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# Genetic characterization of genus *Tephrosia* Pers. based on molecular markers in KSA

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**Abstract**
Illustration the genetic diversity among ten accessions of *Tephrosia* collected from KSA using three molecular markers (RAPD, ISSR & SCoT). Molecular markers produced 99 total bands with 20 monomorphic bands and 79 polymorphic bands. Molecular data generated from RAPD –PCR showed highest polymorphism (92.31%) using ten primers with 10 unique bands. SCoT-PCR marker showed the lowest polymorphism (56.52%) with one unique band. RAPD markers may be more efficient in studying genetic diversity and relationship of *Tephrosia* than ISSR and SCoT markers with regards to polymorphism detection. This result could be explained as RAPD marker covers the whole genome for amplification. Cluster dendrogram and Biplot give the possibility to separate the studied taxa into two groups; first group include only of *Tephrosia purpurea* ssp. *leptostachya* and the second group contain other nine accessions. These results are important to improve the accuracy of resolution of genetic relationships for sustainable conservation as medicinal plant by such as the construction of gene banks of the genus *Tephrosia*.

**Keywords:** *Tephrosia*, genetic diversity, molecular marker, RAPD, ISSR, SCoT, KSA

## Introduction

Kingdom of Saudi Arabia is considered as one of the most area rich in plant diversity comprises a lot of medicinal wild plants and important crops, in addition to presence of a large number of endemic plants (Rahman *et al*., 2004) [21]. Family Leguminosae (Fabaceae) is one of the most important economically and ecologically in Dicotyledonae (Harborne, 1994).Genus *Tephrosia* belongs to family Fabaceae. This genus includes about 400 species, distributed in the tropical, subtropical and arid regions of the world (Willis, 1973; Al-Ghamdi, 2013) [30, 2]. According to taxonomy genus *Tephrosia* was classified into three sub genera based on the morphological traits includes *Marconyx* (includes *T. tenuis*), *Brissonia* (includes *T. candida*), and *Reineria* (includes rest of the species of *Tephrosia)* (Lakshmi *et al*., 2008) [14]. There are about 11 species of *Tephrosia*, which are distributed in the northwestern, western, south and southwestern regions in Saudi Arabia (Chaudhary, 2001 and Osman & Abdein (2019b) [6, 18].

Genus *Tephrosia* was distinguished different biological activities as antifungal, antiviral, antiprotozoal, anti-diabetic, antiulcer, anti-inflammatory, antidiarrheal, antiplasmodial, wound healing and used in many other activities in addition to having highest antioxidant activity (Chen *et al*., 2014) [8].

Molecular markers played a significant role the characterization, evolution and improvement of plant species. Molecular markers used to estimate phylogenetic relationships, biodiversity and in the construction of genetic map within and among plant species (Doddabhimappa *et al*., 2018 [10] and Osman & Abdein (2019a) [17]. Different types of molecular markers such as random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSRs), simple sequence repeats (SSRs) and SCoT polymorphism were used for estimation of genetic diversity in the available germplasm of plants (Zhang *et al*., 2015; Abdein 2018 and Arie *et al*., 2018) [32, 1, 4].

Inter-simple sequence repeats (ISSR) utilized to study and design of hereditary variety in plants Qian *et al.* (2001) [21], SSR to illustrate developmental relationships Zhou *et al.* (2005) [33] and Chen *et al.* (2006). El-Kholy *et al.* (2011) [11] used RAPD molecular markers to evaluate genetic variability among different population of *Nepta* species in Saint Katherine Protectorate. Molecular markers techniques have been used in plant systematics and genetic diversity studies (Shahlaei *et al*., 2014) [25]. Start Codon Targeted (SCoT) polymorphism, the novel marker system based on the short conserved region in plant genes surrounding the ATG translation start codon, has been successfully employed in determining the genetic diversity analysis and finger printing the population (Mulpuri *et al*., 2013; Gao *et al.,* 2014; Satya *et al*., 2015; Raina *et al.* (1986) [15, 12, 24, 22] studied cytogenetic variations (based on G+C Content) in eight species of *Tephrosia*. The RAPD fingerprinting technique was used to reassess the systematic positions of the genus *Tephrosia* (Lakshmi *et al.,* 2008) [14].

