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# Research Article
# Genetic Diversity of Five *Lathyrus* Species using RAPD, ISSR and SCoT Markers

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## Abstract

**Background and Objective:** *Lathyrus* (Leguminosae, Papilionoideae) is a member of Viciae tribe, which consists of about 150~160 annual and perennial species, it has an importance as foodstuffs worldwide. The main goal of this study was to determine the genetic diversity of 5 *Lathyrus* species (*L. articulates* L., *L. hierosolymitanus* Boiss, *L. latifolius* L. (France), *L. pseudocicera* pamp (Israel) and *L. tuberosus* L. (Soviet union)) by using different molecular markers as Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR) and Start Codon Targeted (SCoT). **Materials and Methods:** The DNA of the 5 *Lathyrus* species was used to amplify different fragments via Polymerase Chain Reaction (PCR) by using 6 RAPD, 8 ISSR and 8 SCoT primers. **Results:** The ISSR markers gave a maximum values of polymorphism (96.81%), Polymorphic Information Content (PIC) (0.305) and gene diversity (0.387) while the minimum values of polymorphism (94.2%), PIC (0.301) and gene diversity (0.379) documented in RAPD markers. The SCoT markers gave a high values of polymorphism (96%), PIC (0.302) and gene diversity (0.381) but slightly less than ISSR values. Also Unweighed Pair Group Arithmetic (UPGMA) results of collective data nearly similar to the UPGMA of ISSR marker. These results indicated that ISSR and SCoT markers considered a powerful marker for discrimination and identification of 5 studied *Lathyrus* species. **Conclusion:** The phylogenetic relationship among the studied species in descending order is *L. tuberosus* is more related to *L. hierosolymitanus* then comes *L. latifolius* and then *L. articulates*, whereas *L. pseudocicera* at a distant position from the other species but still related to *L. hierosolymitanus*.

Key words: *Lathyrus*, genetic diversity, polymorphism, molecular markers, similarity matrix

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

In higher plants, Fabaceae (Leguminosae) is the third largest family after Gramineae and Brassicaceae. This family contains more than 650 genera and 18,000 species. The tribe Vicieae includes three genera such as *Vicia* L., *Lathyrus* L. and *Pisum* L.[1].

The sweet, genus *Lathyrus* L. (Leguminosae, Papilionoideae) considers a member of Viciae tribe, which consists of about 150~160 annual and perennial species and grouped into 12-13 sections, this genus has an importance as foodstuffs worldwide[2]. Grass pea (*Lathyrus sativus* L.) is a food and forage legume cultivated in Central, South and Eastern Europe, West Asia, North Africa and Ethiopia. The crop is tolerant to extremely dry conditions in areas susceptible to drought and it is also tolerant to excessive floods[3].

Diversity was known as the variation between species of different organisms. There are 3 levels of diversity: diversity at genetical level (variation in genes and genotypes), diversity at species level (species richness) and ecosystem diversity (communities of species and their environment[4].

Molecular markers are considered as useful tools in genetic analysis of plants. There are several types of molecular markers, which differ in reproducibility, abundance in the genome, degree of polymorphism, locus specificity and technical demand[5]. Molecular methods such as RAPDs, ISSRs, SCoT, AFLPs or SSRs were considered as important tools for studying the genetic diversity. It is essential to understand that different markers have numerous properties and will reflect different aspects of genetic diversity[6]. Random Amplified Polymorphic DNA (RAPD) was widely used as DNA marker, this marker generated by the random amplification of DNA sequences using 10-mer primers[7]. Inter Simple Sequence Repeats (ISSR) marker is more reliable than the RAPD marker because this marker generates larger numbers of polymorphisms per primer. The ISSRs have been used in many crop species including legumes and have proved their usefulness in genetic mapping and marker assisted selection[8-12].

In the recent years, an alternative novel and capable marker techniques have been developed, such as Start Codon Targeted (SCoT) marker, which is considered a reproducible marker. The SCoT technique is depended on the short conserved region surrounding the ATG translation start (or initiation) codon in plant genes. SCoT markers were used to evaluate genetic diversity and structure, identify cultivars and for Quantitative Trait Loci (QTL) mapping and DNA fingerprinting in different plant species[13-15]. SCoTs have an advantage more than RAPDs and ISSRs as a constructing marker assisted breeding programs. This marker has some properties as fast and easy to be applied[16]. There are several studies used SCoT markers with many crop plant species such as cowpea[17] and soya beans[18].

The goal of this research was to determine the effectiveness of each molecular markers RAPD, ISSR and Scot to find out the phylogenetic relationships among different *Lathyrus* species.

## MATERIALS AND METHODS

All experiments of this research was carried out in the laboratories of Department of Genetics and Cytology, Genetic Engineering and Biotechnology Research Division, National Research Centre, Giza, Egypt from January-September, 2019.

**Plant materials:** Seeds of 5 *Lathyrus* species (*L. articulates* L., *L. hierosolymitanus* Boiss, *L. latifolius* L. (France), *L. pseudocicera* pamp (Israel) and *L. tuberosus* L. (Soviet union) were obtained from Leibniz Institute of Plant Genetics and Crop Plant Research (IPK, Gatersleben, Germany).

**DNA extraction:** Genomic DNA was extracted and purified from young leaves of different *Lathyrus* and *Pisum* species using Gene Jet Plant Genomic DNA Purification Mini Kit (Thermo-Scientific, K0791, made in Germany). The final concentration of DNA was adjusted to 50 ng $\mu L^{-1}$. All the DNA samples were stored at -20°C.

**RAPD-PCR amplification:** RAPD-PCR was performed by using 6 random primers (Table 1). PCR amplification for isolated DNA was performed in 0.2 mL PCR Eppendorf containing 25 µL consisted of 12.5 µL DreamTaq Green PCR Master Mix 2X (Thermo scientific K1081), 1 µL primer 10 pmol (Metabion, German) and 1 µL template DNA (50 ng $\mu L^{-1}$) then completed to 25 µL by water (nuclease-free). Thermocycler (Bio-Rad) was programmed as follows: 94°C for 5 min (one cycle) then 94°C for 1 min, 38°C for 45 sec and 72°C for 45 sec (35 cycles) then 72°C for 5 min (one cycle) then held at 4°C. Then 100 bp DNA ladder H3 RTU (GeneDirex, Cat No. DM003-R500) and 5 µL of DNA amplified PCR product were loaded in each well of 1% agarose stained with ethidium bromide, which placed in 1X TAE buffer and run at 100 V for about 2 h. The gel was photographed by gel documentation (Bio-Rad).

