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Leaf water content and biochemical parameters as affected by salinity and their impacts on yield of selected okra (*Abelmoschus esculentus* L.) cultivars

Jeyapraba J, Mahendran S, Sujirtha N

[1]Department of Agricultural Biology, Faculty of Agriculture, Eastern University, Sri Lanka.

Abstract
Salinization of underground water resource is a major problem that contributes in fixing the agricultural productivity. Most plants are salt sensitive with either a relatively low salt tolerant or severely inhibited growth at low salinity levels so differ in the growth response to salinity. Okra plant is more sensitive to salinity as it affects water relations and nutrient uptake of plants. Almost all parts of okra plant are consumed, like fresh okra pods are used as vegetable, roots and stems are used for making fibre and ropes. By considering this feature, an experiment was conducted at the Agronomy farm of the Eastern University, Sri Lanka in the '*Yala*' 2014 to investigate the salinity stress responses of selected okra cultivars on certain Physiological and Biochemical attributes. The okra cultivars 'MI 5', 'Haritha' and 'EUOK 2' were used for this study. The experiment was laid out in the Completely Randomized Design with 2 × 3 factor factorial arrangement having six treatments and four replications. The okra cultivars were grown in polyethylene bags filled with sandy regosols as the growth medium. These plants were subjected to salt stress after 30 days of germination with 100 mM NaCl concentration. The control plants were irrigated with distilled water. Salt stress significantly reduced the Relative Water Content (RWC) of leaves of the selected okra cultivars. The highest RWC (73.5%) was obtained in the 'EUOK 2' while the lowest one (58.7%) was found in the 'MI 5'. Salt stress also significantly reduced the Total Soluble Solids (TSS) of okra pods. The highest TSS (5.8% brix) were obtained in the 'EUOK 2' and the lowest ones (3.1% brix) were found in the 'MI 5'. Salt stress significantly reduced the Ascorbic Acid contents of okra pods. The highest Ascorbic Acid content (10 mg 100g-1) was obtained in the 'MI 5' and the lowest value (3.3 mg 100g-1) was found in the 'EUOK 2'. Salt stress significantly reduced the fresh pod yield of okra cultivars. The highest pod yield (7.4 t ha-1) was obtained in the 'EUOK 2' and the lowest one (4.3 t ha-1) was found in the 'MI 5'. From the above results it was inferred that okra cultivar 'EUOK 2' had the ability to resist salinity stress better than the other two cultivars. Hence, 'EUOK 2' was identified as the most salt tolerant okra cultivar among the tested ones.

Keywords: Ascorbic acid, Okra, Relative Water content, Total Soluble Solids, Yields

1. Introduction
Abiotic stresses like heat, cold, drought and salinity affect the plant growth and productivity but, salt stress exerts more drastic effects in terms of low productivity [1]. High salt contents reduce the growth and production by affecting physiological processes including modification of ion balance, water status, mineral nutrition, Stomatal behaviour and photosynthetic efficiency. Most plants are salt sensitive with either a relatively low salt tolerance or severely inhibited growth at low salinity levels so differ in the growth response to salinity [2]. Salt stress affects plant physiology at both whole plant and cellular levels through osmotic and ionic stress. High concentration of salts in the root zone decreases soil water potential and the availability of water. 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 [3]. Reduced photosynthesis under salinity is not only attributed to stomatal closure leading to a reduction of intercellular CO2 assimilation but also to non – stomatal factors like reduction in green pigments and leaf area.
Okra (Abelmoschus esculentus L.) is recognized as an annual herbaceous plant grown in tropical and sub – tropical areas and serves as a source of carbohydrates, fats, vitamins and various minerals [4]. In spite of having good nutritional value, it's per hectare yield is very low. 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 [5]. High ratios of salts in root zone affect different processes like root density, root turgor pressure and its growth and ultimately create hinderance in water absorption [6]. Okra plant at earlier growth stages is more sensitive to salinity 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. Salinity changes photosynthetic parameters [7] including osmotic and water potential, transpiration rate, leaf temperature and relative leaf water content [8].
The present study was conducted with the objectives of investigating the effects of salt stress on the relative leaf water contents, total soluble solids and ascorbic acid contents of selected okra cultivars, finding out the extent of effects of these parameters, determining the effects of salt stress on the yield and finally identifying the most salt tolerant okra cultivar that could be grown in salt prone areas of the sandy regosols in Batticaloa district.

