were used for this study. Sodium chloride (100 mM) was used to create the salinity while, distilled water was used as the control. Salt stress significantly reduced the shoot and root length of all the tested okra cultivars. The lowest reduction (39.1 cm shoot length and 21.7 cm root length) was found in the 'EUOK 2' cultivar. 'MI 5' showed the highest reduction (31.6cm shoot length and 15.6 cm root length). Salt stress also significantly reduced the shoot and root fresh weights of all the okra cultivars. The highest reduction (11.9 g shoot weight and 4.0g root weight) was found in the 'MI 5' and 'EUOK 2' showed the lowest (16.3 g shoot weight and 6.6 g root weight) reduction. Salt stress also significantly reduced the amounts of chlorophylls 'a' and 'b' contents of okra cultivars. The highest reduction (0.73 mg g^{-1} chlorophyll 'a' and 0.03mg g^{-1} chlorophyll 'b') was obtained in the 'MI 5' cultivar. 'EUOK 2'showed the lowest (1.17 mg g^{-1} chlorophyll 'a' and 0.11mg g^{-1} chlorophyll 'b') reduction. From these observations it was arrived that 'EUOK 2' cultivar of okra was able to maintain the physiological attributes relatively better than the other tested cultivars under salinity situation. 'EUOK 2' therefore could be selected as the most salt tolerant okra cultivar which could thrive and perform successfully in the salt affected areas of the sandy regosols.

Key words: Chlorophyll, growth physiology, okra, salinity stress, sandy regosols.

1.INTRODUCTION

Most of cultivated plants are sensitive to salt stress in which NaCl-salinity involves modification of the morphological, physiological and biochemical processes [1]. These changes usually result in reduction of shoot [2] and restricted rooting [3]. Salt stress affects plant physiology at both whole plant and cellular levels through osmotic and ionic stress [4]. High concentration of salts in the root zone decreases soil water potential and the availability of water [5]. This deficiency in available water under saline condition causes dehydration at cellular level and ultimately osmotic stress occurs. The most important process that is affected by salinity is photosynthesis [6]. Reduced photosynthesis under salinity is not only attributed to stomatal closure leading to a reduction of intercellular CO_2 assimilation but, also to non-stomatal factors like reduction in green pigments and leaf area. There is increasing evidence that salts affect photosynthetic enzymes, chlorophylls and ionic contents [7]. Okra (*Abelmoschus esculentus L.)* is a popular vegetable among both the consumers and farmers because it is rich in vitamins and minerals [8]. Although the area under okra has progressively increased during last few years, there is a decreasing trend in its yield per hectare [9]. This decline in optimum yield is due to the drastic effects of salts which are deposited in soil by the use of brackish underground water. Salinization of soils is one of the serious problems for irrigated agriculture and the situation is most severe in tropical regions [10]. High ratios of salts in root zone affect different processes like root density, root turgor pressure and its growth and ultimately create hindrance in water absorption [11]. Okra plant at earlier growth stages is more sensitive to salinity [12], as it affects water relations and nutrient uptake of plants. While later on, the ionic stress in turn reduces leaf expansion. During long term exposure to salinity, plants experience ionic stress, which can lead to premature senescence of adult leaves and thus reduction in photosynthetic rate is a common observation [13].

The present study was conducted to determine whether the shoot and root length, shoot and root fresh weight, number of leaves and flowers and chlorophylls 'a' and 'b' contents could be used to differentiate between cultivars differing in apparent salt tolerance, to deal with the physiological responses exhibited as a result of salinity stress and to suggest the most salt tolerant okra cultivar that could be grown under salinity situation.

2. MATERIALS AND METHODS

This experiment was conducted at the Agronomy farm of the Eastern University which is located at an elevation of 75 m above mean sea level in the Eastern Province of Sri Lanka. Studies were conducted during the '*Yala*' season of the year 2014. The climate is warm (28-32°C) with an average annual rain fall of 1250 mm.

Okra (*Abelmoschus esculentus* L.) cvs. 'Haritha', 'EUOK 2' and 'MI 5' were used for this study. The seeds were surface sterilized with sodium hypochlorite 0.5% (v/v) for 20 min. washed repeatedly with distilled water and were allowed to germinate in polyethylene bags filled with fine sand as growth medium. A number of three seeds per bag were sown initially but, after 15 days of germination, the plants were thinned out to one.

