International Journal of Plant & Soil Science
9(5): 1-5, 2016; Article no.IJPSS.22877
ISSN: 2320-7035

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Growth Physiology and Membrane Permeability of Okra (*Abelmoschus esculentus* L.) Seedlings as Affected by Salinity

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Authors' contributions

This work was carried out in collaboration by all three authors. The authors of this manuscript worked together to design, conduct, analyze and interpret the findings of this experiment. All the authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2016/22877
Editor(s):
(1) Sławomir Borek, Faculty of Biology, Department of Plant Physiology, Adam Mickiewicz University, Poland.
Reviewers:
(1) Petigrosso, Lucas Ricardo, Universidad Nacional de Mar del Plata, Argentina.
(2) Edward Missanjo, Malawi College of Forestry and Wildlife, Malawi.
Complete Peer review History: http://sciencedomain.org/review-history/12622

Original Research Article

Received 2nd November 2015
Accepted 24th November 2015
Published 10th December 2015

ABSTRACT

Aims: Among the abiotic stresses, salinity is one of the most important environmental constraints which provokes grave threats for agriculture and environment. It is a growing threat to agricultural crops and its impacts have already started becoming visible as expansion in the affected areas and deteriorating soil fertility and crop productivity. By considering this feature, a study was conducted to determine the salinity responses of selected okra cultivars on certain physiological and biochemical attributes.
Study Design: The experiment was laid out in a Completely Randomized Design with six treatments and four replications.
Place and Duration of Study: A study was conducted at the crop farm of the Eastern University, Sri Lanka during the yala season of the year 2014.
Methodology: The seeds of okra cultivar "Haritha" were surface sterilized with sodium hypochlorite 0.5% (v/v) and were allowed to germinate in petri dishes having filter papers saturated with distilled water as control and NaCl solutions of 25, 50, 75, 100 and 125 mM. The germinated seeds in distilled water and in saline solutions were transferred to plastic pots filled with soil/compost mixture

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(2:1V/V). The pots were irrigated with distilled water or saline solution for two weeks. The response of okra plants to salinity stress with respect to physiological growth attributes, water content and membrane permeability were investigated.

Results: Salinity stress did not show significant ($P > 0.05$) effect on shoot dry weight. However, root dry weight decreased with an increase of NaCl concentration. The root water content and shoot/root ratio increased significantly ($P < 0.05$) with an increase of salt concentration (25, 50, 75, 100 and 125 mM). It was observed that there was an increase in membrane permeability with associated electrolyte leakage when okra seedlings were exposed to high levels of salt particularly 100 and 125 mM NaCl.

Conclusion: The property of salinity tolerance depends on different physiological interactions. The growth physiological attributes presented by the plant in response to salinity may not be enough to determine its effect.

Keywords: Dry weight; electrolyte leakage; okra; salinity; water content.

1. INTRODUCTION

Abiotic stresses like heat, cold, drought and salinity affect the plant growth and productivity but, the salt stress exerts more drastic effects [1]. Despite the essentiality of chloride as a micro-nutrient for all higher plants and of sodium as mineral nutrient for many halophytes, salt accumulation may reduce local biodiversity, limit growth and reproduction of plants and may lead to toxicity in non-salt-tolerant plants. Most of the cultivated plants are sensitive to salt stress with either as relatively low salt tolerant or severely inhibited growth at low salinity levels [2]. This change results in reduction of shoot and restricted rooting. Germination and seedling establishment constitute the most critical periods in the life cycle of plants. The conductivity test, based on solute leakage has been proposed as a good indicator of salt tolerance in plants [3]. Blokhina [4] pointed out that the cell membrane often suffered injury associated with the increases in permeability.

Okra plants have tap roots 2 inch thick and 4.5 feet deep. The stem is semi-woody and sometimes pigmented with green or reddish tinged colour. It is erect, variable in branching with many short branches. The woody stem bears leaves that are lobed and are generally hairy. Leaves are cordate, simple, usually palmately 3-7 lobed and veined. The flowers are borne vertically only on the orthotropic axis every two or three days. They are large, around 2 inches in diameter with five white to yellow petals with a red or purple spot at the base of each petal. The fruit is an elongated, conical capsule comprising for the most part and five cavities containing ovules. The fruit actually a long pod is generally ribbed developing in the leaf axil.