**ISSR-PCR amplification:** ISSR-PCR was performed by using 8 ISSR primers (Table 2). PCR amplification for isolated DNA (50 ng $\mu L^{-1}$) was performed in 0.2 mL PCR Eppendorf

Table 1: Sequence of RAPD primers

| Primers | Sequence (5'-3') | Annealing temperature (°C) |
|---|---|---|
| OPA1 | CAGGCCCTTC | 38 |
| OPA4 | AATCGGGCTG | 38 |
| OPA11 | CAATCGCCGT | 38 |
| OPB3 | CATCCCCCTG | 38 |
| OPH7 | CTGCATCGTG | 38 |
| OPH12 | ACGCGCATGT | 38 |

Table 2: Sequence of ISSR primers

| Primers | Sequence (5'-3') | Annealing temperature (°C) |
|---|---|---|
| ISSR2M | (CA) 8 AAGCT | 61 |
| 807 | (AG) 8 T | 55 |
| 812 | (GA) 8 A | 54 |
| 818 | (CA) 8 G | 55 |
| 842 | (GA) 8 CTG | 58 |
| 848 | (CA) 8AAGG | 61 |
| 857 | (AC) 8 CTG | 54 |
| 866 | (CTC) 6 | 61 |

Table 3: Sequence of SCoT primers

| Primers | Sequence (5'-3') | GC (%) |
|---|---|---|
| SCoT-1 | CAACAATGGCTACCACCA | 50 |
| SCoT-3 | CAACAATGGCTACCACCG | 56 |
| SCoT-6 | CAACAATGGCTACCACGC | 56 |
| SCoT-9 | CAACAATGGCTACCAGCA | 50 |
| SCoT-10 | CAACAATGGCTACCAGCC | 56 |
| SCoT-11 | AAGCAATGGCTACCACCA | 50 |
| SCoT-14 | ACGACATGGCGACCACGC | 67 |
| SCoT-15 | ACGACATGGCGACCGCGA | 67 |

containing (25 µL) consisted of 12.5 µL dream *Taq* green PCR master mix 2X (Thermo scientific K1081), 1 µL primer 10 pmol (Metabion, German) and 1 µL Template DNA (50 ng $\mu L^{-1}$) then completed to 25 µL by water (nuclease-free). Thermocycler (Bio-Rad) was programmed as follows: 93°C for 20 sec (one cycle) then 94°C for 20 sec, annealing temperature (Tm°C) for 1 min and 72°C for 20 sec (40 cycles) then 72°C for 6 min (one cycle) then held at 4°C. After that, 100 bp DNA Ladder H3 RTU (GeneDirex, Cat No. DM003-R500) and 5 µL of DNA amplified PCR product were loaded in each well of 1.5% agarose stained with ethidium bromide, which was placed in 1X TAE buffer in horizontal electrophoresis apparatus manufactured by Cleaver, UK, then it run at 100 V for about 2 h. The gel was photographed by gel documentation (Bio-Rad).

**SCoT-PCR amplification:** SCoT-PCR was performed by using eight SCoT primers (Table 3). PCR amplification for isolated DNA (50 ng $\mu L^{-1}$) was performed in 0.2 mL PCR Eppendorf containing (25 µL) consisted of 12.5 µL dream *Taq* green PCR master mix 2X (Thermo scientific K1081), primer 10 pmol $\mu L^{-1}$ (Metabion, German) and 1 µL template DNA (50 ng $\mu L^{-1}$) then completed to 25 µL by water (nuclease-free). Thermocycler (Bio-Rad) was programmed as follows: 94°C for 3 min (one cycle) then 94°C for 1 min, 50°C for 1 min and 72°C for 2 min (35 cycles) then 72°C for 5 min (one cycle) then held at 4°C. After that, 100 bp DNA ladder H3 RTU (GeneDirex, Cat No. DM003-R500) and 5 µL of DNA amplified PCR product were loaded in each well of 1.2% agarose stained with ethidium bromide, which was placed in 1X TAE buffer in horizontal electrophoresis apparatus manufactured by Cleaver, UK, then it run at 100 V for about 2 h. The gel was photographed by gel documentation (Bio-Rad).

**Statistical analysis:** Images of RAPD, ISSR and SCoT assays were analyzed by total lab program to find out the molecular weight of each band and that to compare the presence and absence of the band among species and this data was imported in MVSP (Multi Variant Statistical Package[19]) to find the similarity matrix and dendrogram (UPGMA, suing Jaccard's coefficient) which reflect the relationships among the studied species.

Gene diversity referred to as Polymorphic Information Content (PIC) values were calculated with the following formula[20]:

$$PICi = 1 - \sum_{f=1}^{n} (Pij)^2$$

where, n is the number of marker alleles for marker i and Pij is the frequency of the jth allele for marker i. Power marker version 3.25 was used to analyze the number of alleles per locus, the major allele frequency, gene diversity, Polymorphism Information Content (PIC) values[21].

## RESULTS

In this research, it has been obtained the characteristic banding patterns for *Lathyrus* species by RAPD, ISSR and SCoT fingerprints to study their phylogenetic and genetic diversity.

**Random Amplified Polymorphic DNA (RAPD) analysis:** The results of the amplification of RAPD primers were used in this investigation are mentioned in Table 4. The 6 primers produced a good reproducible patterns that reflected the polymorphisms between the 5 *Lathyrus* species (Fig. 1).

A total number of 69 bands were detected, among which 65 bands were polymorphic. The percentage of polymorphic bands ranged between 83.33% with OPA11 and 100% with OPA4, OPB3 and OPH7. The average percentage of polymorphism was 94.2%. Polymorphic Information

Table 4: Total number of amplicons and percentage of polymorphism as revealed by RAPD markers between the 5 *Lathyrus* species

| Primers | Total of amplicons | Polymorphic amplicons | Monomorphic amplicons | Polymorphism (%) | Unique band | | Gene diversity | PIC | Allele size range (bp) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Positive | Negative | | | |
| OPA1 | 9 | 8 | 1 | 88.90 | 1 | 0 | 0.372 | 0.292 | 190-1000 |
| OPA4 | 9 | 9 | 0 | 100.00 | 4 | 2 | 0.373 | 0.300 | 280-1310 |
| OPA11 | 12 | 10 | 2 | 83.33 | 3 | 0 | 0.346 | 0.272 | 140-720 |
| OPB3 | 13 | 13 | 0 | 100.00 | 3 | 0 | 0.423 | 0.342 | 150-730 |
| OPH7 | 13 | 13 | 0 | 100.00 | 5 | 3 | 0.381 | 0.305 | 150-3100 |
| OPH12 | 13 | 12 | 1 | 92.30 | 5 | 0 | 0.380 | 0.299 | 185-2730 |
| Total | 69 | 65 | 4 | - | 21 | 5 | - | - | - |
| Average | 11.5 | 10.83 | 0.66 | 94.20 | - | - | 0.379 | 0.301 | - |

