حسام مغربى

شبكة المعلومات الجامعية

بسم اللّه الرحمن الرحيم

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شبكة المعلومات الجامعية

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

حسام مغربى

شبكة المعلومات الجامعية

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

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نقسم باللّه العظيم أن المادة التي تم توثيقها وتسجيلها علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

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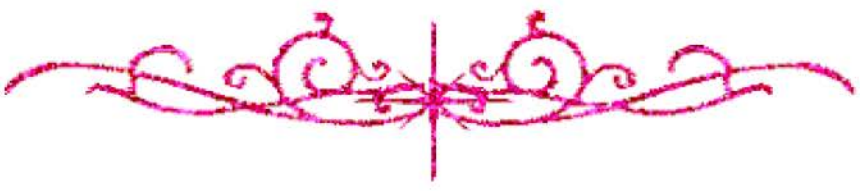

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شبكة المعلومات الجامعية

بالرسالة صفحات لم ترد بالأصل

Genetic Fingerprinting of seven *Lathyrus* species

By
HODA BADRY MOHAMED

B.Sc. Agric., Biochemistry, Cairo University
(1988)
M. Sc. Agric., Genetics, Cairo University
(1994)

THESIS

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2002

SUPERVISORS SHEET

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UNDER THE SUPERVISION OF

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ABSTRACT

The present study was carried out to find out the phylogenetic relationships of seven *Lathyrus* species (*L. aphaca,* section Aphaca; *L. clymenum,* and *L. ochrus,* sect. Clymenum; *L. annuus, L. cicera, L. sativus* and *L. tingitanus* sect. Lathyrus) by using different parameters: protein profiles, isozyme polymorphisms, amino acid contents, analysis of random amplified polymorphic DNA (RAPD), and Karyotype features.

A- <u>Protein profiles</u>: -

Water-soluble protein (WSP) in the seven *Lathyrus* species showed a total number of 29 bands. Based on UPGMA calculation, it was found that the relationships between the seven *Lathyrus* species were as follows: *L. ochrus, L. clymenum, L. sativus, L. aphaca, L. cicera, L. tingitanus* and *L. annuus*. On the water-non soluble protein level, a total number of 25 bands was found in the seven *Lathyrus* species. The relationships between the seven *Lathyrus* species were as follows: *L. ochrus, L. clymenum, L. aphaca, L. annuus L. cicera, L. tingitanus* and *L. sativus*. A total number of 32 bands for seed albumin protein were detected in the seven *Lathyrus* species. The relationships between the seven *Lathyrus* species were as follows, *L. ochrus, L. aphaca, L. clymenum, L. tingitanus, L. annuus, L. sativus* and *L. cicera*. A total number of bands was 23 in the seven *Lathyrus* species on the level of seed globulin proteins. The relationships between the seven *Lathyrus* species were as follows: *L. ochrus, L. clymenum, L. aphaca, L. annuus, L. cicera, L. sativus* and *L. tingitanus*

Analysis the data of all seed proteins together (water soluble, water non-soluble, seed albumins and seed globulins) reflected the following relationships:

L. ochrus, L. clymenum, L. aphaca, L. annuus, L. cicera, L. tingitanus and *L. sativus*.

B - Isozyme polymorphisms: -

Glutamine Oxalacetate Transferase (GOT), Esterase (EST), and Xanthine oxidase isozymes indicated the relationships among the seven *Lathyrus* species to be as follows: *L. ochrus*, *L. aphaca*, *L. annuus*, *L. sativus*, *L. clymenum*, *L. cicera* and *L. tingitanus*.

C - Amino acid concentrations:

The highest amino acid concentration was found in *L. tingitanus* and *L. annuus* (206.8 and 204.8 mg/g), whereas the lowest one was found in *L. sativus* (146.4 mg/g). *L. ochrus*, *L. clymenum*, *L. cicera*, and *L. aphaca*, recorded the amino acids concentration in descending order viz. 193.6, 182.4, 172.8, and 162.0 mg/g, respectively. The relationships between the tested *Lathyrus* species were as follows: *L. tingitanus*, *L. annuus*, *L. ochrus*, *clymenum*, *L. cicera*, *L. aphaca* and *L. sativus*.

D- Randomly Amplified Polymorphic DNA (RAPD) analysis: -

Eighteen primers out of twenty primers were used to screen the differences among the *Lathyrus* species, where amplified a high number of polymorphic DNA products (253 markers), all of them were used to construct the dendrogram using Jaccard's coefficients. The total number of amplified RAPDs products by each primer varied from a minimum number of eight amplified products by MPA-03 to a maximum of twenty amplified products by MP-10. In this case the relationships between the species could take the following regular order: *L. ochrus*, *L clymenum*, *L. aphaca*, *L. annuus*, *L. cicera*, *L. sativus* and *L. tingitanus*.

