GENETIC IMPROVEMENT OF SOME IMPORTANT ACTIVE INGREDIENTS IN SOME MEDICINAL PLANTS

By

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A thesis submitted in partial fulfillment
of
the requirements for the degree of

DOCTOR OF PHILOSOPHY

in
Agricultural Science
(Genetics)

Department of Genetics
Faculty of Agriculture
Ain Shams University

2012

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II. INTRODUCTION

Medicinal plants have been used to treat illness and disease for thousands of years; even now they are economically important, being used in the pharmaceutical, cosmetic, perfumery, and food industries. In recent years we've witnessed an explosion in the popularity of natural products and cosmetic products containing natural extracts. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Medicinal plants provide very little amount of the active constituents, so large amounts of the plant material shall be harvested to maintain the desired amount, which leads to the risk on the plants biodiversity and on the ecosystem. The global market value of medicinal plants exceeds 60 billion USD annually, so protection of wild life ecosystem diversity is crucial for the continued collection of medicinal plants. Half of the ca 20000 medicinal plants used today are threatened with extinction.

Jatropha curcas is becoming an increasingly popular plant for its proposed value in the biodiesel, biopharmaceuticals, cosmetics and biopesticides industry **(*Gubitz et al., 1999; Kumar & Sharma, 2008).*** Meager genotypic characterization, limited information on the genome of *J. curcas* and a scarce number of its isolated genes require major research initiatives in agronomy, breeding and molecular biotechnology of *J. curcas* for it to live up to its potential (***Popluechai et al., 2009, Basha*** and ***Sujatha (2007)***.

For a long time the interest in Ribosome-inactivating proteins (RIPs) has been focused on developing antitumor drugs that selectively target to tumor cells. RIPs are enzymes which damage ribosomes in an irreversible manner by removing one or more adenine residues from rRNA. They also depurinate other polynucleotide's. Curcin, is a protein of type I Ribosome-inactivating proteins (RIPs), which was first isolated from seeds of *Jatropha curcas* and could inhibit the growth of some tumor cells. The antitumor activity is related to N-glycosidase

action, which cleaves the N-glycosidic bond of adenine A4234 of 28S rRNA. This makes ribosome unable to bind the elongation factors 1 or 2, consequently arresting protein synthesis. It is also used as an external applicant for skin diseases, rheumatism, livestock sores, and piles and as an antidote for certain snake-bites. In addition, the ether extract shows antibacterial properties against *Staphylococcus aureus* and *Escherichia coli* and even antiviral activity against plant, fungal and animal viruses.

The objectives of this study are:

1. Identifying the different plant species and identify the existed variability among them as the first step in any improvement strategy using RAPD (Random Amplified Polymorphic DNA) and ISSR (Inter Simple Sequence Repeats) molecular markers.
2. Isolating the curcin gene from *Jatropha curcas* ecotypes from the different locations and compare it to those found in the gene bank.
3. Screening for the presence or absence of this gene in other related plant species.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude and appreciation to **Professor Mohamed Abdelsalam Rashed** for his supervision, guidance, criticism, friendship and encouragement through all the stages of this study.

I also would like to express my sincere gratitude to **Professor Aly Zain Elabidin Abdelsalam** for his supervision, support and encouragement throughout my PhD study.

I would like to express my gratitude to **Professor Ahmed Bahieldin** for his valuable guidance, his initial support and collaboration.

I would like to thank **Dr. Mohamed Mustafa** and **Dr. Hala Eissa** for their help and financially supporting part of the work. I also appreciate the help and pleasant working atmosphere from members of **AGERI** research team and their sincere friendship specially **Dr. Ahmed Ramadan, Dr. Ahmed Shoukry, Dr. Sameh Hassanein** and **Yasser Bahaa ElDin**.

Many thanks to all staff members of **Faculty of Biotechnology Misr University for Science and Technology** especially **Aly Farouk** and friends in **Genetics department** and **(ACGEBFAG)** for their sincere helping, fruitful advising and faithful encouragement during the study.