Table 5: Number of positive and negative unique RAPD markers recorded in the 5 *Lathyrus* species

| Species | Positive unique bands | | | Negative unique bands | | |
|---|---|---|---|---|---|---|
| | Primer | Size of band (bp) | Total of marker | Primer | Size of band (bp) | Total of marker |
| *L. articulates* L. | OPA11 | 390 | 1 | OPH7 | 500 | 1 |
| *L. hierosolymitanus* Boiss | OPA1 | 760 | 8 | OPH7 | 270 | 1 |
| | OPA11 | 540, 720 | | | | |
| | OPB3 | 730 | | | | |
| | OPH7 | 200, 700, 850, 1290 | | | | |
| *L. latifolius* L. (France) | OPA4 | 600 | 4 | OPH7 | 360 | 1 |
| | OPH7 | 3100 | | | | |
| | OPH12 | 1400, 2730 | | | | |
| *L. pseudocicera* pamp (Israel) | OPA4 | 1055 | 4 | OPA4 | 280 | 1 |
| | OPH12 | 500, 730, 850 | | | | |
| *L. tuberosus* L. (Soviet union) | OPA4 | 400, 885 | 4 | OPA4 | 330 | 1 |
| | OPB3 | 550, 580 | | | | |
| Total | - | - | 21 | - | - | 5 |

Table 6: Similarity matrix among studied *Lathyrus* species as computed according to Jaccard's coefficient as revealed by RAPD marker

| Species | *L. articulates* L. | *L. hierosolymitanus* | *L. latifolius* L. | *L. pseudocicera* | *L. tuberosus* L. |
|---|---|---|---|---|---|
| *L. articulates* L. | 1 | | | | |
| *L. hierosolymitanus* | 0.327 | 1 | | | |
| *L. latifolius* L. | 0.359 | 0.245 | 1 | | |
| *L. pseudocicera* | 0.341 | 0.309 | 0.333 | 1 | |
| *L. tuberosus* L. | 0.240 | 0.339 | 0.313 | 0.408 | 1 |

Content (PIC) and gene diversity ranged from 0.272-0.346 in OPA11-0.342 and 0.423 in OPB3 with an average of 0.301 and 0.379, respectively (Table 4).

Each studied species revealed a positive and a negative unique bands, all species had 21 positive unique and 5 negative unique bands. A maximum number of positive unique bands were 8 bands found in *L. hierosolymitanus* Boiss, while a minimum number was 1 band found in *L. articulates* L. but each other species had 4 positive unique bands (Table 5).

The highest similarity value 0.408 was reported among *L. pseudocicera* and *L. tuberosus* L., these indicating that, these 2 species are closely related. While, the lowest values recorded were 0.24 and 0.245 between *L. articulates* L. and *L. tuberosus* L. and between *L. hierosolymitanus* and *L. latifolius* L., respectively this indicating that these were distant species (Table 6). The dendrogram gave 2 main clusters, the first cluster contained *L. articulates* L. and *L. latifolius* L., while the second cluster contained the other three species, which was further split into two sub-clusters, the first sub-cluster contained *L. pseudocicera* and *L. tuberosus* L. while the second sub-cluster contained *L. hierosolymitanus* only (Fig. 2).

**Inter Simple Sequence Repeats (ISSR) analysis:** The results of ISSR amplification were reveled in Table 7. The eight primers gave a reproducible patterns, that were screened the polymorphisms among studied *Lathyrus* species (Fig. 3).

The eight primers produced 94 bands, 91 bands out of them were polymorphic. The percentage of polymorphic bands ranged from 75% with 848 and 100% with ISSR 2 M, 812, 818, 857 and 866. The average percentage of polymorphism was 96.81%. The maximum Polymorphic Information Content (PIC) and gene diversity were 0.346 and

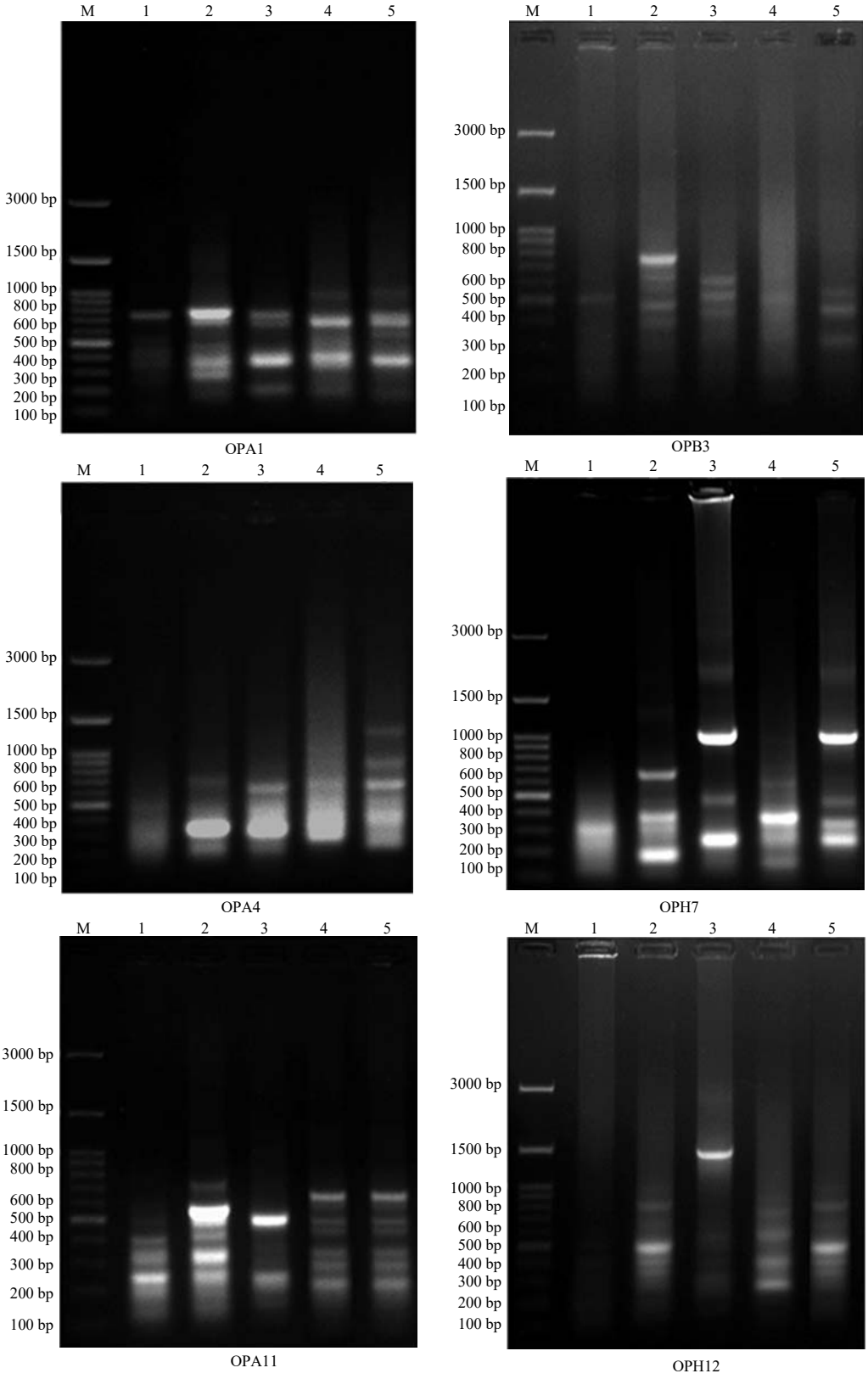


Fig. 1: Electrophoretic banding pattern of RAPD amplification for different *Lathyrus* species