Okra (*Abelmoschus esculentus* L.) is recognized as an annual herbaceous plant and it is one of the most important warm season fruit vegetables grown in tropical and sub-tropical parts of the world and valued for its edible green pods that are popular vegetable in Sri Lanka. Okra requires a long, warm and humid growing period. It can successfully be grown in hot humid areas. It is sensitive to frost and extremely low temperatures. For normal growth and development, a temperature between 24 and 28ºC is preferred. At higher temperatures, the plants grow faster. Temperatures beyond 40-42ºC, flowers may desiccate and drop causing yield losses. Okra is grown on sandy to clay soils but, due to its well developed tap root system, relatively light, well – drained rich soils are ideal. As such, loose, friable, well manured loamy soils are desirable. A pH of 6-6.8 is ideally suited.

Okra serves as a source of carbohydrates, fats, vitamins and minerals [5]. In spite of having good nutritional value, its per hectare yield is very low. This decline in optimum yield may be due to the drastic effects of salts. High ratios of salts in root zone affect root density, root turgor pressure and its growth [6]. Okra plant at earlier growth stages is more sensitive to salinity, as it affects water relations of plants.

The present studies were conducted to evaluate the growth physiological attributes of okra seedlings to saline conditions. By measuring the seedling growth and membrane permeability, the degree of tolerance of okra plants to different salinity levels was investigated.

2. MATERIALS AND METHODS

Okra seeds cv. 'Haritha' were collected from the Agronomy farm of the Eastern University of Sri

Lanka. The seeds were surface sterilized with sodium hypochlorite 0.5% (V/V) for 20 min; washed repeatedly with distilled water and then sown in petri dishes having filter papers saturated with distilled water (control) or NaCl solutions (25, 50, 75, 100 and 125mM NaCl) at room temperature.

The seeds after eight days of germination were shifted to plastic pots filled with soil/compost mixture (2:1 V/V). The pots were irrigated with distilled water or saline solution for two weeks. There were six treatments and each one was replicated four times in a factorial arrangement laid out in a Completely Randomized Design.

2.1 Growth Measurements

A number of five plants were randomly collected from each replicate of the treatments, uprooted and washed with distilled water. A number of twenty seedlings per treatment were partitioned into hypocotyls, cotyledons and roots for the measurement of Fresh Weight (FW). The Dry Weight (DW) was recorded after drying the samples in an oven at 80℃ for 24hrs. The (hypocotyls + cotyledons) dry weight/root dry weight ratio was determined. In addition, cotyledons and hypocotyls (aerial part) water content and root water content were estimated based on (FW- DW)/DW ratio.

2.2 Membrane Permeability

Electrolyte leakage was used to assess the membrane permeability as described by [7]. The cotyledons were cleaned and washed with distilled water and were cut into discs of uniform size. These discs were placed in test tubes containing 10 ml of de-ionized water and kept at room temperature for 24h and the electrical conductivity of the solution (EC1) was recorded. The samples were autoclaved at 120℃ for 20 min and the final electrical conductivity (EC2) was measured. The Electrolyte Leakage (EL) was calculated by using the formulae EC1/EC2.

2.3 Statistical Analysis

The data were statistically analyzed and the differences between treatment means were compared using DMRT.

3. RESULTS AND DISCUSSION

3.1 Growth Attributes

The growth parameters such as hypocotyls + cotyledons and root dry weights were measured for different NaCl salinity stress levels (Table 1). It was observed that the shoot dry weight did not change much although in different NaCl concentrations.

The values were between 0.043 and 0.054g for 125 and 0 mM NaCl respectively. However, there was a significant (P < 0.05) reduction in the root dry weight with increasing salt concentration. This has resulted in an increase in the aerial part/root ratio. Hence, salt stress significantly inhibited the root growth. The shoot growth was not significantly (P>0.05) affected by increased NaCl concentration. As pointed out by Jamil [8] salt stress inhibited the growth of root greater than shoot in four vegetable species. According to Saha [9] the roots of mungbean seedlings were more sensitive to salinity than the aerial parts. Roots are directly in contact with growth media containing toxic salts that stop the long term root growth. Under saline condition, CO2 assimilation of plant becomes decreased. It is the major energy source for growth and development so, root growth decreases. According to Renault [10], reduction in seedling dry weight in response to salinity is due to decline in weight of mobilized