Finally, I wish to express my special thanks to my **parents** and family for their love, understanding, support and encouragement. There is no word to express my gratitude to my wife **Dr. Asmaa AbuShady** and to my sons **Mohamed** and **Aly** for their continuous and ever support, understanding and patience during the past years.

V. SUMMARY

The present study was carried out in the Department of Genetics, Faculty of Agriculture, Ain Shams University and Faculty of Biotechnology, Misr University for Science and Technology.

The aim of the study was to isolate the curcin gene from *Jatropha curcas* ecotypes from different location and screen for it's presence in *Jatropha multifida* and *Jatropha integerrima*. Moreover to characterize the genetic variability within the three species under study on the molecular level based on RAPD and ISSR analysis as a first step towards the understanding and development of an improving strategy.

Genomic DNA extracts from five different plants within each species were used as templates for RAPD and ISSR amplifications, as well as the isolation of the curcin gene fragment. Eight preselected random primers (OPA-20, OPC-13, OPC-14, OPC-18, OPC-19, OPC-20, OPD-19, and OPZ-10) and ten ISSR primers (17898A, 17898B, 844A, 844B, HB8, HB9, HB10, HB11, HB13 and HB15) were used to study the genetic variability in *Jatropha* samples.

Data obtained by RAPD analysis among different *Jatropha* species revealed that the eight selected RAPD primers together yielded 234 fragments, of which 217 (93 %) were found to be polymorphic. All the primers produced polymorphic bands, however, the extent of percent polymorphism varied with each primer ranging from 86 % to 100 %. The analysis showed that the strongest relationship was scored between *J. curcas* and *J. integerrima* with an average of 38%, while *J. curcas* and *J. multifida* were shown to be the most genetically distant with an average of 36%.

Based on ISSR data analysis among the different *Jatropha* species, the ten selected primers together yielded 193 fragments, of which 163 (84%) were found to be polymorphic. All the primers produced polymorphic bands, however, the extent of percent

polymorphism varied with each primer ranging from 63% to 100%. The strongest relationship was scored between *J. curcas* and *J. integerrima* with an average of 52%, while *J. curcas* and *J. multifida* were shown to be the most genetically distant with an average of 41%.

And across all scored markers including RAPDs and ISSRs markers revealed that the strongest similarity was observed between *J. curcas* and *J. integrima* with an average of 44 %. While *J. curcas* and *J. multifida* were shown to be the most genetically distant with an average of 38%, thus demonstrating the distinctiveness of the genetic background of the *J. multifida* form *J. curcas* and *J. integrima*.

The results of curcin gene analysis using curcin CDS1 and CDS2 primers showed that the two curcin gene fragments were present in all *J. curcas* plants from different locations and produced fragments with molecular sizes of approximately 1525 and 1820 bp respectively. These fragments were not present in *J. multifida*, but surprisingly, they could be isolated from *J. integerrima*, and gave a fragments of the same expected size. Despite mentioning that there is no data in the gene bank or any literature that discusses the presence of the curcin gene in *J. integerrima*. Therefore, it is a new discovery of the presence of this sequence in that close related species to the original one.

Analysis of the obtained nucleotide sequences from the different species revealed high sequence identity to the *Jatropha curcas* curcin gene, complete CDS (GenBank Accession No. EU395775) and to the *Jatropha curcas* curcin-L precursor, gene, complete CDS (GenBank Accession EU195892) ranging from 93-98%. Searching for conserved domains using the deduced nucleotide sequences of *J. integerrima* curcin gene fragments high similarity of the amino acid sequence with the RIP superfamily was revealed, which indicates that the two fragments isolated from *J. integerrima* appears to be a new member of the RIP gene superfamily.

ABSTRACT

Osama Ahmed Mohamed Said: Genetic Improvement of Some Important Active Ingredients in Some Medicinal Plants. Unpublished Ph.D. Thesis. Department of Genetics, Faculty of Agriculture, Ain Shams University, 2012.