M: DNA ladder 100 bp 1: *L. articulates* L., 2: *L. hierosolymitanus* Boiss, 3: *L. latifolius* L. (France), 4: *L. pseudocicera* pamp (Israel), 5: *L. tuberosus* L. (Soviet union)

Table 7: Total number of amplicons and percentage of polymorphism as revealed by ISSR markers between the 5 *Lathyrus* species

| Primers | Total of amplicons | Polymorphic amplicons | Monomorphic amplicons | Polymorphism (%) | Unique band | | Gene diversity | PIC | Allele size range (bp) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Positive | Negative | | | |
| ISSR2M | 9 | 9 | 0 | 100.00 | 3 | 3 | 0.373 | 0.301 | 170-700 |
| 807 | 15 | 14 | 1 | 93.33 | 4 | 4 | 0.363 | 0.289 | 170-1220 |
| 812 | 18 | 18 | 0 | 100.00 | 10 | 1 | 0.382 | 0.306 | 150-1200 |
| 818 | 12 | 12 | 0 | 100.00 | 6 | 0 | 0.400 | 0.317 | 280-1100 |
| 842 | 16 | 15 | 1 | 93.75 | 6 | 0 | 0.390 | 0.306 | 210-1650 |
| 848 | 4 | 3 | 1 | 75.00 | 0 | 1 | 0.320 | 0.249 | 240-480 |
| 857 | 10 | 10 | 0 | 100.00 | 1 | 3 | 0.416 | 0.326 | 230-1150 |
| 866 | 10 | 10 | 0 | 100.00 | 2 | 0 | 0.448 | 0.346 | 190-1050 |
| Total | 94 | 91 | 3 | - | | 32 | 12 | - | -- |
| Average | 11.75 | 11.375 | 0.375 | 96.81% | - | - | 0.387 | 0.305 | - |

Table 8: Number of positive and negative unique ISSR markers recorded in the five *Lathyrus* species

| Species | Positive unique bands | | | Negative unique bands | | |
|---|---|---|---|---|---|---|
| | Primer | Size of band (bp) | Total of marker | Primer | Size of band (bp) | Total of marker |
| *L. articulates* L. | ISSR2M | 440 | 4 | ISSR2M | 470 | 3 |
| | 812 | 150 | | 807 | 370 | |
| | 842 | 210 | | 848 | 480 | |
| | 866 | 190 | | | | |
| *L. hierosolymitanus* Boiss | 807 | 170 | 5 | 857 | 560 | 1 |
| | 812 | 440, 1200 | | | | |
| | 842 | 750 | | | | |
| | 866 | 1050 | | | | |
| *L. latifolius* L. (France) | ISSR2M | 410 | 9 | 807 | 440 | 3 |
| | 807 | 410 | | 812 | 230 | |
| | 812 | 650 | | 857 | 230 | |
| | 818 | 320, 520, 650, 780, 1100 | | | | |
| | 842 | 800 | | | | |
| *L. pseudocicera* pamp (Israel) | ISSR2M | 665 | 6 | ISSR2M | 170 | 2 |
| | 812 | 180, 290, 780 | | 807 | 800 | |
| | 842 | 1000, 1650 | | | | |
| *L. tuberosus* L. (Soviet union) | 807 | 640, 1220 | 8 | ISSR2M | 190 | 3 |
| | 812 | 470, 530, 920 | | 807 | 580 | |
| | 818 | 1000 | | 857 | 285 | |
| | 842 | 950 | | | | |
| | 857 | 1150 | | | | |
| Total | | | 32 | | | 12 |

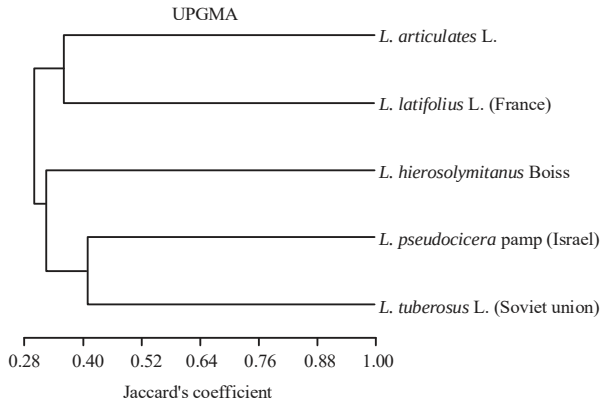


Fig. 2: Dendrogram for the 5 *Lathyrus* species constructed from RAPD polymorphism data using Unweighed Pair Group Arithmetic (UPGMA) and similarity matrices computed according to Jaccard's coefficient

0.448, respectively with 866 while the minimum PIC and gene diversity were 0.249 and 0.32, respectively with 848. The average of PIC and gene diversity were 0.305 and 0.387, respectively (Table 7).

All studied *Lathyrus* species had a positive and a negative unique bands, all species had 32 positive unique and 12 negative unique bands. A maximum number of positive unique bands were 9 bands found in *L. latifolius* L. (France), while a minimum number was 4 band found in *L. articulates* L., whereas the maximum number of negative unique bands were 3 bands found in *L. articulates* L., *L. latifolius* L. and *L. tuberosus* L., while a minimum number was 1 band found in *L. hierosolymitanus* (Table 8).

The highest similarity value 0.358 was reported between *L. hierosolymitanus* and *L. latifolius* L. which indicates that these 2 species are closely related. While, the lowest value