Plants were grown in Hoagland solution under non saline conditions for 30 days after germination. Afterwards, the salt treatment was imposed. Sodium chloride was dissolved in distilled water to obtain the concentration of 0 (control) and 100mM and this solution was applied to create the salinity while half strength Hoagland solution was applied as nutrient medium. The treated plants were grown under saline condition. Irrigation along with half strength Hoagland solution was applied to the selected treatments according to the need of the plants by regularly observing the wetness extent of sand.

The experiment was carried out with six treatments and five replications and the treatments were as follows:

T_1 = 'Haritha' cultivar of okra irrigated with distilled water (Control)

T_2 = 'Haritha' cultivar of okra irrigated with saline water (100 mM NaCl)

T_3 = 'EUOK 2' cultivar of okra irrigated with distilled water (Control)

T_4 = 'EUOK 2' cultivar of okra irrigated with saline water (100 mM NaCl)

T_5 = 'MI 5' cultivar of okra irrigated with distilled water (Control)

T_6 = 'MI 5' cultivar of okra irrigated with saline water (100 mM NaCl).

The experiment was laid out in the Completely Randomized Design with 2 x 3 factor Factorial arrangements.

Growth Attributes

A number of four plants were randomly selected from each replicate of the treatments and were uprooted from the polyethylene bags. Measurements such as root length, shoot length, shoot weight (fresh) and root weight (fresh) were made. Root length was measured from the base of the shoot to the tip of the tap root and shoot length was measured from the base of the shoot to the tip of the plant. Fresh weights of the shoot and root were weighed in grams by a digital balance. Number of leaves and flowers were counted manually.

Chlorophyll Determination

Four leaves representing four plants were randomly collected from each replicate of the treatments for the determination of chlorophyll. These leaves were sampled from the control and treated plants on the 15th day of salinity application. A quantity of 1g of fresh sub-sample leaves from the above sample was placed in a clean mortar. A quantity of 40ml of 80% (v/v) acetone was added and the tissues were ground to a fine pulp. The Extract was filtered by a Whatman No.1 filter paper.

The pulp was ground repeatedly with fresh 30ml aliquot of 80% acetone. The second extract was filtered into the flask containing the first extract using a filter paper. The final volume of the filtrate was adjusted to 100ml by adding sufficient amount of 80% acetone. The optical density of the chlorophyll extract was recorded by a spectrophotometer (Camspec, M330BT) using 10 mm cuvettes. The wavelengths used were 645nm and 663nm. A quantity of 80% acetone was used as the solvent blank.

The amount of chlorophyll present in the extract was calculated on the basis of milligrams of chlorophyll per gram of leaf tissue by using the following equations:

$$\text{mg chlorophyll a/g tissue} = [12.7(D_{663}) - 2.69(D_{645})] \text{ X } \frac{V \qquad V}{1000 \text{ x} W 1000 \text{ x} W}$$

$$\text{mg chlorophyll b/g tissue} = [22.9(D_{645}) - 4.68(D_{663})] \text{ X } \frac{V \qquad V}{1000 \text{ x} W 1000 \text{ x} W}$$

Where,

D = Optical density reading of the chlorophyll extract at the specific wavelength

V = Final volume of the 80% acetone – chlorophyll extract

W = Fresh weight of the tissue (g)

Statistical Analysis

The data were statistically analyzed using Analysis of Variance to determine the significance if any at the treatment level. The differences between treatment means were compared using DMRT.

3. RESULTS AND DISCUSSION

It was found that there were significant differences between treatments in the shoot and root length, shoot and root weight, number of leaves and flowers, chlorophylls 'a' and 'b' contents of leaves of the selected okra cultivars.

Shoot And Root Length

In the treatments where the salinity stress was imposed on plants, the shoot and root length on the 15th day of salinity application was significantly lower than the control values (Table 1).

It was also found that there were significant differences in the shoot and root length of salinity stressed okra cultivars. The highest shoot and root length were obtained in the 'EUOK 2' cultivar followed by 'Haritha' and 'MI 5'. The lowest value was found in the 'MI 5' cultivar. From these observations it could be stated that salinity stress decreased the length of shoot and root of selected okra cultivars.

