CONTENTS

LIST OF TABLES

Page

Title

CHAPTER FIVE

SUMMARY

The present investigation was carried out during 2011, 2012 and 2013 at the Tissue Culture Laboratory of Agriculture Research Center. El-Sabhya, Alexandria, Central Laboratory at the Faculty of Science, University of Alexandria and Institute of Genetic Engineering & Biotechnology Research. Sadat City University

Sugarcane (*Saccharum officinarum* L.) is an important sugar crop for produced the sweetener and sugar. Commercial production of sugar from sugarcane began in India and China approximately 2500 years ago and spread to Western Europe in the eighteenth century.

Today, sugar production, sugarcane are used as raw materials for fuel production, chemicals, bio-fertilizers, paper and pulp. Sugarcane is an important agro-industrial sugar crop, contributing about 70% world sugar production. Globally, it occupies more than 23.98 million hectares of land worldwide, generating 1.71 billion tons of harvested cane in 2010

Tissue culture system is useful for the evaluation of tolerance to environmental stresses because the stress conditions can be easily controlled *in vitro*. Moreover, *in vitro* culture provides a uniform population of synchronously developing plant cells without involving regulatory mechanisms that naturally repaired at the whole plant level.

The present investigation was undertaken to fill in some of lacunae with the following objectives:

1- To evaluate three sugarcane (*Saccharum officinarum* L.) genotypes for their capabilities for callus induction, day of callus initiation and embryogenic calli,

2- To study the effect of drought stress by using different concentration of mannitol on calli to determine the tolerance of each genotypes,

3- To study effect of relative growth rate (RGR), Water content (WC), accumulation of Na^+ and K^+ ions and determine the proline content at different concentration of mannitol on callus,

4- To identify the genetic variation among sugarcane genotypes via RAPD-PCR,

5- To study the effect of drought stress on shoot and root formation.

The sugarcane genetic materials used in this study namely G 84-47, Ph8013 and with the commercial Genotype GT 54-9. They are under the genus

Saccharum. Three Sugarcane genotypes ph 8013, GT 54-9 and G 84 - 47 were selected and tested in the present research.

Stem sections containing two lateral buds were planted in plastic pots containing soil in greenhouse conditions until reaching ~6 months. The explants from 6-8 month old, healthy, disease free were cut the shoot tip which used in our study. Stem sections of sugarcane genotypes (GT 54-9, G 84–47 and ph 8013) were used as starting materials on MS containing 3 mgL^{-1} 2,4-D for callus induction.

The explants were induced to develop callus at all genotypes. The results clearly indicated that the degree of callus proliferation varied from 70 - 86%.

Analysis of variance indicated high significant difference between three genotypes with LSD=3.88 in relation to percentage of callus induction. The highest frequency (86±3.16) was recorded to GT 54-9 compared to the other two genotypes (70±1.87, 80±20) in respect.

Although the two genotypes GT 54-9, G 84–47 had the same day to callus initiation in average 10 days, GT 54-9 was the highest one compared with others in mean 14±2.0 and LSD=2.38. While the highest day to callus initiation was 14 days for ph 8013 and showed the second value in callus induction.

The results showed the high embryogenic callus percentages ~ 80%. While no significant difference was observed between GT 54-9 and G 84–47 ($L.S.D._{0.05}$) which gave the best response compared to the other genotype.

The results showed that the maximum RGR were obtained for GT 54-9 (2.22± 0.15) while the lowest was 0.73 ± 0.20 for ph8013. On the other hand our results showed that control callus gained the highest RGR in mean 2.83 ± 0.011 for GT 54-9 followed by mannitol treated callus

Among the treated samples, callus treated with 100mM mannitol showed the highest RGR in mean 2.56 ± 0.022 for GT 54-9 while, with 300mM callus recorded the lowest RGR by mean 0.09 ± 0.002 for ph8013.

The results clearly indicated that, by increasing the mannitol concentration, the RGR of callus decreased.

Data showed that significant effect between the three genotypes and treatments. The result showed that the maximum callus RGR in control and treated genotypes were decreased by increasing the concentrations of mannitol.

Concerning to percentage of water content callus fresh weight and dry weight were recorded after four weeks of treatment.

Results showed that the highest %WC was 77.952 ± 2.36 for GT 54 -9 while, the lowest was 76.12 ± 3.38 for ph8013.

Comparison between the means of genotypes results indicated that genotypes showed no significant variations in callus water content between GT 54-9 and G 84-47.

Genotype GT 54 -9 recorded the highest water content after all treatment except with 100 mM mannitol concentration. While, ph8013 recorded the lowest % WC value in mean 54.45 ± 0.796 after treatment with 100mM mannitol.

In the current study, five oligonucleotide primers (100%) used in the RAPD analysis gave unique markers. Out of the five primers, five detected for GT 54-9 and 8 for G 84-47. Data showed the similarity matrix of the five RABD-PCR markers.

Cluster analysis of the current research, divided the three sugarcane genotypes into two main groups in similarity percentage 59%. The first group includes G 84-47 by the three concentrations 100, 200 and 300 mM mannitol by similarity 70%. While, control with the other concentrations by 68%. On the second group, the genotypes divided into two sub groups on 65% similarity. The two sub group includes the genotypes ph 8013 and GT 54-9.

The main consolation of the present study indicated that, GT 54-9 genotype considers the promising genotypes other than G 84-47 and ph-80-13, in respect, in all the morphological and molecular studies.

LIST OF FIGURES

Page

Title

LIST OF PLEATS

page

Title

ABBREVIATIONS

%	: Percentage
µl	: Micro liter
µM	: Micro molar
2,4-D	: 2,4- Dichlorophenoxy Acetic Acid
AC	: Activated Charcoal
bp	: Base Pair
Cv(s)	: Cultivar(s)
CW	: Coconut Water
DW	: Dry Weight
EC	: Embryogenic Callus
FW	: Fresh Weight
hrs	: Hours
K^+	: Potassium
Kin	: Kinetin
M	: Molar
Mg l^{-1}	: Milligram per liter
ml	: Milliliter
mM	: Milli molar
MS	: Murashige and Skoog
Na^+	: Sodium
NAA	: 1-Nphthylacetic acid
NaOH	: Sodium Hydroxide
NO.DRF	: Number of Day for Root Formation
NO.R	: Number of Rroot
NO.SF	: Number of Shoot Formation
^oC	: Degree Celsius
PCR	: Polymerase Chain Reaction
PEG	: Poly Ethylene Glycol
pH	: - Log $[H^+]$
PVP	: Poly Vinyl Pyrrolidone
RAPD	: Random Amplified Polymorphic DNA
RGR	: Relative Growth Rate
SE	: Somatic Embryogenesis
WC	: Water Content