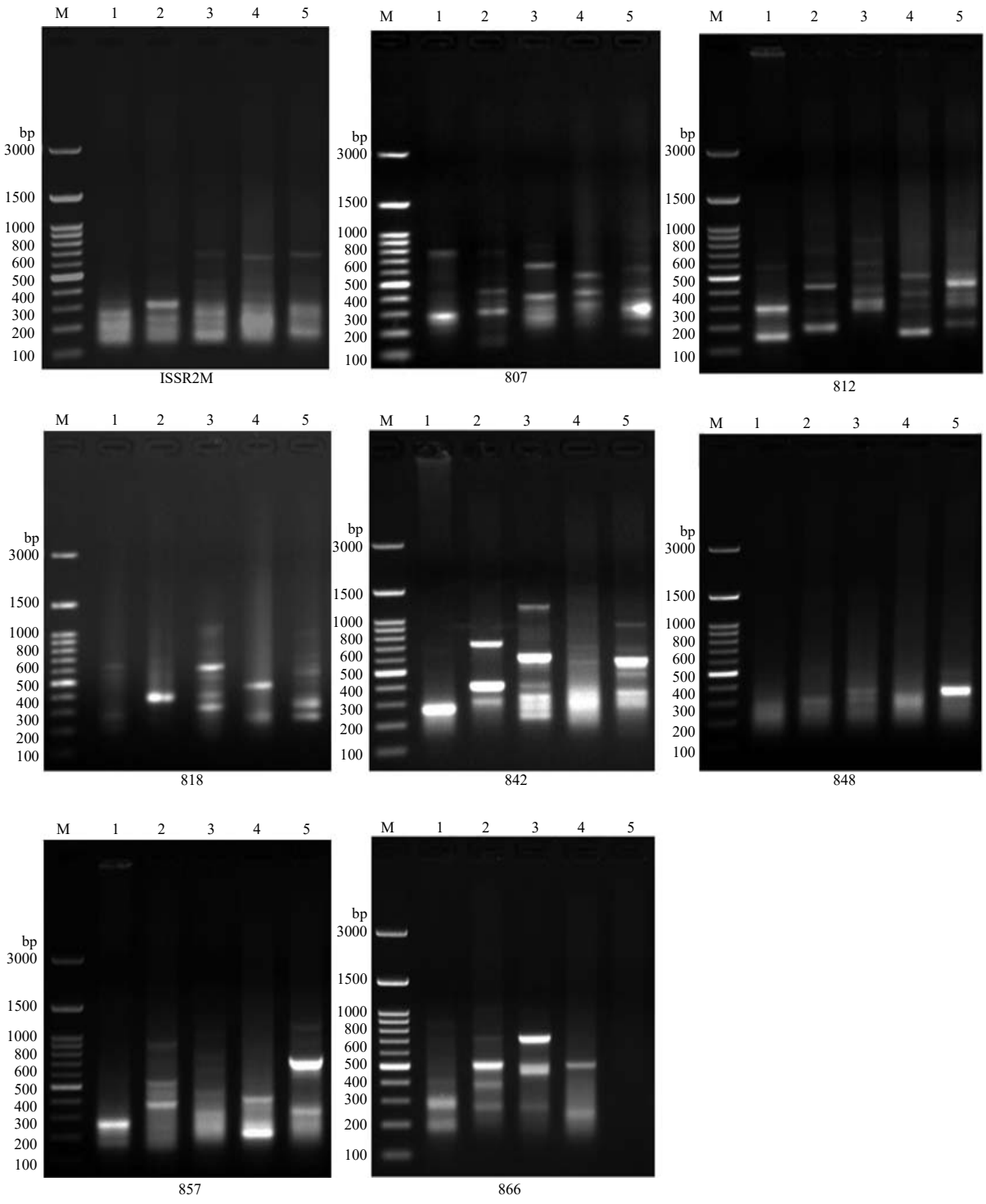


Fig. 3: Electrophoretic banding pattern of ISSR amplification for the 5 *Lathyrus* species

M: DNA ladder 100 bp 1: *L. articulates* L. 2: *L. hierosolymitanus* Boiss, 3: *L. latifolius* L. (France) 4: *L. pseudocicera* pamp (Israel) 5: *L. tuberosus* L. (Soviet union)

recorded was 0.254 between *L. articulates* L. and *L. latifolius* L. and 0.258 between *L. latifolius* L. and *L. pseudocicera*, this indicates that these species were distant species (Table 9). The dendrogram gave two main clusters, the first cluster includes only *L. articulates* L., while the second cluster includes all other species. The second cluster was split into 2 sub-clusters, the first sub-cluster contained *L. pseudocicera* only, while the second sub-cluster contained the other

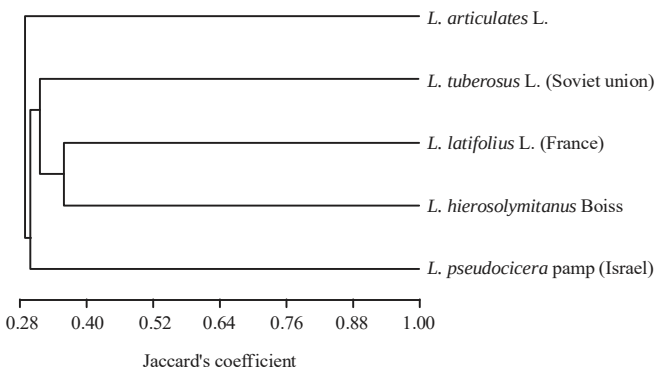


Fig. 4: Dendrogram for the five *Lathyrus* species constructed from ISSR polymorphism data using Unweighed Pair Group Arithmetic (UPGMA) and similarity matrices computed according to Jaccard's coefficient

Table 9: Similarity matrix among studied *Lathyrus* species as computed according to Jaccard's Coefficient as revealed by ISSR marker

| Species | *L. articulates* L. | *L. hierosolymitanus* | *L. latifolius* L. | *L. pseudocicera* | *L. tuberosus* L. |
|---|---|---|---|---|---|
| *L. articulates* L. | 1 | | | | |
| *L. hierosolymitanus* | 0.339 | 1 | | | |
| *L. latifolius* L. | 0.254 | 0.358 | 1 | | |
| *L. pseudocicera* | 0.293 | 0.344 | 0.258 | 1 | |
| *L. tuberosus* L. | 0.265 | 0.310 | 0.324 | 0.288 | 1 |

Table 10: Total number of amplicons and percentage of polymorphism as revealed by SCoT markers between the 5 *Lathyrus* species

| Primers | Total number of amplicons | Polymorphic amplicons | Monomorphic amplicons | Polymorphism (%) | Unique band | | Gene diversity | PIC | Allele size range (bp) |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Positive | Negative | | | |
| SCoT-1 | 13 | 13 | 0 | 100.00 | 4 | 2 | 0.406 | 0.321 | 500-2950 |
| SCoT-3 | 13 | 13 | 0 | 100.00 | 4 | 4 | 0.382 | 0.306 | 200-1330 |
| SCoT-6 | 17 | 15 | 2 | 88.23 | 5 | 1 | 0.367 | 0.288 | 250-2000 |
| SCoT-9 | 17 | 17 | 0 | 100.00 | 8 | 2 | 0.386 | 0.308 | 310-2075 |
| SCoT-10 | 14 | 12 | 2 | 85.71 | 5 | 0 | 0.354 | 0.278 | 300-1570 |
| SCoT-11 | 18 | 18 | 0 | 100.00 | 9 | 2 | 0.382 | 0.306 | 210-1650 |
| SCoT-14 | 19 | 19 | 0 | 100.00 | 7 | 1 | 0.413 | 0.324 | 120-935 |
| SCoT-15 | 14 | 13 | 1 | 92.86 | 8 | 0 | 0.354 | 0.284 | 200-985 |
| Total | 125 | 120 | 5 | - | 50 | 12 | - | - | - |
| Average | 15.625 | 15 | 0.625 | 96.00 | - | - | 0.381 | 0.302 | - |

3 species. The second sub-cluster split into 2 groups, the 1st group contained *L. tuberosus* L. only while the second group contained *L. hierosolymitanus* and *L. latifolius* L. species (Fig. 4).