Table 1: Effects of salinity stress on the shoot and root length of selected okra cultivars on the 15th day of salinity application

Cultivars	Shoot length (cm)		Root length (cm)	
	Control	Salt stress	Control	Salt stress
'Haritha'	(T_1) 46.8a	(T_2) 35.3d	(T_1) 26.8a	(T_2) 18.1 c
'EUOK 2'	(T_3) 42.3 b	(T_4) 39.1c	(T_3) 24.2b	(T_4) 21.7 b
'MI 5'	(T_5) 38.2 c	(T_6) 31.6 e	(T_5) 23.1b	(T_6) 15.6 d

*Values in the same column followed by the same letter do not differ significantly (P<0.05).

*Values are the means of 20 plants in 5 replications.

As pointed out by [14], salinity affects plant growth most dramatically during developmental stages. According to [15], salt stress significantly reduced the root and shoot length in all the tested okra cultivars.

The highest shoot and root length observed in the 'EUOK 2' shows its ability to withstand saline condition far better than the other two cultivars. Tolerance of this cultivar to salinity stress would have been an intrinsic characteristic feature. The lowest value obtained in the 'MI 5' shows its susceptibility to salinity stress.

Shoot And Root Fresh Weight

In the treatments where the salinity stress was imposed on plants, the shoot and root fresh weights on the 15th day of salinity application were significantly lower than the control values. It was also found that there were significant differences in the shoot and root fresh weights of salinity stressed okra cultivars (Table 2).

The highest shoot and root weights were obtained in the 'EUOK 2' followed by 'Haritha' and 'MI 5' cultivars. The lowest value was found in the 'MI 5'. From these results it could be stated that salinity stress reduced the shoot and root fresh weights of okra cultivars. As stated by [16], salt stress significantly reduced the shoot and root fresh weights, transpiration rate and net CO_2 assimilation of okra. [17] pointed out that the fresh and dry weights of the shoot system are affected, either negatively or positively by changes in salinity concentration, type of salt present or type of plant species.

Table 2 Effects of salinity stress on the shoot and root fresh weight of selected okra cultivars on the 15th day of salinity application

Cultivars	Shoot weight (fresh) (g)		Root weight (fresh) (g)	
	Control	Salt stress	Control	Salt stress
'Haritha'	(T_1) 21.7a	(T_2) 14.4c	(T_1) 5.8c	(T_2) 5.1c
'EUOK 2'	(T_3) 19.6 a	(T_4) 16.3b	(T_3) 7.9a	(T_4) 6.6b
'MI 5'	(T_5) 23.1a	(T_6) 11.9d	(T_5) 6.7b	(T_6) 4.0d

*Values in the same column followed by the same letter do not differ significantly (P<0.05).

*Values are the means of 20 plants in 5 replications.

The highest weight observed in the 'EUOK 2' cultivar shows its ability to withstand salinity stress relatively better than the other two cultivars. 'MI 5' shows its weak affinity towards salinity stress. The performance of 'Haritha' was in between the above two cultivars.

Number Of Leaves And Flowers

In the treatments where the salinity stress was imposed on plants, the number of leaves and flowers on the 15th day of salinity application were significantly lower than the control values (Table 3).

Table 3: Effects of salinity stress on the number of leaves and flowers of selected okra cultivars on the 15th day of salinity application

Cultivars	Number of leaves		Number of flowers	
	Control	Salt stress	Control	Saltstress
'Haritha'	(T_1) 14.3 a	(T_2) 8.2b	(T_1) 9.7b	(T_2) 7.1c
'EUOK 2'	(T_3) 16.1 a	(T_4) 14.5a	(T_3) 11.3a	(T_4) 9.8b
'MI 5'	(T_5) 9.7 b	(T_6) 5.6c	(T_5) 6.2c	(T_6) 4.7d

*Values in the same column followed by the same letter do not differ significantly (P<0.05).

*Values are the means of 20 plants in 5 replications.

It was noted that there were significant differences in the average number of leaves and flowers of the salinity stressed okra cultivars. According to [18], the harmful influence of salinity on leaf number increases with the increase in sodium chloride concentration. As stated by [19], the treatment of sodium chloride reduced the number of leaves compared with control plants. The decrease of leaf numbers may be due to the accumulation of sodium chloride in the cell walls and cytoplasm of the older leaves. At the same time, their vacuole sap cannot accumulate more salt and thereby decreases the concentration of salt inside the cells, which ultimately leads to their quick death and cut down.

