IN VITRO EVALUATION OF SOME FIG CULTIVARS UNDER SALINITY STRESS

By

RANA MOHEBELDIN SAAD ELAZAB
B.Sc. Agric. Sci. (Biotechnology), Fac. Agric., Ain Shams Univ., 2011

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SUPERVISION SHEET

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ABSTRACT

The present investigation was carried out at the Biotechnology Laboratory of Pomology Dept., National Research Center, Dokki, Giza, Egypt during the period from 2015 to 2017 to study plant propagation of four fig cultivars (Black Mission, Aboudy, Conadria and Sultani) by using shoot tip and different concentrations of BAP 0.5 , 2 , 4 mg L-1 and Then put this four cultivars into medium supplemented with different concentrations of NaCl to evaluate the effect of Sodium Chloride on *in vitro* regenerated fig cultivars. NaCl concentrations (1000, 2000, 3000, 4000, 5000, 6000 and 7000 mg L-1) were used for salinity stress. Sultani fig cultivar from indirect regeneration was cultured on Murashige and Skoog medium containing different NaCl concentrations (0, 2000, 4000, 6000, 8000 and10000 mgL-1) were studied. Plant length leaf number, chlorophyll, proline and leaf mineral contents were determined. . The obtained data indicated that Murashig & Skoog medium (MS) supplemented with 0.5 mg/l is most suitable for increasing shoot length, number of leaves, number of branches and greening All fig cultivars were negatively affected by salinity with different levels. The addition of NaCl significantly decreased plantlet growth, leaf content of N, P, K and total chlorophyll in all cultivars. Cl- and Na+ as well as proline level increased as increasing NaCl concentrations up to 6000 mg L-1NaCl at 7000 mg L-1 considered as the lethal dose as it caused high mortality rate of all fig cultivars under study. Sultani fig cultivar accumulated more content of N, P, and K and had higher chlorophyll and proline under high salt stress compared with other fig cultivars. Increasing NaCl concentration more than 8000 ppm induced a bad effect on all growth parameters under study up to the lethal concentration by 10000 ppm which caused almost death of the in vitro Regenerated plantlets from subculture1 and subculture 2 of Sultani fig cultivar.

Key Words: Fig- Salinity –Growth Parameters- Proline-Mineral content- Chlorophyll

DEDICATION

I dedicate this work to whom my heartfelt thanks; to the only person supports me after Allah forever my father, my mother and my Sister for all the support they lovely offered along the period of my post- graduation.

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LISTT OF ABBREVIATIONS

Commercial bleach	Sodium hypo chloride (NaOCl)
BAP	6- benzyl amino purine
IBA	Indole-3-butyric acid
MS medium	Murashige and Skoog medium
2,4 D	2,4 Diclorophenoxy acetic acid
Kin	Kinetin
2ip	2- isopentenyladenine
TDZ	Thidizuron

INTRODUCTION

Ficus carica L. (Moraceae), commonly known as Fig plant considered as one of the traditional Mediterranean crops. Fig is deciduous tree or large shrub, growing to a height of 7–10 meters (23– 33ft). Fig has wide variety of chemical constituents, its use in traditional medicine as remedies for many health problems, and its biological activities (Chawla *et al*., 2017). The majority of fig production is still centered in the Mediterranean basin; Turkey is the biggest world producer, followed by Egypt, Algeria, Morocco, and Iran (FAO, 2003). The fig grows successfully in Egypt; more than 50% of the total fig area is located along the north western coast of Alexandria as well as Sinai governorates.

Agriculture currently uses 70% of water resources worldwide and demands in water are still rising. Climate change and an increasing world population will result in even more water needed for food production. Water scarcity is one of the most common environmental limitations that cause significant reduction of growth, development and yield (Jain, 2001), limited water and hot dry climates necessitate irrigation by ground water containing salts and these can cause transient salinity or in many cases lead to a build-up of salinity that limits or eventually prevents crop production.