There were very few reviews available to study cytogenetic and molecular properties of the genus *Tephrosia*. So, due to the medicinal importance of *Tephrosia* species, in this study the main purpose is to illustrate and estimate the genetic diversity and focused on the genetic characterization of this genus based on molecular markers.

## Materials and Methods

### Plant material

Ten species of genus *Tephrosia* were collected from their natural habitat in various region of KSA and identified according Boulus (2009) [5] as shown in Table 1.

**Molecular studies**

**DNA extraction**

Genomic DNA was extracted from the fresh young leaves of studied plant samples according to Dellaporta *et al*. (1983) [9].

**Random Amplified Polymorphic DNA (RAPD-DNA)**

RAPD reactions were performed according to Williams *et al*. (1990) [29] with some modifications PCR amplifications were performed using five primers. The sequence of these primers was given in Table (2).

**Inter-Simple Sequence Repeats DNA (ISSR-DNA)**

A total five primers were tested to amplify the isolated DNA. These primers listed in Table (2).

**Start Codon Targeted Polymorphism (SCoT)**

A total five primers were tested to amplify the isolated DNA. These primers listed in Table (2).

**Data analysis**

All gels were photographed and analyzed using Bio-Rad video documentation system, Model Gel Doc 2000. The presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0 respectively).

Cluster analysis and Biplot mapping were conducted to generate the possible relationships among ten taxa based on molecular attributes using the SYSTAT version 7.0 program (Wilkinson, 1997) [28].

**Results**

Five primers were used for each of RAPD, ISSR and SCoT molecular markers. The size range of bands, total bands, polymorphic bands in addition to the percentage of polymorphism produced from each primer were recorded in Table (2). Gels of banding profiles for RAPD-PCR, ISSR-PCR and SCoT-PCR were illustrated in Fig. (1-3).

RAPD marker produced 39 total bands with the size range varied from 2500-1100 bp, these bands include three monomorphic bands and 36 polymorphic bands, with percentage of polymorphism (92.31%). Primer OPA5 of RAPD produced one unique band with molecular size 200 bp characteristic for *Tephrosia villosa*, where primer OPA15 produced six unique bands present only in *T. purpurea* subsp. *leptostachya*. Primer OPB6 gave one unique bands specific for *T. purpurea* subsp. *apollinea* with molecular size 310 bp. Primer OPB10 produced two unique bands, one band distinguished for *T. purpurea* subsp. *leptostachya* at size 240 bp and the other distinguished for *T. pumila* at size 350 bp. Finally, two unique bands were generated from OPHO3 primer, first band at size 470 found in *T. purpurea* subsp. *leptostachya* and the second band present in *T. pubescens* at size 900 bp.

Regarding, ISSR marker generated 37 total bands with a size ranged from 60-1570 bp with 30 polymorphic bands and seven monomorphic bands and the percentage of polymorphism was 81.08 % as shown in Table 3. Five unique bands produced from ISSR marker, two unique bands were generated from ISSR 3 primer (at 60 bp characteristic for *T. purpurea* subsp. *apollinea* and at 1570 bp characteristic for *T. villosa*, one band at size 1570 bp produced by ISSR 4 primer also found in *T. villosa*. The last two bands were generated from ISSR 6 (at size range 800 bp found in *T. quartiniana* and at size range 1560 found in *T. desertorum*). Highest percentage of polymorphism (100%) was generated using ISSR 3 primer, where the lowest polymorphism (55.56%) was generated from ISSR 4 (Table 2).