The highest number of leaves and flowers were found in the 'EUOK 2'. 'MI 5' showed the lowest number and 'Haritha' was in between these two cultivars. From these observations it could be arrived that 'EUOK 2' was able to retain comparatively more number of leaves and flowers with them than the other two cultivars. This ability would have been a salinity tolerant feature of this cultivar. 'MI 5' was unable to retain more number of leaves and flowers, thereby exhibited salt susceptibility.

Chlorophylls 'a' and 'b'

In the treatments where the salinity stress was imposed on plants, the chlorophylls 'a' and 'b' contents on the 15th day of salinity application were significantly lower than the control values (Table 4).

It has been observed from the results that there were significant differences in the chlorophylls 'a' and 'b' contents of okra plants which were exposed to salinity stress. The highest amounts of chlorophylls 'a' and 'b' were obtained in the 'EUOK 2' and the lowest amount was received by the 'MI 5'. 'Haritha' showed the value in between the above two cultivars.

From these observations it could be stated that salinity stress reduced the chlorophylls 'a' and 'b' contents of okra cultivars. The results regarding a decrease in chlorophylls 'a' and 'b' contents agree with what [20] reported, that the exposure of barley (*Hordeum vulgare* L.) to zero, 120 and 240 mM of sodium chloride led to the decrease in chlorophylls 'a' and 'b' contents. Moreover, [21] in their study on *Paspalum vaginatum* (L) reported that chlorophylls 'a' and 'b' decreased with the increase of salt concentrations.

Table 4 Effects of salinity stress on the chlorophylls 'a' and 'b' contents of selected okra cultivars on the 15th day of salinity application

Cultivars	Chlorophyll 'a' (mg g^{-1})		Chlorophyll 'b' (mg g^{-1})	
	Control	Salt stress	Control	Salt stress
'Haritha'	(T_1) 1.21b	(T_2) 0 .94c	(T_1) 0.09c	(T_2) 0.06d
'EUOK 2'	(T_3) 1.34a	(T_4) 1.17b	(T_3)0.16a	(T_4) 0.11b
'MI 5'	(T_5) 1.07c	(T_6) 0.73d	(T_5) 0.08c	(T_6) 0.03e

*Values in the same column followed by the same letter do not differ significantly (P<0.05).

*Values are the means of 20 plants in 5 replications.

The green pigment chlorophyll is vital for photosy nthesis and absorbs the light. The excessive amount of salt/ions (Na and Cl) within the leaf tissue may disturb the cellular metabolisms and cause degeneration of cell organelles and it leads to the destruction of green pigments.

The highest amounts of chlorophylls 'a' and 'b' found in the 'EUOK 2' under salinity condition exhibits better salt tolerance of this cultivar compared to the others. 'MI 5' was unable to maintain sufficient amounts of chlorophylls 'a' and 'b' contents which perhaps be due to its susceptibility to salinity stress.

4. CONCLUSIONS

This study determined the extent to what the growth physiological attributes such as shoot and root length, shoots and root fresh weight, number of leaves and flowers and chlorophylls 'a' and 'b' contents were affected by salinity stress. The responses were manifested and the degree of salinity tolerance of the selected okra cultivars was evaluated. 'EUOK 2' okra cultivar showed the highest growth attributes compared to the others. The growth performance of 'MI 5' was inferior to 'EUOK 2' and 'Haritha' cultivars. It was therefore concluded that 'EUOK 2' was the most salt tolerant okra cultivar among the tested ones which could be recommended for cultivation in the salt prone areas of the sandy regosols.

REFERENCE

[1] Tester, M. and Davenport, R. "Na^+ tolerance and Na^+ transport in higher plants". Annals of Botany, 91, pp 503-527, 2003.

[2] Misra, M., Das, N. and. Misra, A.N. "Sodium chloride salt stress induced changes in protein content and protease activity in callus culture of pearl millet (*Pennisetum glaucum* L.R. br.)". Acta Physiological Plant, 17, pp. 371-374, 1995.

[3] Lopez, M. V. and Satti, S. M. E. "Calcium and potassium-enhanced growth and yield of tomato under sodium-chloride stress". Plant Science, 114, pp. 19-27, 1996.

[4] Murphy, K. S. T. and Durako, M. J. "Physiological effects of short term salinity changes on *Ruppia maritime*". Aquatic Botany, 75, pp. 293-309, 2003.