Salinity is a chief factor limiting the growth of many plant species throughout the world and it has been estimated that around 831 million hectares were affected by salinity in the world. An

increase in the salinity of soil water inhibits growth of most plants because it reduces water uptake and reduce water use efficiency and inhibits of K, Ca and NO_3 uptake by plant roots. It has been shown that salinity reduces gas exchange, growth traits, yield and yield components and causing a reduction in leaf chlorophyll and photosynthesis.

The more tolerate plant species elevates antioxidant enzymes and proline content as a stress response to deal with increased levels of Reactive Oxygen Species (ROS) (Gill and Tuteja, 2010). As well as most plant species are unable to discriminate between the ions K+ and Na+ and in saline conditions take up and accumulate high levels of Na+ to the detriment of the necessary quantities of K+ leading to loss of function of K+ dependent reactions and leading to Na+ induced toxicity (Zhang *et al.,* 2002).

In light of shortage in water resources and agricultural land, Egyptian ministry of agriculture followed a new strategy to maximize utilization of these resources; by focusing on crops with low water requirements. One of these crops is *Ficus carica* L., (Mustafa *et al.,* 2013). It is recognized to have the ability to tolerate water deficit and moderate salinity stress, making it a suitable species for cultivation in semi-arid environments such as the Mediterranean and Middle-East where it is an important crop plant.

Tissue culture has become an important technique for propagation and breeding of woody plants. Cell and tissue culture systems have been used for selection of plant tolerance to abiotic

stresses including salinity, drought, and other stresses.

Resent progress in genetic manipulation of plant cells has opened new possibilities in crop improvement. *In vitro* technique is used for biochemical and physiological studies in response to stress at the cellular level (Liu et al., 2006). *In vitro* studies have been introduced as a reliable alternative screening method to the field experiments (Karimi et al., 2012).

Techniques of *in vitro* tissue culture have been utilized as a valuable tool in the selection of crop cultivars for tolerance to stress factor (Lupotto et al., 1988 and Mongodi et al., 1988). *In vitro* selection is conducted at the protoplast, cell-suspension or callus stages or later during the regeneration of shoots and roots (Widoretno 2003).

In vitro culture techniques minimize environmental variations due to defined nutrient media, controlled conditions and homogeneity of stress application. In addition, the simplicity of such manipulations enables studying large plant population and stress treatments in a limited space and short period of time (Larher et al., 1993).

In order to have effective utilization of salt affected soils it is important to identify fig genotypes which tolerate salinity and produce substantial yield under adverse soil environment and utilize the heritable variation present in the germplasm to propagate resilient genotypes or to utilize these in a breeding programe. Targeted breeding in fig can play a major role in narrowing the gap

between supply and demand through utilizing improved varieties that can be grown under abiotic stress conditions (Metwali *et al*., 2014).

The main objective of the present study was to investigate the effect of different concentrations of sodium chloride on plant growth, total chlorophyll, and proline and leaf mineral content of *in vitro* grown four fig cultivars.

REVIEW OF LITERATURE

In vitro Propagation is a collection of techniques used to maintain or grow plant cells, tissues or organs under sterile conditions on a nutrient culture medium of known composition. Plant tissue culture is widely used to produce clones of a plant in a method known as micropropagation.

Tissue culture is one of the fundamental tools in the field of plant biotechnology. Saltiness and dry season tolerant cultivars can be chosen and assessed utilizing tissue culture. Screening of plantlets by tissue culture under abiotic or biotic pressure of numerous plant and assesses these plants under particular conditions in a brief timeframe. Moreover, *in vitro* culture gives a critical system for consider the physiological impacts of salt at the cell level under known natural conditions. Biotechnology and tissue culture systems are an intense instrument used to select plants under abiotic or biotic pressure and to enhance profitability qualities in agricultural yields. Tissue culture has been successfully used to create new genetic variation in the breeding programs. (Shahzad *et al*., 2017).

1. Micropropagation

a. Establishment Stage

Kumar *et al* (1998) reported that in establishment stage apical bud cultured on MS medium supplemented with 2 mg/l BAP + 0.2 mg/l NAA, started shoot formation from the 12 day. Hemaid *et al.,* (2010) reported that micropropagation of *Ficus Carica* using shoot tips and nodal explants of cultivars (Conadria, Aboudy and Sultani) were the