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' 2014. The climate is warm (28 – 32 ºC) with an average annual rainfall 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 minutes, washed repeatedly with distilled water and were allowed to germinate in polyethylene bags filled with sandy regosols as the growth medium. A number of three seeds per bag were sown initially but, after 15 days of germination, the plants were thinned to one.

Plants were grown in Hoagland nutrient solution [9] under non – saline condition for 30 days after germination. Thereafter, the salt treatment was initiated. Sodium chloride was dissolved in distilled water to obtain the concentration of 0 (control) and 100 mM and this solution was applied to create the salinity while 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 four 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 × 3 factor factorial arrangement.

2.1. Determination of Relative Water Content

A number of five leaves representing five plants were randomly collected from each replicate of the treatments. The leaves were cut into discs (10 discs of 1 cm diameter each) and were weighed immediately after plucking to determine the fresh weight (FW). Leaf discs were immersed in distilled water in petri dishes for four hours. The discs were gently wiped with filter papers (Whatmann No.1) to remove the excess water on their surface. These discs were weighed to determine the turgid weight (TW). The samples were oven dried at 80ºC for 24 hours to find out the dry weight (DW). The RWC was calculated using the following formula:

$$RWC\ (\%) = \frac{FW - DW}{TW - DW} \times 100$$

2.2. Determination of Total Soluble Solids (TSS)

A number of five tender pods representing five plants were randomly collected from each replicate of the treatments and were analyzed for TSS. For measuring the TSS of pod sap, an indirect method was used namely the measurement of the refractive index of the cellular sap after extracting it from the pod. The sap was extracted rapidly by grinding tender pods using mortar and pestle. The measurement of the refractive index of the total soluble solids was made by using a drop of cellular sap placed on a hand refractometer (ATAGO, S – 28E) and the brix value was recorded.

2.3. Determination of Ascorbic Acid content (Vitamin C)

A number of five tender pods representing five plants were randomly collected from each replicate of the treatments and the ascorbic acid contents of the sap were determined by oxidizing vitamin C in acid medium using 2, 6 - dichlorophenol indophenol dye to dehydro ascorbic acid. For standardizing purpose, an amount of 0.01 g ascorbic acid was dissolved in 100 ml metaphosphoric acid containing 3% acetic acid and was titrated against 2, 6 - dichlorophenol indophenol dye until a faint pink colour persisted for a few seconds. The collected pods were blended by a blender (Sumeet type). The sap was extracted and an amount of 10 g was taken and volumerized to 25 ml by using metaphosphoric acid containing 3% phosphoric acid and 3% acetic acid. A quantity of 10 ml from the volumerized sap was taken into the conical flask and was titrated against the dye until a faint pink colour persisted for a few seconds.

Calculations

A volume of 100 ml standard solution contains 0.01 g ascorbic acid. Hence, 10 ml of solution contains 0.001 g ascorbic acid. The volume of dye necessary to react with the standard ascorbic acid solution was V1 ml. Therefore, 1 ml dye solution is equivalent to (0.001/V1) g ascorbic acid.

Hence;

$$\text{The amount of ascorbic acid in 100 g of okra pod} = \frac{0.001 \times 100 \times D \times 100}{V_1 \times V_2 \times W}$$

Where,

V_2 = Volume of dye for titrating okra sap

D = Dilution factor

W = Weight of sample

2.4. Marketable yield

Five plants were randomly selected from each replicate of the treatments. The marketable tender pods from these plants were plucked on alternate days and their weights were determined by an electronic balance. The total marketable yield was found out by summing up the collected weights of pods.

3. Results and discussion

It was found that there were significant differences between treatments in the relative water content of okra leaves, total soluble solids and ascorbic acid content of okra pods.

3.1. Relative water content

Significant ($P < 0.05$) differences were noticed between treatments in the Relative Water Content (RWC) of leaves of the selected okra cultivars (Table 1).

In the treatments where the salinity stress was imposed on plants, the RWC on the 15th day of salinity application was significantly lower than the control values. It was also found that there were significant interactions between salt stress and cultivars.

Table 1: Effects of salt stress on the Relative Water Content of selected okra cultivars

Salt stress	Cultivars	Treatments	Relative Water Content (%)
0 mM	'Haritha'	T_1	86.3 ± 2.4 a
	'EUOK 2'	T_3	84.1 ± 1.3 a
	'MI 5'	T_5	86.7 ± 0.2 a
100 mM	'Haritha'	T_2	67.4 ± 0.3 b
	'EUOK 2'	T_4	73.5 ± 2.2 a
	'MI 5'	T_6	58.7 ± 1.0 c
Salinity level (S) < 0.0001			
Cultivar (V) 0.0013			
Interaction (S*V) <0.0001			

* Values with the same letters within the salt treatment do not differ significantly (P < 0.05).
* Values are the means of 20 plants in four replications.