For SCoT marker, out of a 23 total bands with molecular size varied from 300- 1300 bp, with ten monomorphic bands and 13 polymorphic bands were produced using five primers, the percentage of polymorphism was 56.52% as shown in Table (3). One unique band was produced from SCoT 1 at size range 300 bp recorded in *T. villosa*. Highest polymorphism (100%)was produced by SCoT 8 primer, whereas the lowest polymorphism (75%)was generated using SCoT 6 and primer SCoT9 had no polymorphism (Table 2).

Data analysis

To illustrate the genetic relationships among the studied taxa, genetic distances were measured based on all molecular attributes. Sperman correlation showed that the similarity indices between the studied taxa of *Tephrosia* in KSA. The highest similarity was observed between *T. desertorum* (T4) and *T. nubica* (T5) (0.943). The similarity between *T. apollinea* (T3) and *T. villosa* (T10) (0.110) was recorded as the lowest Table (4).

Cluster analysis for RAPD divided studied ten taxa in two groups, first group includes *T. purpurea* subsp. *leptostachya* and the other nine taxa and the second group, also second group divided into two subgroups, first subgroup contains *T. uniflora* and the second subgroup contains the last eight taxa Fig. (4).

Cluster dendrogram for ISSR marker showed that the ten taxa were separated in two groups; first group comprises *T. desertorum, T. nubica*, *T. pubescens* and *T. villosa.* The second group contains the other six taxa (Fig.5).

For SCoT cluster dendrogram, the studied taxa were divided into two groups; first group contains *T. purpurea* subsp. *Leptostachya* and the second group contains the other nine taxa (Fig.6). Figure (7) showed the cluster analysis for all studied taxa using molecular attributes (RAPD, ISSR and SCoT markers); the cluster separated the taxa into two groups, first group *Tephrosia purpurea* and the second group contain the other nine taxa under study. Biplot based on all molecular attributes showed the importance of OPB10, ISSR9, SCoT 1 and OPA15, TCV, OP-A02 and OP-A09 primers to distinguish the all studied taxa of *Tephrosia* into two groups the first group included only *T. purpurea* subsp. *leptostachya*, the second group included the other nine taxa (Fig. 8).