[5] Lloyd, J., Kriedemann P. E. and Aspinall, D. "Comparative sensitivity of Prior Lisbon lemon and Valencia orange trees to foliar sodium and chloride concentrations". Plant and Cell Environment, 12, pp. 529-540, 1989.

[6] Hayat, S., Hasan, S. A., Yusuf, M., Hayat, Q. and Ahmad, A. "Effect of 28-homobrassinolide on photosynthesis, fluorescence and antioxidant system in the presence or absence of salinity and temperature in *Vigna radiate*". Environmental and Experimental Botany, 69, pp. 105-112, 2010.

[7] Misra, A. N., Sahu, S. M., Misra, M. Singh, P., Meera, I. Das, N., Kar, M. and Shau, P. "Sodium chloride induced changes in leaf growth and pigment and protein contents in two rice cultivars". Biologia Plantarum, 39, pp. 257-262, 1997.

[8] Oyelade, O. J., Ade-Omowaye, B. I. O., Adeomi, V. F. "Influence of variety on protein, fat contents and some physical characteristics of okra seeds". Journal of Food Engineering, Vol. 57, No. 2, pp. 111-114, 2003.

[9] Anonymous. "Fruits, Vegetable and Condiments Statistics". 2006-2007. MINFAL, Islamabad, Pakistan, 2008.

[10] Khan, A., Rao, S. A. and Mc Neilly, T. "Assessment of salinity tolerance based upon seedling root growth response functions in maize (*Zea mays* L.)". Euphytica. Vol. 131, No. 1, pp. 81-89, 2003.

[11] Maggio, A. S. D. Pascale, G. Angelino, C. Ruggiero and Barbieri G. "Physiological response of tomato to saline irrigation in long-term salinized soils". European Journal of Agronomy, 21, pp. 149-159, 2004.

[12] Cerda, A., Pardines, J., Botella, M. A. and Martinez, V. "Effect of potassium on growth, water relations and organic solute contents for two maize grown under saline conditions". Journal of Plant Nutrition, 18, pp. 839-851, 1982.

[13] Cramer, G. R. and Nowak, R. S. "Supplemental Manganese improves the relative growth, net assimilation and photosynthetic rate of salt stressed barley". Physiologia Plantarum, 84, pp. 600-605, 1992.

[14] Shannon, M. C. "Breeding, Selection and Genetics of Salt Tolerance" In: Staples, R. C., Toenniessen, G. A. (Eds.) "Salinity Tolerance in Plants-Strategies for Crop Improvement". John Wiley and Sons, New York, USA. pp. 231-254, 1984.

[15] Abbas,T., Pervez, M A., Ayyub, C. M. and Ahmad, R. "Assessment of the different Morphological, Antioxidant, Biochemical and Ionic Responses of Salt-Tolerant and Salt - Sensitive okra (*Abelmoschus esculentus* L.) under saline Regime". Pakistan Journal of Life and Social Science, Vol. 11, No. 2, pp 147-153, 2013.

[16] Saleem, A., Ashraf, M. and Akram, N. A. "Salt (NaCl)- Induced modulation in some key Physio-Biochemical Attributes in okra (*Abelmoschus esculentus* L.*)*" Journal of Agronomy and Crop Science, Vol. 197, No. 3, pp. 202-213, 2011.

[17] Bayuelo, J. S., Debouk, D. G., Lynch, J. P. (2002). "Salinity tolerance in phaseolus species during early vegetative growth". Crop Science, 42, pp. 2184-2192, 2002.

[18] Qados, A. "Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba*(L.)". Journal of the Saudi Society of Agricultural Sciences, 10, pp. 7-15, 2011.

[19] Raul, L., Andres, O., Armado,L., Bernardo, M., Enrique, T. "Response to salinity of three grain leg umes for potential cultivation in arid areas". Soil Science and Plant Nutrition, Vol. 49, No. 3, pp. 329-336, 2003.

[20] Tort, N. and Turkyilmaz, B. "A physiological investigation on the mechanisms of salinity tolerance in some barley culture forms". Journal of Forage Sciences, 27, pp. 1-16, 2004.

[21] Lee, G., Carrow, R. and Duncan, R. R. "Photosynthetic responses to salinity stress of halophytic seashore *paspalum* ecotypes". Plant Science, 166, pp. 1417-1425, 2004.