Jatropha is a genus of approximately 175 succulent plants, shrubs, and trees, from the family *Euphorbiaceae*. In the present study three different *Jatropha* species were investigated (*Jatropha multifida*, *Jatropha integerrima* and *Jatropha curcas*). *J. curcas* samples were collected from three different locations in Egypt from Sheikh Zoaid (Elariesh), Luxor and from the University Farm in European Reef, wherefrom also the other two species were collected. RAPD and ISSR primers were used to identify the different plant species and evaluate the existed variability among them as the first step in any improvement strategy. Curcin, is a protein of type I Ribosome-inactivating proteins (RIPs), which was first isolated from seeds of *J. curcas* and could inhibit the growth of some tumor cells. The curcin gene was isolated using specific primers from *J. curcas* from the different location and compared it to those found in the gene bank. Also, the presence of the gene was tested and screened on the other two *Jatropha* species.

Keywords: Molecular Fingerprinting, Genetic Markers, Genetic Characterization , Medicinal Plants.

VI. APPENDIX

1. CDS1 reverse sequences

1.1. *Jatropha curcas* CDS1 fragment sequence (Reef Aloroupi) (J.curcasRcds1)

NNNGNNGNNGATNTTTCTTCATTGAGACTTTGTAATTGACTGCATTCAAC
AAGACTCCCATGAGACCTGTTACTTGGGTGACATTGTTCACTAGGATATT
GGTATAGTTTTCACGTTGCAATTGAACTGGCTTCAGAAATACATCATTTA
CAGATTTCTGTATTTGATAAGAGAGGTCTCCCCAGTTGTTCTCAAGGCTA
ATTATGTCACCACCCGGCCTAAAGGTTTTGCTAATTTGAGTTGATATTTT
TTTCTCAATATATTTGAATCTTGCTGCCTCTGGAACCATTTCGATAAAAC
CAACTAGAGGTTTAGCAATGTCTGCTGGCTGAGAACTTTTTTCAAGTGTA
TATATGTAATTATCTAATGCCTGCACCCCTAAATCCACTTCCTCTCTGTG
TACGTTTGCCCTAGATAGAAAATCTGCATAGCTACCAGTAAATGCTAGCG
TTTGTTGGTTTGTGTCTGTGAAAAGATTTTTTTTTGCATCAGCCAAAGAT
TCCGGATCGTTAAAGAAATAGGAAGTACCTCCTACCTTATAAGCCACTAA
ATATGCATTAACGACGTTTAATCCTAATGATACTTCTAAGTTCCCTACAT
TTATGACTTTGGCTACAATAAATTTCTGATTTGCAGCAACCGTGGCCCGT
AAGACTGGTATTTCATGGCTTGAATAACTGAAGCCAAATGCTTCTCTTAG
ATCTTTAATGAACTGGGCGTAGTTTTTCTTATCAGTAGTAGCGTCATAAG
TAATGGTTAAAGTTGGAGGGGAACCAGCTTTGTAGTTTTGGTTTGATGAG
AATGGACAAACTATTTCCCTAGCCGATGCCCATCCGAATATAATACTACT
CCAGCAAAACCATGCAGCCACCATAATGGAGAGGATTCATCTTTCCACCT
TTCATATTGATTTCACCTGTCCAGTTGTATGAAGAAACACAAATATCATT

1.2. *Jatropha integerrima* CDS1 fragment sequence (J.integerrimacds1)

CCCTGATGATATTTCTTCACTGAGACTTCTGTAATTGGCTGCATTCAACA
AGACTCCCATGAGACCTTTTACTTGGGTGACATTGGTCACTAGGATATTG
GTATAGTTTTCACGTTGCAATTGAACTGGCTTCAGAAATACACCATTTTC
AGATTTCTGTATTTGATAAGAGAGGTCTCCCCAGTTGTTCTCAAGGCTAA
TTATGTCACCACGCGGCCTAAATGTTTTGCTAATTTGAGCTATTACTTTT
TTCTCAATATATTTGAATCTTGCTGCCTCTGGAACCATTTCGATAAAACC
AACTAGAGGTTTAGCAATGTCTGCTGGTTGAGAACTTTTTTCAAGTGTAT
ATATGTAATTATCTAATGCCAGCACCCCTAAATCCACTTCCTCTCTGTGT