Table 1. The values of the shoot (cotyledons + hypocotyls) dry weight (DW), root dry weight and the shoot/root ratio of okra cv. 'Haritha' exposed to different NaCl concentrations during the seedling stage

NaCl (mM)	shoot DW (g)	Root DW (g)	shoot / root ratio
0	0.054±0.016^{a}	0.015±0.013^{a}	3.60±0.376^{a}
25	0.052±0.014^{a}	0.013±0.007^{b}	4.00±0.362^{ab}
50	0.049±0.016^{a}	0.011±0.003^{c}	4.45±0.357^{ab}
75	0.046±0.015^{a}	0.009±0.019^{d}	5.11±0.581^{bc}
100	0.045±0.014^{a}	0.007±0.010^{e}	6.43±0.660^{bc}
125	0.043±0.015^{a}	0.005±0.022^{f}	8.60±0.741^{c}

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 four replications

seed reserve. The depressed growth of plants may be due to the toxic effect of Na+ and Cl- ions present in NaCl and low water potential in the rooting medium.

Okra plants treated with salt significantly ($P<0.05$) affected the shoot/root ratio. The 75 and 100 mM treatments increased this ratio to 5.11 and 6.43 respectively. The highest value of shoot/root ratio was obtained in plants treated with 125 mM NaCl and it was 8.6. As pointed out by Dkhil and Denden [11], salt stress resulted in a significant increase in cotyledons + hypocotyls/ root ratio which was considered as the direct effect of the root dry weight reduction of treated seedlings. The magnitude of root growth reduction was highly dependent on NaCl concentrations. The root dry weight was reduced by approximately 33% at the highest salt concentration (125 mM NaCl) compared to control (Table 1).

It was observed that there was a decrease in the water content of hypocotyls and cotyledons with increasing salt concentration (Table 2). The aerial part water content was limited to certain extent with the increase in salt stress. But, the root water content significantly increased with increasing NaCl concentration. 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 [12].

3.2 Membrane Permeability

It was observed that the quantity of electrolyte leakage increased under salt stress (Table 3). The extent of this increase was significant at 100 and 125 mM NaCl concentrations. It was found that the electrolyte leakage was slightly altered with 50 and 75mM NaCl salinity. The range was between 20.86 and 22.37% respectively. The highest % leakage found at 125 mM NaCl salinity indicated membrane damage. As pointed out by Kaya [13] increase in membrane permeability at seedling stage was lower than at vegetative stage at high salinity. This indicates a strong relationship between time of exposure to high salinity and membrane permeability.

Salt stress induced electrolyte leakage has also been observed in maize [14]. It is now generally accepted that the first deteriorating change during stress injury is an alteration in the structure and function of cell membranes [15]. Based on the results, it was suggested that salinity levels imposed did not create high membrane damage.

Table 2. The water content in aerial part (cotyledons + hypocotyls) and root of okra cv. 'Haritha' exposed to different NaCl concentrations during the seedling stage

NaCl (mM)	Aerial part water content (g/g) (FW-DW)/DW	Root water content (g/g) (FW-DW)/DW
0	15.13 ± 1.42^{a}	11.32 ± 1.36^{d}
25	12.57 ± 1.53^{ab}	13.47 ± 1.67^{cd}
50	13.44 ± 1.47^{ab}	13.43 ± 1.72^{cd}
75	10.28 ± 1.40^{b}	16.36 ± 1.93^{b}
100	9.50 ± 1.36^{b}	14.86 ± 2.10^{bc}
125	9.41 ± 1.39^{b}	21.16 ± 2.98^{a}

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 four replications

Table 3. Effects of NaCl treatments on electrolyte leakage (%) at seedling stage of okra cv. 'Haritha'

NaCl (mM)	Electrolyte leakage (%)
0	19.74 ± 0.713^{a}
25	19.32 ± 0.709^{a}
50	20.86 ± 0.658^{ab}
75	22.37 ± 0.633^{ab}
100	26.62 ± 0.615^{b}
125	27.08 ± 0.638^{b}

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 four replications

4. CONCLUSION

The property of salinity tolerance depends on different physiological interactions. The growth physiological attributes presented by the plant in response to salinity may not be enough to determine its effect. However, the okra seedlings were able to maintain the physiological growth attributes considerably well in the presence of relatively high degree of salinity stress during the seedling stage.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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