**Start Codon Targeted polymorphism (SCoT) analysis:** The results of SCot amplification were reveled in Table 10. The eight primers gave a reproducible patterns that showed the polymorphisms among studied *Lathyrus* species (Fig. 5).

The number of obtained bands were 125 bands, among which 120 bands were polymorphic bands. The percentage of polymorphic bands ranged from 85.71% with SCoT-10 and 100% with SCoT-1, SCoT-3, SCoT-9, SCoT-11 and SCoT-14. The average percentage of polymorphism was 96%. The maximum Polymorphic Information Content (PIC) and gene diversity were 0.324 and 0.413, respectively with SCoT-14, while the minimum PIC were 0.278 with SCoT-10 and the minimum gene diversity were 0.354 with SCoT-10 and SCoT-15. The average of PIC and gene diversity were 0.302 and 0.381, respectively (Table 10).

The studied species had a positive and a negative unique bands, all species had 50 positive unique and 12 negative unique bands. A maximum number of positive unique bands were 15 bands found in *L. tuberosus* L., while a minimum number was 6 band found in *L. articulates* L. and *L. latifolius* L., while a maximum number of negative unique bands were 4 bands found in *L. pseudocicera*, whereas a minimum number was one band found in *L. tuberosus* L. (Table 11).

The highest similarity value 0.363 was reported between *L. hierosolymitanus* and *L. latifolius* L. which indicated that these 2 species were closely related. While, the lowest value recorded was 0.198 between *L. pseudocicera* and *L. tuberosus* L. which showed that these 2 species were distant species (Table 12). The dendrogram gave 2 main

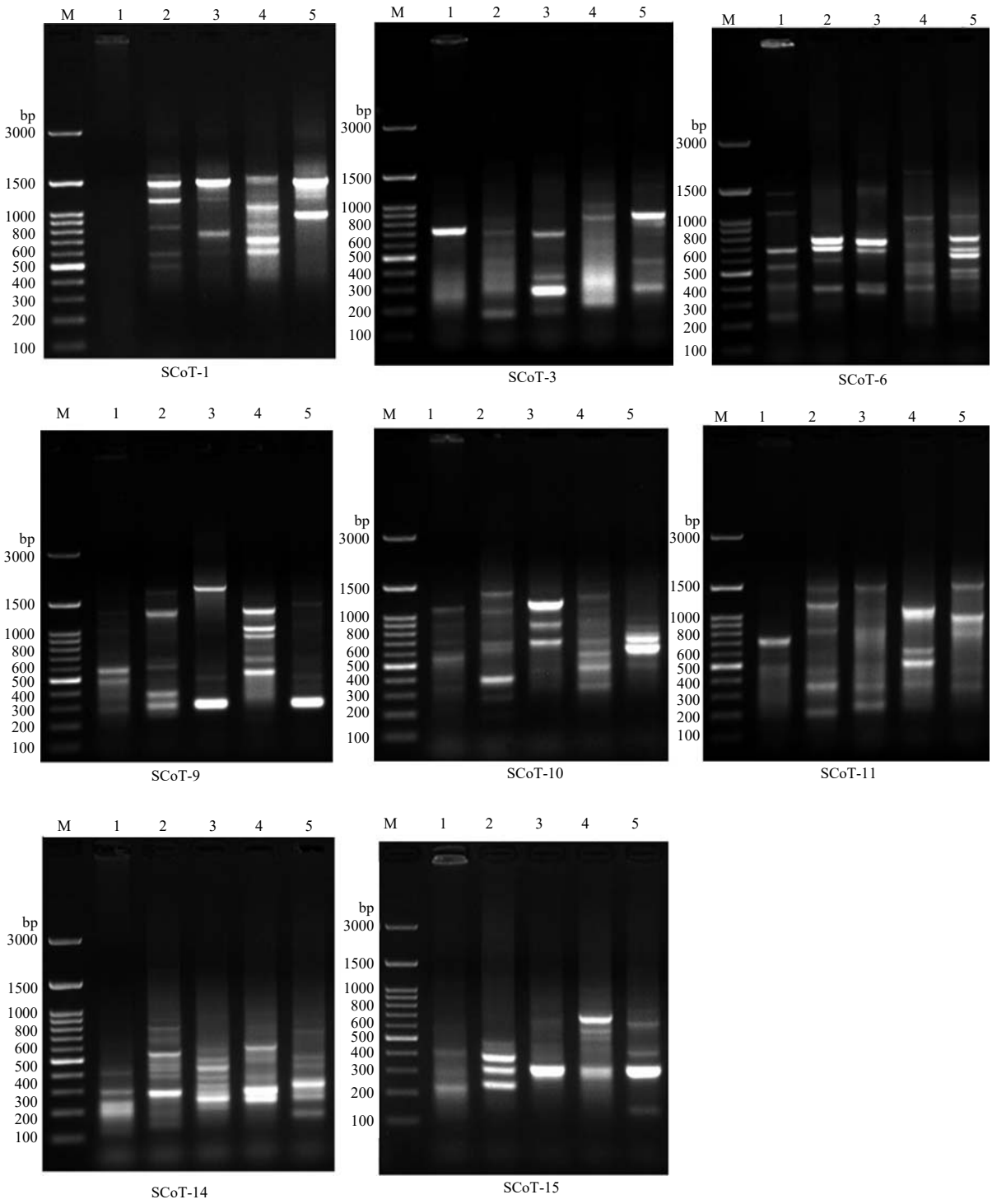


Fig. 5: Electrophoretic banding pattern of SCoT amplification for the 5 *Lathyrus* species

M: DNA ladder 100 bp 1: *L. articulates* L., 2: *L. hierosolymitanus* Boiss, 3: *L. latifolius* L. (France) 4: *L. pseudocicera* pamp (Israel) 5: *L. tuberosus* L. (Soviet union)

clusters, the first cluster includes *L. pseudocicera* only, while the second cluster includes all other species. The second cluster was split into 2 sub-clusters, the first sub-cluster contained *L. articulates* L., while the other sub-cluster contained the other three species, which was split into 2 groups, the 1st group contained *L. tuberosus* L. whereas the 2nd group contained *L. hierosolymitanus* and *L. latifolius* L. (Fig. 6).

