Kafrelsheikh University
Faculty of Agriculture
Horticulture Department

Induction of Genetic Variations of Some Grape Rootstocks

By
Asmaa Mohy El-dien Ibrahim Salama
B.Sc. Agric.(pomology) Kafrelsheikh University ,2007
M.Sc. Agric. (pomology) Kafrelsheikh University,2011

Thesis
Submitted in Partial Fulfillment of the requirements for the Degree of
DOCTOR OF PHILOSOPHY
In
Agricultural Sciences
(Pomology)

Horticulture Department
Faculty of Agriculture
Kafrelsheikh University

2020

Induction of Genetic Variations of Some Grape Rootstocks

By
Asmaa Mohy El-dien Ibrahim Salama

Abstract

This work was carried out during two growing seasons of 2015 and 2016 at experimental farm of Faculty Agricultural Kafrelsheikh University, Egypt. on four grapevine rootstocks Ramsey, Harmony, Freedom, So4 and Thompson seedless as a cultivar. The objectives of this study was to induce mutations by gamma rays in five grapevine genotypes including commercial variety Thompson seedless to generate promising salt tolerant mutant grapevine rootstocks. In this study molecular markers were used to detect mutations induced and to test gene expression associated with salinity tolerance in grapevine.

Results showed that both doses of 10 and 20 Gy were effective in obtaining mutations in all five grapevine genotypes including Thompson seedless. Mutations were confirmed by morphological markers such as bud burst percentage, shoot length and number of leaves, and by genetic markers using RAPD technique. Six RAPD markers were able to generate polymorphic bands ranged from 1 to 9 among irradiated and un-irradiated grapevine genotypes. The similarity coefficients detected by RAPD markers ranged from 0.24 to 0.87, which revealed clear genetic variation among irradiated and un-irradiated grapevine genotypes. According to phylogenetic analysis, genotypes were divided into two clusters, where all five un-irradiated genotypes and irradiated Freedom genotype with 10 Gy were placed in one cluster and second cluster included the rest of nine irradiated genotypes. Furthermore, there was significant upregulation of *VviERF073* gene expression level in the leaves of irradiated Thompson seedless and four rootstock plants compared to un-irradiated plants under various salinity concentration (50, 100, 150 mM). *VviERF073* gene expression levels were increased in all genotypes significantly as a result of irradiation and salinity recording higher expression (10.68-folds) with Thompson seedless at 30 Gy, and 150 Mm.. This study suggests use of mutation breeding by gamma rays in grapevine rootstock improvements for salinity tolerance, and use molecular markers for detecting mutations and genetic diversity among grapevine genotypes.

LIST OF CONTENTS

PAGE

INTRODUCTION ……. 1

REVIEW OF LITERATURE ……...4

1. Influence of gamma rays on horticultural crops.……..... **4**

2. Influence of salinity on horticultural crops....……..….....**9**

MATERIALS AND METHODS.…… 13

1. Plant materials..……...…….. **13**

2. Gamma irradiation and salinity treatments... …… **14**

2.1. Vegetative growth….……..……. **15**

2.2 Chemical analysis..…….... **16**

3. Molecular analysis. ……..….**17**

3.1. DNA isolation ……...……...**17**

3.2. Random Amplification of Polymorphic DNA analysis (RAPD)…...**18**

3.3 RNA analysis.……..... **19**

3.4 Statistical analysis of molecular marker data….……..... **22**

RESULTS AND DISCUSSION. ……...…...23

1. The effect of some grape genotype and, doses of gamma ray on:…...…… **23**

1. 1. Bud Burst (%). …….……..**23**

1. 2. Shoot length (cm) ……...……**27**

1. 3. Number of leaves. ……...……...……**30**

1. 4. Survival (%). ……...…...……....**35**

1.5 Chemical analysis:. ……...……...……**38**

2. Confirmation of mutation-induced and genetic diversity among grapevine genotypes by RAPD analysis. ……**42**

3. The effect of some grape variety, doses of gamma ray and their interaction between them under salinity conditions on ……..… **49**

3. 1. Shoot length (cm)…….....……. **49**

3. 2. Number of leaves…….……...**53**

3.3. Survival (%)……..... **58**

3.4. chemical analysis: …….. ……..….... **61**

4. Expression analysis of ERF transcription factor VviERF073 gene in irradiated and un-irradiated grapevine rootstocks under salinity condition ……**86**

SUMMARY and CONCLUSION……...……..93

Annex……...……..... 102

REFERENCES…… **106**

ARABIC SUMMARY