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EFFECT OF NACL SALINITY ON COTTON (*GOSSYPIUM ARBOREUM* L.*)* GROWN ON MS MEDIUM AND IN HYDROPONIC CULTURES

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ABSTRACT

Salinity is a major environmental stress and is a substantial constraint to cotton production in Pakistan and worldwide. In the present study, cotton (*Gossypium arboreum* L.) cv. FDH-786 seeds were exposed to different salinity (100, 200, 1000 mM NaCl) levels. In the first experiment, effect of salinity on germination of seeds was observed. Increasing salt concentration resulted in a parallel inhibitory effect on total germination percentage and the rate of germination of cotton seeds. Germination was delayed and significantly reduced by 300-700 mM solution of NaCl. At highest salt levels of 800–1000 mM, germination was completely arrested. The second experiment was conducted to study the effect of different salinity levels on survival of cotton seedlings. The seedlings were grown on MS-medium as well as in hydroponic cultures. Survival percentage was significantly reduced by all the concentrations of the salt solution. Cotton seedlings showed more susceptibility to salt stress in hydroponic cultures than to MS medium. None of the cotton seedlings survived beyond 60 and 40 mM concentration on MS medium and hydroponic cultures, respectively.

Key words: Cotton, germination, growth, hydroponic cultures, MS medium, salinity.

INTRODUCTION

Salinity in topsoil and subsoil is one of the major abiotic environmental stresses to crop production (Grewal, 2010). Worldwide, soil salinity is becoming a serious threat to agricultural productivity (Cha-um *et al.,* 2006). About 20% of the world's cultivated area and nearly half of the world's irrigated lands are affected by salinity (Zhu, 2001). Various plant growth and development processes viz. seed germination, seedling growth, flowering and fruiting are adversely affected by salinity, resulting in reduced yield and quality (Jampeetong and Brix, 2009; Gorai *et al.,* 2010). The problem of water-logging and salinity in Pakistan is typical for irrigated agriculture where adequate drainage is not provided. Soil salinity in Pakistan is a product of climatic conditions, original soil chemistry, land use, irrigation practices, and the shallow depth of the water table.

Cotton is an important cash crop worldwide. It plays a pivotal role in the agriculture-based economy of Pakistan. Pakistan is the fourth largest cotton producing country after China, USA and India (Ahmad *et al.,* 2009). In addition to fiber, cotton is also an important source of vegetable oil in Pakistan. It generates significant proportion of foreign exchange. It is grown on an area of 3.1 million hectares with production of about 12.4 million bales in Pakistan (Anonymous, 2007). Although cotton is classified as a salt tolerant crop, it is often adversely affected by soil salinity especially during emergence and seedling growth (Ashraf, 2002). The present study was carried out to investigate the effect of salinity on germination and seedling growth of cotton (*Gossypium arboretum* L.) cv FDH-786 using MS solid medium and hydroponics cultures.

MATERIALS AND METHODS

Surface sterilization of seeds: The experiment was conducted at the Center of Excellence in Molecular Biology, University of the Punjab, Lahore Pakistan. Cotton var. FDH-786 was selected because of its best germination and growth in previous experiments. Cotton seeds were delinted with concentrated commercial HCl or H_2SO_4 at the rate of 100 mL kg^{-1} of cotton seeds. The seeds were continuously stirred with the help of spatula for 10-15 min until the surface of seeds became shiny. Seeds were washed 5 times with tap water to remove acid completely. The seeds which float over the water were removed.

Seeds were sterilized using autoclaved magenta boxes. For the purpose of sterilization of seeds, an empty flask was autoclaved. Then in 50 mL of water, 5% murcuric chloride and 0.5 mL of 10% SDS (sodium dodecyl sulphate) were added. One hundred cotton seeds were added to this flask constantly shacked for 5-7 minutes. The solution was discarded and the seeds were rinsed with autoclaved distilled water for 5-7 times till no foam was present in the flask. Three mililitre of water was added in the flask and kept it at 30°C in an incubator for germination after wrapping with cotton.