Table 11: Number of positive and negative unique SCoT markers recorded in the 5 *Lathyrus* species

| | Positive unique bands | | | Negative unique bands | | |
|---|---|---|---|---|---|---|
| Species | Primers | Size of band (bp) | Total of marker | Primers | Size of band (bp) | Total of marker |
| *L. articulates* L. | SCoT-6 | 1150 | 6 | SCoT-1 | 1230, 1500 | 4 |
| | SCoT-9 | 1320 | | SCoT-3 | 460 | |
| | SCoT-11 | 300, 730 | | SCoT-11 | 800 | |
| | SCoT-14 | 120 | | | | |
| | SCoT-15 | 200 | | | | |
| *L. hierosolymitanus* Boiss | SCoT-9 | 1800 | 9 | | | |
| | SCoT-10 | 300, 400 | | | | |
| | SCoT-11 | 210, 1650 | | | | |
| | SCoT-14 | 170, 560, 720 | | | | |
| | SCoT-15 | 460 | | | | |
| *L. latifolius* L. (France) | SCoT-6 | 370 | 6 | | | |
| | SCoT-9 | 910, 1920 | | | | |
| | SCoT-10 | 1210 | | | | |
| | SCoT-14 | 340 | | | | |
| | SCoT-15 | 430 | | | | |
| *L. pseudocicera* pamp (Israel) | SCoT-1 | 1100 | 14 | SCoT-3 | 320, 410, 710 | 7 |
| | SCoT-3 | 640, 870, 1050 | | SCoT-6 | 1550 | |
| | SCoT-6 | 2000 | | SCoT-9 | 310, 500 | |
| | SCoT-9 | 800 | | SCoT-11 | 480 | |
| | SCoT-10 | 600 | | | | |
| | SCoT-11 | 400, 520, 640 | | | | |
| | SCoT-14 | 935 | | | | |
| | SCoT-15 | 560, 790, 985 | | | | |
| *L. tuberosus* L. (Soviet union) | SCoT-1 | 1300, 1870, 2950 | 15 | SCoT-14 | 400 | 1 |
| | SCoT-3 | 1330 | | | | |
| | SCoT-6 | 520, 650 | | | | |
| | SCoT-9 | 440, 1450, 2075 | | | | |
| | SCoT-10 | 1000 | | | | |
| | SCoT-11 | 1320, 1550 | | | | |
| | SCoT-14 | 360 | | | | |
| | SCoT-15 | 600, 735 | | | | |
| Total | | | 50 | | | 12 |

Table 12: Similarity matrix among studied *Lathyrus* species as computed according to Jaccard's coefficient as revealed by SCoT marker

| Species | *L. articulates* L. | *L. hierosolymitanus* | *L. latifolius* L. | *L. pseudocicera* | *L. tuberosus* L. |
|---|---|---|---|---|---|
| *L. articulates* L. | 1 | | | | |
| *L. hierosolymitanus* | 0.282 | 1 | | | |
| *L. latifolius* L. | 0.309 | 0.363 | 1 | | |
| *L. pseudocicera* | 0.215 | 0.275 | 0.265 | 1 | |
| *L. tuberosus* L. | 0.222 | 0.352 | 0.286 | 0.198 | 1 |

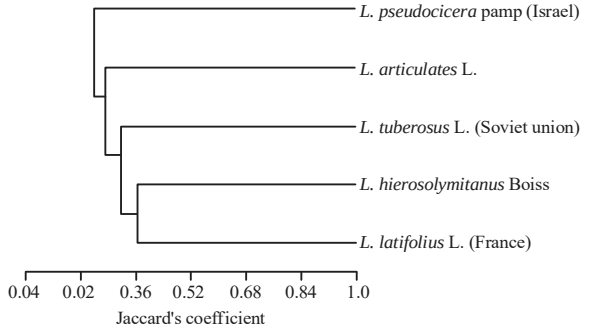


Fig. 6: Dendrogram for the 5 *Lathyrus* species constructed from ScoT polymorphism data using Unweighed Pair Group Arithmetic (UPGMA) and similarity matrices computed according to Jaccard's coefficient

**Genetic similarity analysis:** It was observed from the comparison of data of three markers RAPD, ISSR and SCoT that the maximum polymorphism, PIC and gene diversity were detected with ISSR marker, while the minimum polymorphism, PIC and gene diversity were detected with RAPD marker (Table 13).

The highest similarity value 0.335 was reported among *L. hierosolymitanus* and *L. tuberosus* L. species these indicating that, these 2 species with high similarity were closely related. While, the lowest value recorded was 0.241 among *L. articulates* L. and *L. tuberosus* L. species, this indicating that these 2 species were distant species (Table 14). The dendrogram gave 2 main clusters, the first cluster includes *L. articulates* L. only, while the second cluster

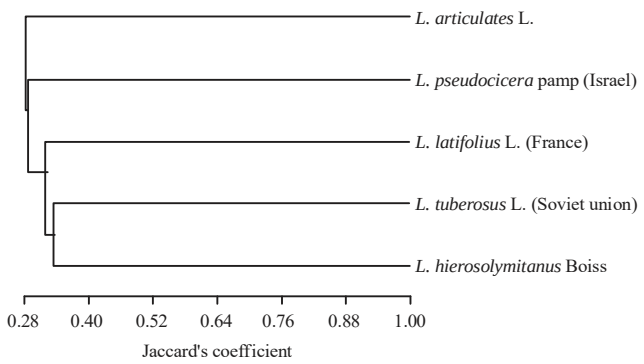


Fig. 7: Dendrogram for the 5 *Lathyrus* species constructed from the collective data of RAPD, ISSR and ScoT polymorphism using Unweighed Pair Group Arithmetic (UPGMA) and similarity matrices computed according to Jaccard's coefficient

Table 13: Comparison of genetic parameters between RAPD, ISSR and SSR analysis

| | Resultant values | | |
|---|---|---|---|
| Molecular parameters | RAPD | ISSR | ScoT |
| Total number of amplicon | 69.00 | 94.00 | 125.00 |
| Number of polymorphic amplicon | 65.00 | 91.00 | 120.00 |
| Positive unique bands | 21.00 | 32.00 | 50.00 |
| Percentage of polymorphism per assay | 94.20 | 96.81 | 96.00 |
| PIC | 0.301 | 0.305 | 0.302 |
| Gene diversity | 0.379 | 0.387 | 0.381 |

Table 14: Similarity matrix among studied *Lathyrus* species as computed according to Jaccard's Coefficient as revealed by RAPD, ISSR and SCoT markers

| Species | *L. articulates* L. | *L. hierosolymitanus* | *L. latifolius* L. | *L. pseudocicera* | *L. tuberosus* L. |
|---|---|---|---|---|---|
| *L. articulates* L. | 1 | | | | |
| *L. hierosolymitanus* | 0.312 | 1 | | | |
| *L. latifolius* L. | 0.299 | 0.330 | 1 | | |
| *L. pseudocicera* | 0.271 | 0.304 | 0.278 | 1 | |
| *L. tuberosus* L. | 0.241 | 0.335 | 0.305 | 0.275 | 1 |

includes all other species. The second cluster was split into 2 sub-clusters, the first sub-cluster contained *L. pseudocicera* only, while the second sub-cluster contained the other three species. The second sub-cluster split into two groups, the first group contained *L. latifolius* L. Only while the 2nd group contained *L. hierosolymitanus* and *L. tuberosus* L. species (Fig. 7). Also, it was observed that UPGMA results of collective data similar to the UPGMA of ISSR marker, this indicate that ISSR marker is a powerful marker for discrimination and identification of different species.