Effects of cultivars on RWC were significantly affected by salt stress at 5% probability level. At 0 mM salt stress, there were no significant differences in the RWC among the cultivars but, at 100 mM salt stress, significant differences were observed in the RWC among the cultivars. Hence, salinity has significantly reduced the RWC of all the tested cultivars. The highest RWC was obtained in the 'EUOK 2' and the lowest one was found in the "MI 5'. Changes in water relations of plants that are stressed by salinity could be seen in certain studies which confirmed that many plants undergo reduced plant water status when they are exposed to salt stress [10]. Appraisal of water relations in plants grown under stress conditions including saline stress is necessary to ascertain that up to what extent cellular water content is maintained because almost all metabolic activities within the cell are dependent on the availability of sufficient amount of water therein [11]. As stated by [12], salt stress significantly reduced the relative water content of *Solanum melongena* L.

Based on this observation, it could be stated that 'EUOK 2' okra cultivar was able to maintain relatively high RWC than the other two cultivars under saline condition. This is a favourable feature with regard to salt tolerance of this cultivar. Cultivars which were believed to be more salt resistant usually maintain higher leaf RWC under salinity stress. The lowest RWC found in the 'MI 5' cultivar exhibits it's susceptibly to salt stress. Salinity alters plant water relations due to decreased availability of water from soil solution as a result of lowered osmotic potential [1].

3.2. Total soluble solids

It was found that there were significant ($P < 0.05$) differences between treatments in the Total Soluble Solids (TSS) of pods of the selected okra cultivars (Table 2). It was also observed that there were significant interactions between salt stress and cultivars.

Effects of cultivars on the TSS were significantly affected by salt stress at 5% probability level. At 0 mM salt stress, there were no significant differences in the TSS among the cultivars but, at 100 mM salt stress, significant differences were observed in the TSS among the cultivars. Salinity therefore has significantly reduced the TSS of all the tested cultivars. The highest TSS was obtained in the 'EUOK 2' whereas the lowest one was found in the 'MI 5'. Based on this observation, 'EUOK 2' okra cultivar was able to maintain relatively high amount of TSS than the other two cultivars under salinity situation. Salt resistant cultivars usually maintain high TSS under salinity stress. The lowest TSS observed in the 'MI 5' cultivar exhibits its susceptibility to salt stress. Mohamed [13] stated that fruit quality was affected by salinity as the soluble solid content was significantly reduced due to NaCl treatment. Fruit soluble solids were positively correlated with the K % and negatively correlated with the Na %.

Table 2: Effects of salt stress on the Total Soluble Solids of pods of selected okra cultivars

Salt stress	Cultivars	Treatments	Total Soluble Solids (% brix)
0 mM	'Haritha'	T_1	6.8 ± 0.03 a
	'EUOK 2'	T_3	6.4 ± 0.05 a
	'MI 5'	T_5	7.1 ± 0.01 a
100 mM	'Haritha'	T_2	4.3 ± 0.04 a
	'EUOK 2'	T_4	5.8 ± 0.03 b
	'MI 5'	T_6	3.1 ± 0.04 c
Salinity level (S) < 0.0001			
Cultivar (V) < 0.0001			
Interaction (S*V) < 0.0001			

* Values with the same letters within the salt treatment do not differ significantly (P < 0.05).
* Values are the means of 20 plants in four replications.

3.3. Ascorbic Acid content

It was found that there were significant (P < 0.05) differences between treatments in the ascorbic acid contents of pods of the selected okra cultivars (Table 3).