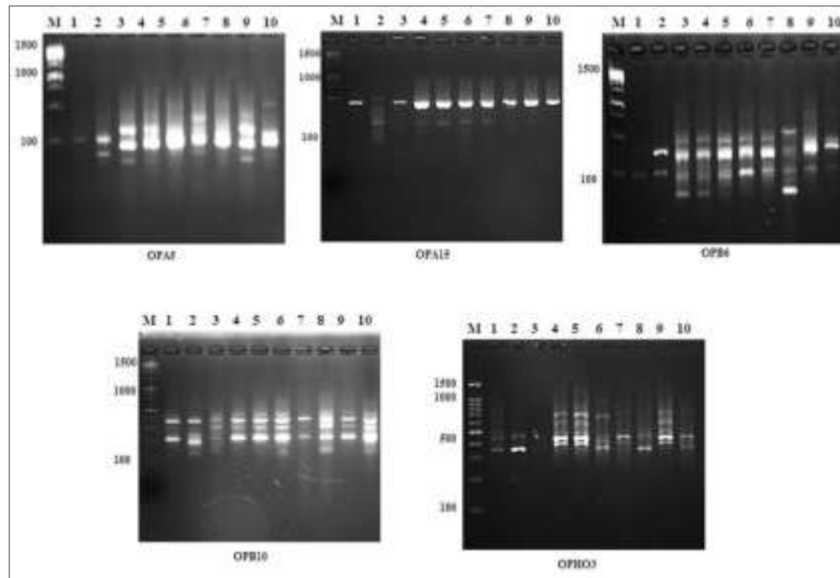


**Fig 1:** RAPD fingerprinting profile produced by five primers in the examined taxa of *Tephrosia*.

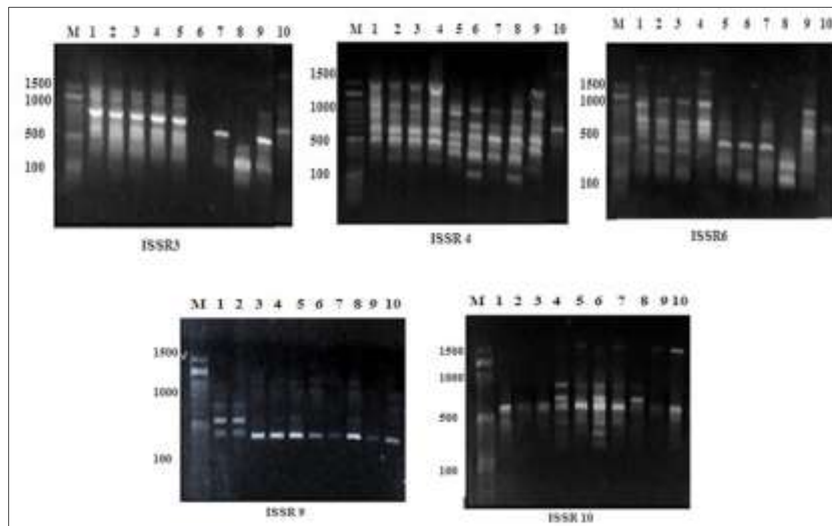


**Fig 2:** ISSR fingerprinting profile produced by five primers in the examined taxa of *Tephrosia*

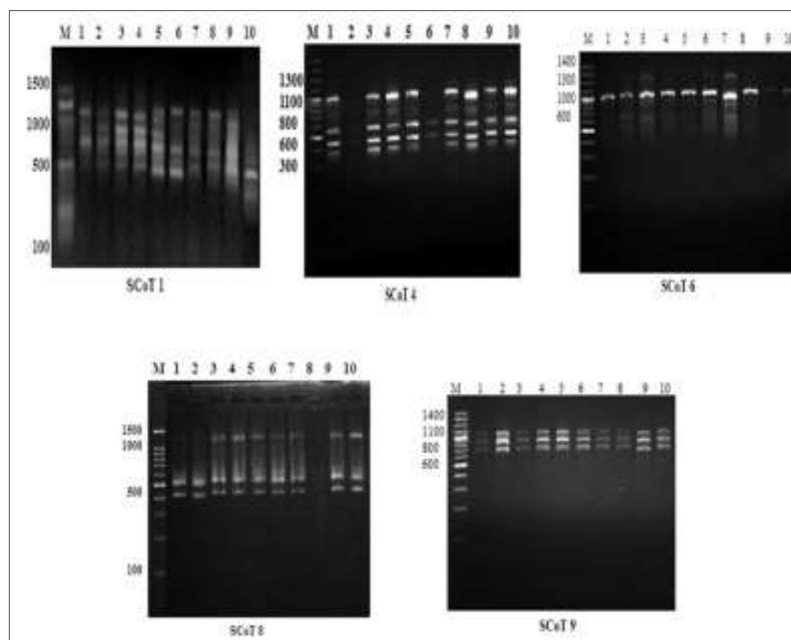


**Fig 3:** SCoT fingerprinting profile produced by five primers in the examined taxa of *Tephrosia*.

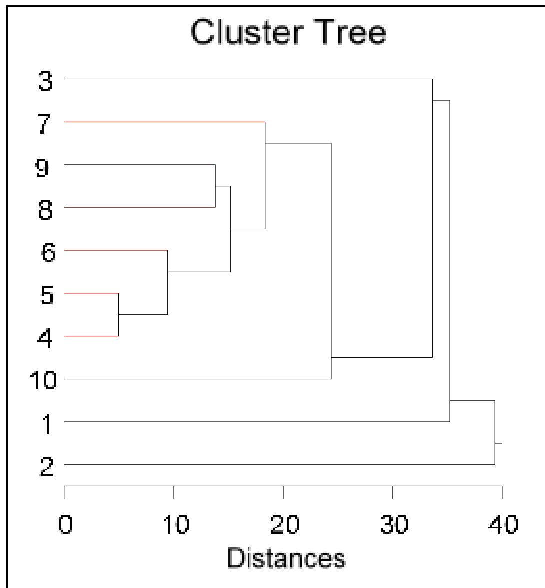


**Fig 4:** Cluster analysis among ten taxa of *Tephrosia* based on RAPD molecular marker

1- *Tephrosia uniflora* Pers.
2- *Tephrosia purpurea* subsp. *leptostachya* (DC.) Brummitt
3- *Tephrosia apollinea* (Delile) DC.
4- *Tephrosia desertorum* Scheele
5- *Tephrosia nubica* (Boiss.) Baker
6- *Tephrosia pubescens* Ewart & Morrison