Germination bioassays: Surface sterilized cotton seeds were placed on a double layer Whatman No.1 filter paper seedbed and covered with a double layer of filter paper in sterilized Petri plates. Stock solution of 5 M NaCl was prepared in distilled water. Three mililitre of NaCl solution of different concentrations (100, 200, 1000 mM) was applied to each Petri dish. The control treatment was treated with the same quantity of distilled water. Each treatment was replicated thrice. Petri dishes were wrapped in aluminum foil and were placed in an incubator at 30°C to allow germination. Number of seeds germinated were counted for each treatment. A seed was considered germinated when both plumule and radicle were emerged.

Growth bioassays on MS medium: For the growth of selected cotton cultivar seedlings MS (Murashige and Skoog) medium was prepared. The medium was prepared by adding 4.33 g of MS basal medium, and 30 g of sucrose in distilled water. Both were dissolved and pH was adjusted at 5.8. Then 3 g of phytagel was added. No NaCl (sodium chloride) solution was added to the control treatment. For different treatments of salinity (100, 200 1000 mM), MS medium was supplemented with appropriate quantity of stock solution of NaCl to make the medium of required concentrations. Medium was autoclaved and was left at room temperature for some time and then poured into the test tubes. The cotton seeds which were properly germinated on wet filter papers were transplanted into MS medium test tubes. The experiment was designed in a completely randomized design of one control and 10 salt treatments. There were three replicates of each treatment with 12 test tubes in each replicate. Test tubes were plugged with cotton and kept in test tube rack. Data regarding seedling survival were recorded for 1 week

Growth bioassays in hydroponics: Hydroponics nutrient solution was prepared by adding 4.33 g of MS basal medium and 30 g of sucrose in distilled water to prepare 1.0 L of solution. For the ten salt treatments medium was supplemented with appropriate quantity of NaCl to prepare 100, 200, 1000 mM salt solutions. Medium was autoclaved, cooled at room temperature and poured into test tubes.

Seedlings were taken from the 1-week old cultures of MS solid medium, at 2-3 leaf stage. All the seedlings were dipped in autoclaved water and washed thoroughly to remove solid medium. Roots were dried properly on autoclaved filter papers. A wick was made by an autoclaved filter paper and placed into the test tube with the help of sterilized forceps. Seedling were carefully shifted into hydroponics nutrient solution in the way that that all roots were immersed through the hole in the wick into the nutrient hydroponics broth. There were three replicates of each treatment with 12 test tubes in each replicate. Data regarding percentage of survivals of the seedlings were recorded. All the data were subjected to statistical analysis by applying analysis of variance followed by Duncan's Multiple Range Test to delineate the treatment means (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

Effect of salinity on germination of cotton seeds: Data regarding the effect of different concentrations of NaCl solution on germination of cotton seeds is presented in Fig. 1. All the concentrations of NaCl solution significantly reduced the germination of cotton seeds. In general, the adverse effect of the salt solution on germination was increased with an increase in salt concentration. There was 17–100% reduction in germination due to different concentrations of the salt solution. Solutions of concentration 800 mM and higher completely arrested the germination of the seeds. These results are in agreement with the finding of earlier workers who reported reduced seed germination in wheat, *Medicago ruthenica*, *Diplotaxis harra* and other plant species under salt stress (Tlig *et al.*, 2008; Guan *et al.*, 2009; Zheng *et al.*, 2009).

Effect of salinity on survival of seedlings: Data regarding the effect of different concentrations of NaCl solution on survival of cotton seedling on MS medium is shown in Fig. 2. Survival percentage of the cotton seedlings was seedling reduced by all the concentrations of salt solution. Survival percentage was gradually decreased as the salt concentration in the solution was increased from 100–600 mM. The concentrations higher than 600 mM resulted in death of all plants.