ACGTTTGCCCTAGATAGAAAATCTGCATAGCTACCAGTAAATGACAGCGT
TTGTTGCTTTGTGTCTGTGAAAAGATATGTTTTTGCTTCAGCCAAAGATT
CGGGATCGTTAAACAAATAGGAAGTACCTCCTACCTTATAAGCCACTAAA
TACGCATTAACGACGTTTAATCCTAATGATACTTCTAAATTCGCTACATT
TATGACTTTGGCTACAATAAATTTCTGATTTGCAGCAACCGTGGACCGTA
AGACTGGTATTTCATGACTTGAACAACTGTACCCTAATGCTTCTCTTAGA
TCTTTAATGAACTTCGCGTATTTATGCTTATCAGCATCAGCGTCATCAAT
CCTGGTTACCGTTGGAGTGAAATCAGCTTTGTACTATTGCTCCGCTGACA
ATCCACATCCTAATACCCTATCCCATGCCCATCCGTCCATACTACTACTC
CAGCATTCCATGCATTCTCATACCGATCAGATCCTAATACCCACTGCACA
TCCTATTCCTGTCCAGTGTTGAAAAACTCTAGATCTTTTACGATACGATA
TTTAATTCATTCCAAAAATTTTAAAAAATTCAATATACTACTTTCACAAC
TCACCAA

1.3. *Jatropha curcas* CDS1 fragment sequence (Luxor) (J.curcasLcds1)

CCCTGTTGATATTTCTTCATTGAGACTTTGTATTGACTGCATTCAACAAG
ACTCCCATGACACCTGTTACTTGGGTGACATTGTTCACTAGGATATTGGT
ATAGTTTTCACGTTGCAATTGAACTGGCTTCAGAAATACACCATTTACAC
ATTTCTGTATTTGATAAGAGAGGTCTCCCCAGTTGTTCTCAAGGCTAATT
ATGTCACCACCCGGCCTAAAGGTTTTGCTAATTTGACTTAATACTTTTTT
CTCAATATATTTGAATCTTGCTGCCTCTGGAACCATTTCGATAAAACCAA
CTAGAGGTTTAGCAATGTCTGCTGGCTGAGAACTTTTTTCAAGTGTATAT
ATGTAATTATCTAATGCCAGCACCCCTAAATCCACATCCTCTCTGTGTAC
GTTTGCCCTAGATAGAAAATCTGCATAGCTACCAGTAAATGATAGCGTTT
GTTGCTTTGTGTCTGTGAAAAGATATGTTTTTGCATCAGCCAAAGATTCC
GGATCGTTAAAGAAATAGGAAGTACCTCCTACCTTATAACCCACTAAATA
TGCATTAACGACGTTTAATCCTAATGATACTTCTAAATTCGCTACATTTA
TGACTTTGGCTACAATAAATTTCTGATTTGCAGCAACTGTGGCCCGTAAG
ACTGGTATTTCATGGCTTGAATAACTGAAGCCAAATGCTTCTCTTAGATC
TTTAATGAACTGGGCGTAGTTTTTCTTATCAGTAGTAGCGTCATAAGTAA
TGGTTAAAGTTGGAGTGGAACCAGCTTTGTAGTTTTGGTTTGATGAGAAT
GGACAAACTATTTCCCTAGCCGATGCCCATCCGAATATAATACTACTCCA
GCAAAACCATGCAGCCACCATAATGGAGAGATTCATCTTTCCACCTTTCA
TATTGATTTCACCTGTCCAGTTGTATGAAGAAACACAAATATCATTATAC