## DISCUSSION

The phylogenetic relationship was detected by using different types of molecular markers at genomic DNA level[22]. These methods detected the polymorphism by assaying subsets of total amount of DNA sequence variation in a genome[23].

The different molecular markers such as RAPD, ISSR and SCoT markers were used to analyze a numerous segments of the genome, then revealed different genetic information. Positive and negative unique bands for each marker could be potential for species-specific markers[24]. RAPDs proved to be a high resolution technique for the detection of genetic variation among and within populations of *Lathyrus sativus*[25]. RAPD and ISSR targeted different regions of genome, these differences may also be attributed to marker sampling errors and/or the level of polymorphism detected reinforcing the importance of number of loci and their coverage of the whole genome for obtaining reliable estimates of genetic relationships among species. It has been reported that the ability to resolve genetic variation may be more directly related to the degree of polymorphism detected by the marker system[26]. Start Codon Targeted (SCoT) polymorphism was considered as a novel molecular marker, it based on a conserved sequence such as ATG that surrounding the start codon of translation[27].

From the obtained result in current study, ISSR markers gave a maximum values of polymorphism, PIC and gene diversity while the minimum values of polymorphism, PIC and

gene diversity documented in RAPD markers. This agreement with Ashraf *et al*.[28], who mentioned that ISSR markers are more discriminating than RAPD to evaluate the genetic diversity and relationship among species. The highest similarity value 0.363 was reported between *L. hierosolymitanus* and *L. latifolius* L. these indicating that these 2 species were closely related to each other, while, the lowest value recorded was 0.198 among *L. pseudocicera* and *L. tuberosus* L. species this indicating that these 2 species were distant species by using SCot marker (Table 12). It was noticed from collective data that UPGMA results of collective data approximately similar to the UPGMA of ISSR marker, which indicated that ISSR marker is an efficient marker to differentiate and identify the different studied species (Fig. 6, 7). Huangfu *et al*.[29] documented that the high products of ISSR bands comparing with RAPD could be due to the higher annealing temperature and longer used primers. Belaid *et al*.[30] have used ISSR marker for detecting the genetic variation and the relationships among different populations from many of geographical origins, representing *L. sativus*, *L. cicera* and *L. ochrus* of the genus *Lathyrus*. The data provided evidence of a large genetic diversity among and within the studied populations. Datta *et al*.[31] used 10 RAPD primers to find out the genetic diversity among 20 grass pea genotypes, they depended on different parameters such as Resolving Power (RP), Marker Index (MI), Polymorphism Information Content (PIC) and Diversity Index (DI).

Also, in the current study it has been observed that SCoT markers gave a high values of polymorphism (96%), PIC (0.302) and gene diversity (0.381) but slightly less than ISSR values this indicated that SCoT markers also a powerful marker for discrimination and identification of different species. This agreement with Etminan *et al*.[32], who studied the genetic diversity and relationship among durum wheat genotypes and they found that SCoT marker results such as polymorphic fragment percentage and number of polymorphic bands were similar to ISSR marker result, this indicated that both ISSR and SCoT markers were considered an efficient techniques to estimate the genetic variation. There are several studies on the usage of SCoT marker to evaluate the genetic relationship with Egyptian Glycine max cultivars, *Elymus sibiricus* and *Vign aunguiculata*[17-18,33]. The phylogenetic relationships of cultivated peanut genotypes and Egyptian soyabean cultivars was determined by Xiong *et al*.[27] using SCoT marker, they found that not all genotypes related to the same variety were classified in the same group. Aboulila and Mansour[34] investigated the genetic relationships of some barley accessions using SCoT marker and they documented that the efficient technique for detecting a new fingerprint of *Hordeium vulgare* was SCoT technique. Also SCoT marker was used for discrimination and identification of different wheat cultivars that collected from different location from North Africa, these cultivars were classified into many groups according to the resulting dendrogram[35].

Phylogenetic relationships among the 5 *Lathyrus* species under this study was figured out by using three molecular markers, RAPD, ISSR and Scot. By evaluating the RAPD observation, it was found that the most related species were *L. tuberosus* and *L. pseudocicera* (40.8%), this relationship has been not verified by ISSR and SCot (*L. pseudocicera* was more related to *L. hierosolymitanus* by 34.4 and 27.5% by using ISSR and Scot markers, respectively). But the RAPD, ISSR and SCot results reflected the closely relationships among *L. tuberosus*, *L. hierosolymitanus*, *L. latifolius* and verified that the most related species to *L. latifolius* is *L. articulates*, the percentage of similarity was 35.9% by RAPD and 30.9% by SCot.

By combining the similarity matrix among studied *Lathyrus* species as computed according to Jaccard index. Coefficient from these three markers (Table 14), it could be concluded that *L. hierosolymitanus* is similar to *L. tuberosus*, *L. latifolius* and *L. articulates* by a percentage of 33.5, 33.0 and 31.2%, respectively and the most related species to *L. latifolius* is *L. articulates* (29.9%), whereas the most related species to *L. pseudocicera* was *L. hierosolymitanus* (30.4%). According to these observations, the phylogenetic relationship among the studied species in dissenting order is *L. tuberosus* is more related to *L. hierosolymitanus* then comes *L. latifolius* and then *L. articulates*, whereas *L. pseudocicera* at a distant position from the other species but still related to *L. hierosolymitanus*.

## CONCLUSION

The phylogenetic relationships among the 5 *Lathyrus* species was studied using three molecular markers, RAPD, ISSR and SCot. It was found that, ISSR and SCoT markers considered a powerful marker for discrimination and identification of 5 studied *Lathyrus* species. In addition, it was found that, the phylogenetic relationship among the studied species in dissenting order is *L. tuberosus* is more related to *L. hierosolymitanus* then comes *L. latifolius* and then *L. articulates*, whereas *L. pseudocicera* at a distant position from the other species but still related to *L. hierosolymitanus.*

## SIGNIFICANCE STATEMENT

This study discovers the importance of the molecular markers like, RAPD, ISSR and SCoT in plant, that can be beneficial to clarify the genetic relationships among 5 *Lathyrus* species. This study will help the researcher to uncover the critical areas of genetic diversity of the different *Lathyrus* species that many researchers were unable to explore. Thus a new theory in *Lathyrus* taxonomy and marker-assisted programs may be arrived at.

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