Data regarding the effect of different concentrations of NaCl solution on survival of cotton seedling in hydroponic cultures is illustrated in Fig. 3. Cotton seedlings were more sensitive to salinity in hydroponic cultures than on MS medium. Survival percentage of the seedlings was gradually decreased by increasing the concentration from 100–400 mM. Salt concentrations higher than 400 mM resulted in death of all the plants. Soil salinity affects plant growth and development by way of injurious effects of toxic ions, osmotic stress, reduced water use efficiency and the resulting nutrient imbalance (Sairam and Tyagi, 2004; Grewal, 2010). Moreover, saline-induced stress in plants can imbalance reactive oxygen species, such as superoxide, hydrogen peroxide, and the hydroxyl radical, producing oxidative damage to lipids, proteins, and nucleic acids (Schwanz et al., 1996; Halliwell and Gutteridge, 1999).

The present study concludes that cotton cv. FDH-786 is highly sensitive to NaCl salinity. The response of seedlings of this cultivar to salinity can be best studied in hydroponic cultures.

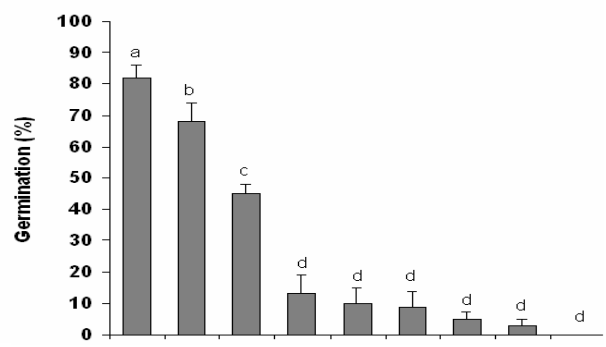

Fig. 1: Effect of different concentrations of NaCl solution on germination of cotton seeds. Vertical bars show standard errors of means of three replicates. Values with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

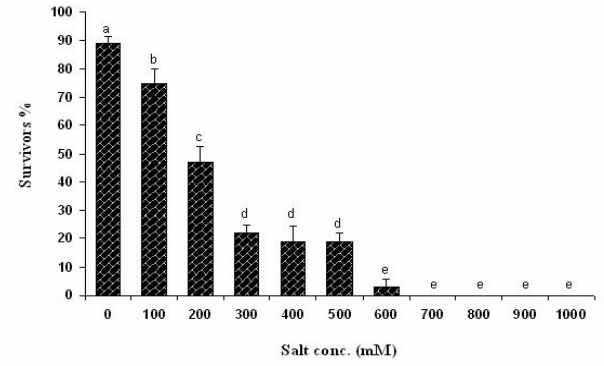

Fig. 2: Effect of different concentrations of NaCl solution on growth of cotton seedlings on MS Medium.Vertical bars show standard errors of means. Values with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

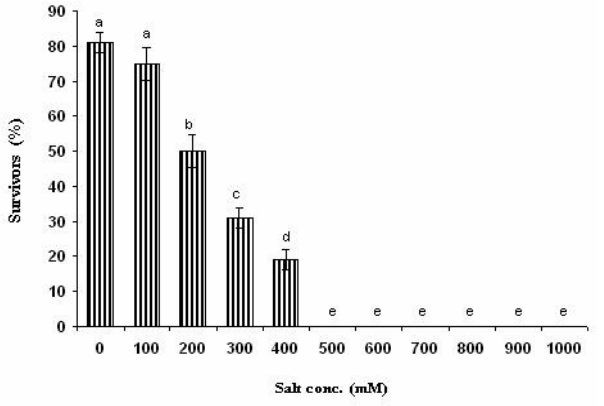

Fig. 3 - Effect of different concentrations of NaCl solution on survival of cotton seedlings in hydroponic cultures. Vertical bars show standard errors of means. Values with different letters show significant difference (P≤0.05) as determined by Duncan's Multiple Range Test.

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