E- Karyotype features :-

The somatic chromosome number of the seven species is 2n = 14. The longest chromosome (no. 1) of *L. ochrus*, *L. aphaca*, *L. annuus* and *L. cicera* was characterized by the presence of the secondary constriction region on their long arm whereas *L. tingitanus* has the secondary constriction on the short arm. *L. sativus* has two constrictions, one on the long arm of chromosome no. 3 and the other on the short arm of chromosome no. 5, whereas *L. clymenum* has it on the long arm of chromosome no 4. Karyotype analysis based on the total length of the complement for the seven *Lathyrus* species revealed the relationships between the studied species as follows: (81.70, 59.88, 58.63, 57.56, 48.80, 43.43, and 40.38 for *L. tingitanus*, *L. sativus*, *L. annuus*, *L. cicera*, *L. ochrus*, *L. aphaca*, and *L. clymenum*, respectively).

Conclusion: RAPD analysis, karyotype features and protein profiles almost revealed the same relationships, whereas amino acid concentrations and isozyme polymorphisms gave different relationships. So that, it could be concluded that the phylogenetic sequence of the seven investigated species in ascending order of specialization is as follows: *L. clymenum*, *L. ochrus*, *L. aphaca*, *L. annuus*, *L. cicera*, *L. sativus*, and then *L. tingitanus*.

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CONTENTS

INTRODUCTION

INTRODUCTION

The analysis of genetic diversity and relatedness between or within different species, populations, and individuals is a central task for many disciplines of biological science. For some years the term 'fingerprinting' has been used for electrophoresis and chromatographic characterization of proteins and, more recently, of deoxyribonucleic acid (DNA) molecules. With DNA being the only basis of genetic differences between distinct organisms, DNA fingerprinting is presently the ultimate method of biological individualization. In principle, genetic uniqueness is brought about by two factors, inheritance and new mutation, and since all genetic differences between individuals are laid down in the primary sequence of their genomic DNA, the most straightforward method of identifying an individual would be to determine this sequence for genomes under comparison (**Krawczak and Schmidtke, 1994**).

Morphological markers, with their complex and undefined genetic control, were used for individual identification. Morphological markers which may be affected by environmental factors and growth practices. In contrast to morphological markers, proteins and DNA-based data are available from genetically based descriptions. Apart from identification, they can also be used in tests of parentage, in genetic mapping and in the measurement of genetic diversity (**Chapparo *et al.* 1994; Iqbal *et al.* 1997**). The environment, however, can interfere with the quality and quantity of proteins in seed (**Higgins 1984**). Proteins are growth-stage, tissue specific and in seeds they show also interaction with other compounds, which are found in roots and leaves, protein is also. In the late 1960's allozymes became available to examine levels of genetic variation and they have since been successfully used in numerous plant species studies. However, in some cases they have been shown not to be the perfect tool. Their inactivity when the tissue is not fresh, and the small number of loci that are polymorphic can readily be visualized.

The researchers have been curious about the precise relationship between the physical features of a chromosome and its genetic map. One way to show this relationship is by using cytogenetic map (**Harper and Cande, 2000**). In the last 20 years, new tools have become available to make dense cytogenetic mapping possible, particularly the advent of genetically mappable molecular markers such as restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNA (RAPD), and the technique for *in situ* hybridization (FISH).

With the development of DNA-based techniques, new tools have become available for studying the genetic structure of populations of most organisms. DNA fingerprinting technology had relied for years on restriction fragment length polymorphism (RFLP) analysis, which has been applied to several plant species to study the evolution, population genetic, phylogenetic relationships and genome mapping (**Shappley *et al.* 1996, Yu *et al.* 1997, Zhang *et al.* 2000, Duval *et al.* 2001**).

As DNA is much stable than are proteins or enzymes, therefore the polymerase chain reaction (PCR) was established, as, PCR has provided a new and powerful tool for DNA fingerprinting since its development (**Williams *et al.* 1990**). According to the earlier PCR-based approaches, fingerprints are generated through the selective amplification of hypervariable loci such as mini- or microsatellites (**Jeffreys *et al.* 1990**). More recently, a novel PCR-based strategy involving the use of arbitrary primers to amplify random genomic DNA fragments (RAPD) has been developed. Resently, random amplification methodology has become very popular due to its ability to easily and rapid generate polymorphic markers using a very small amount of starting DNA, independently of any prior knowledge of the target DNA sequence. This feature makes random amplification technology a tool potentially useful in many areas of genetic research such as gene mapping, individual and strain identification, population genetics and phylogenetics (**Thormann *et al.* 1994,**