

ABSTRACT

TAHA SAYED TAHA HUSSEIN. EVALUATION OF SOME PLANT GENETIC RESOURCES USING BIOCHEMICAL AND MOLECULAR MARKERS. Unpublished Ph.D. thesis, Genetics Dept., Fac. of Agric., Ain Shams Univ., 2004.

The aim of this study was to characterize the genetic diversity within 11 wild *Astragalus* genotypes belonging to five species (*A. hamosus*, *A. peregrinus*, *A. mareoticus*, *A. hispidulus* and *A. boeticus*) on the biochemical and molecular levels as a first step towards the development of a complete gene map of the most diverse genotypes in the north coastal region of Egypt. Seed storage protein profile showed polymorphism among the studied genotypes, however it was not efficient to discriminate the five species of *Astragalus* under investigation, but it was helpful in characterization of accession within the same species. The RAPD-PCR as well as ISSR analyses provided more conclusive results regarding genetic relationships among different accessions and/or species, when compared to protein SDS-PAGE analysis.

Key Words:

Astragalus, Genetic resources, genetic relationships, SDS-PAGE, RAPD, ISSR.

CONTENTS

| | Page |
|--|------|
| INTRODUCTION | 1 |
| REVIEW OF LITERATURE | 4 |
| 1- Genetic diversity among wild plant species on the basis of seed storage protein | 4 |
| 2- Genetic diversity on the basis of molecular techniques. | 10 |
| A- Randomly amplified polymorphic DNAs (RAPDs) analysis | 10 |
| B- Inter simple sequence repeats (ISSRs) analysis. | 16 |
| MATERIALS AND METHODS | 20 |
| A- Materials | 20 |
| I- Plant materials | 20 |
| B- Methods | 21 |
| I- SDS- polyacrylamide gel electrophoresis (SDS-PAGE). | 21 |
| a- Reagents | 21 |
| b- Preparation of samples | 26 |
| c- Application of samples | 26 |
| d- Gel preparation | 27 |
| e- Electrophoresis | 27 |
| f- Detection of protein bands | 27 |
| II – Molecular methods | 28 |
| a- Extraction and purification of genomic DNA | 28 |
| b- Estimation of DNA quantity and quality | 28 |
| c- Randomly amplified polymorphic DNAs (RAPDs) | 28 |
| 1. RAPD-PCR reactions | 28 |
| 2. Thermocycling profile | 29 |
| d- Inter Simple Sequence Repeats (ISSRs) | 29 |
| 1. Thermocycling profile | 30 |

| | |
|--|----|
| 2. Gel electrophoresis and visualization of DNA bands | 30 |
| e- Photography of agarose gels | 31 |
| f- Band nomenclature | 32 |
| g- Data analysis | 32 |
| RESULTS AND DISCUSSION | 33 |
| A- Protein fingerprints of <i>Astragalus</i> species | 33 |
| B- Molecular fingerprints of <i>Astragalus</i> species based on RAPDs. | 40 |
| C- Molecular fingerprints of <i>Astragalus</i> species based on ISSRs. | 60 |
| SUMMARY | 81 |
| REFERENCES | 86 |
| ARABIC SUMMARY | |