7- *Tephrosia pumila* (Lam.) Pers.
8- *Tephrosia purpurea* subsp. *apollinea* (Delile) Hosni & Z.A.R.El-Karemy
9- *Tephrosia quartiniana* Cufod. ex Greuter & Burdet
10- *Tephrosia villosa* (L.) Pers.

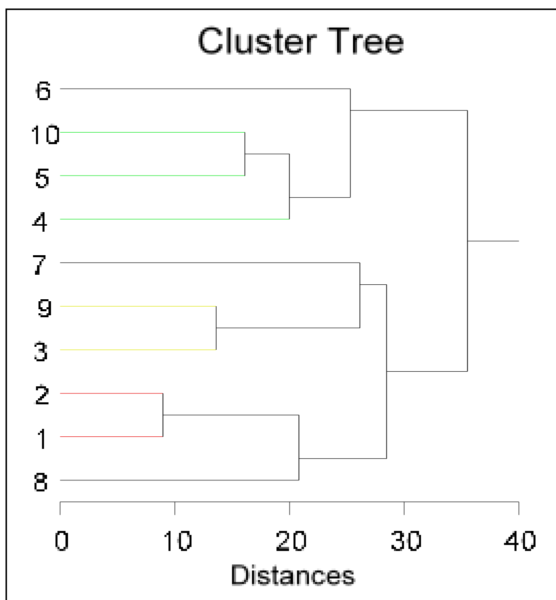


**Fig 5:** Biplot mapping among ten taxa of *Tephrosia* based on ISSR molecular marker

1- *Tephrosia uniflora* Pers.
2- *Tephrosia purpurea* subsp. *leptostachya* (DC.) Brummitt
3- *Tephrosia apollinea* (Delile) DC.
4- *Tephrosia desertorum* Scheele
5- *Tephrosia nubica* (Boiss.) Baker
6- *Tephrosia pubescens* Ewart & Morrison
7- *Tephrosia pumila* (Lam.) Pers.
8- *Tephrosia purpurea* subsp. *apollinea* (Delile) Hosni & Z.A.R.El-Karemy
9- *Tephrosia quartiniana* Cufod. ex Greuter & Burdet
10- *Tephrosia villosa* (L.) Pers.