Table 3: Effects of salt stress on the Ascorbic Acid contents of pods of selected okra cultivars

Salt stress	Cultivars	Treatments	Ascorbic Acid contents (mg 100 g^{-1})
0 mM	'Haritha'	T_1	9.3 ± 0.67 a
	'EUOK 2'	T_3	9.1 ± 0.63 a
	'MI 5'	T_5	10.0 ± 0.12 a
100 mM	'Haritha'	T_2	6.7 ± 0.31 b
	'EUOK 2'	T_4	3.3 ± 0.27 c
	'MI 5'	T_6	10.0 ± 0.53 a
Salinity level (S) < 0.0001			
Cultivar (V) < 0.0001			
Interaction (S*V) < 0.0001			

* Values with the same letters within the salt treatment do not differ significantly (P < 0.05).
* Values are the means of 20 plants in four replications

In the treatments where the salinity stress was imposed on plants, the ascorbic acid contents of the pods were significantly lower than the control values. It was also found that there were significant interactions between salt stress and cultivars. Effects of cultivars on the ascorbic acid contents were significantly affected by salt stress at 5% probability level. At 0 mM salt stress, there were no significant differences in the ascorbic acid contents of the pods among the cultivars but, at 100 mM salt stress, significant differences were observed in the ascorbic acid contents among the cultivars. Salinity therefore has significantly reduced the ascorbic acid contents of pods of all the tested cultivars. The highest ascorbic acid content was obtained in the 'MI 5' and the lowest one was found in the 'EUOK 2'. From this observation it could be stated that 'MI 5' cultivar of okra was able to maintain high level of ascorbic acid content compared to the other two cultivars under saline condition. Retention of high level of ascorbic acid content of 'MI 5' okra cultivar under salinity condition would have been a characteristic feature of this cultivar. The lowest ascorbic acid content exhibited in the "EUOK 2' indicates its susceptibility to salt stress. Mohamed [13] stated that ascorbic acid is a very important component of fruit quality. They further stated that ascorbic acid was substantially affected by salinity as the content was changed due to saline treatment. According to [14] ascorbic acid is involved in counteracting adverse effects of salt stress in tomato.

3.4. Yield

It was found that there were significant ($P < 0.05$) differences between treatments in the fresh pod yield of okra cultivars (Table 4).

Table 4: Effects of salt stress on the fresh pod yields of selected okra cultivars

Salt stress	Cultivars	Treatments	Marketable yield (t ha^{-1})
0 mM	'Haritha'	T_1	9.8 ± 0.41 ab
	'EUOK 2'	T_3	10.0 ± 0.60 a
	'MI 5'	T_5	8.6 ± 0.25 b
100 mM	'Haritha'	T_2	5.7 ± 0.13 b
	'EUOK 2'	T_4	7.4 ± 0.23 a
	'MI 5'	T_6	4.3 ± 0.12 c
Salinity level (S) < 0.0001			
Cultivar (V) < 0.0001			
Interaction (S*V) < 0.0162			

* Values with the same letters within the salt treatment do not differ significantly ($P < 0.05$).
* Values are the means of 20 plants in four replications

In the treatments where the salinity stress was imposed on plants, the fresh pod yields were significantly lower than the control values. It was also observed that there were significant interactions between salt stress and cultivars on the yield of okra pods.

Effects of cultivars on the yield were significantly affected by salt stress at 5% probability level. At 0 mM salt stress, there were no significant differences in the yield among the cultivars but at 100 mM salt stress, significant differences were observed in the yield among the cultivars. Hence, salinity has significantly reduced the fresh pod yields of all the tested cultivars. The highest fresh pod weight (7.4 tha^{-1}) was obtained in the 'EUOK 2' and the lowest one (4.3 tha^{-1}) was found in the 'MI 5'. As stated by [14] reduction in crop yield in saline and saline – sodic soils is associated with osmotic and specific ion effects and the degree and extent of the adverse effect is further exacerbated when saline water is used for irrigation. Based on this observation it could be stated that 'EUOK 2' was able to produce substantially high yield under saline condition. Thus, salinity has caused less impact on the yield of 'EUOK 2' and therefore it could be considered as a salt tolerant crop compared to the others.

4. Conclusions

Salt stress reduced the water status of the selected okra cultivars by reducing the Relative Water Content. Okra cultivar 'EUOK 2' was able to maintain high RWC under salinity condition. The Total Soluble Solids (TSS) and Ascorbic Acid contents of the okra pods were reduced by salt stress. 'EUOK 2' showed the highest TSS than the other two cultivars under saline condition. The highest Ascorbic Acid content was obtained in the 'MI 5' okra cultivar. 'EUOK 2' produced the highest fresh pod yield followed by 'Haritha' and 'MI 5'. By considering the tested parameters, it was concluded that all these parameters were affected by salinity and okra cultivar 'EUOK 2' was identified as the most salt tolerant okra cultivar among the tested cultivars which was able to maintain certain of these parameters to a substantially high level under salt stress condition and thus could be suitable for salt prone environment of the sandy regosols.

5. References

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