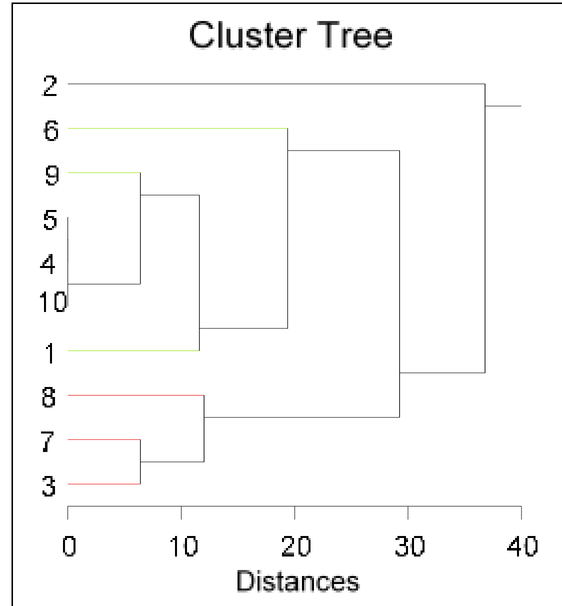


**Fig 6:** Biplot mapping among ten taxa of *Tephrosia* based on SCoT molecular marker

1- *Tephrosia uniflora* Pers.
2- *Tephrosia purpurea* subsp. *leptostachya* (DC.) Brummitt
3- *Tephrosia apollinea* (Delile) DC.
4- *Tephrosia desertorum* Scheele
5- *Tephrosia nubica* (Boiss.) Baker
6- *Tephrosia pubescens* Ewart & Morrison
7- *Tephrosia pumila* (Lam.) Pers.
8- *Tephrosia purpurea* subsp. *apollinea* (Delile) Hosni & Z.A.R.El-Karemy
9- *Tephrosia quartiniana* Cufod. ex Greuter & Burdet
10- *Tephrosia villosa* (L.) Pers.

**Table 1:** Ten taxa *Tephrosia* species collected from Wadi Arar of KSA

| Taxa | Taxa name |
|---|---|
| 1 | *Tephrosia uniflora* Pers. |
| 2 | *Tephrosia purpurea* subsp. *leptostachya* (DC.) Brummitt |
| 3 | *Tephrosia apollinea* (Delile) DC. |
| 4 | *Tephrosia desertorum* Scheele |
| 5 | *Tephrosia nubica* (Boiss.) Baker |
| 6 | *Tephrosia pubescens* Ewart & Morrison |
| 7 | *Tephrosia pumila* (Lam.) Pers. |
| 8 | *Tephrosia purpurea* subsp. *apollinea* (Delile) Hosni & |
| 9 | *Tephrosia quartiniana* Cufod. ex Greuter & Burdet |
| 10 | *Tephrosia villosa* (L.) Pers. |

**Table 2:** List of Primers of RAPD, ISSR and SCoT molecular markers, number of total bands, polymorphic bands and percentage of polymorphism of each primer generated used in this study

| Marker | Primer name | Sequence( 5- -3-) | size range(bp) | Polymorphic bands | Total bands | % polymorphism |
|---|---|---|---|---|---|---|
| RAPD | OPA5 | AGG GGT CTT G | 40-200 | 7 | 7 | 100 |
| | OPA15 | TTC CGA ACC C | 80-400 | 9 | 9 | 100 |
| | OPB6 | TGCTCTGCCC | 30-310 | 6 | 7 | 85.71 |
| | OPB10 | CCGTTGCCT | 120-430 | 6 | 8 | 75 |
| | OPHO3 | AGACGTCCAC | 350-900 | 8 | 8 | 100 |
| ISSR | ISSR3 | GTGTGTGTGTGTGG | 60-1570 | 9 | 9 | 100 |
| | ISSR4 | GAGAGAGAGAGACC | 100-1570 | 5 | 9 | 55.56 |
| | ISSR6 | CACCAGCACGC | 170-1560 | 9 | 10 | 90 |
| | ISSR9 | CACACACACACAAG | 400-900 | 3 | 4 | 75 |
| | ISSR10 | CTCTCTCTCTCTCTCTGC | 300-1500 | 5 | 6 | 83.33 |
| SCoT | SCoT1 | | 300-1100 | 5 | 7 | 71.42 |
| | SCoT4 | | 380-1100 | 4 | 5 | 80 |
| | SCoT6 | CAATGGCTACCACTACAG | 600-1300 | 3 | 4 | 75 |
| | SCoT8 | ACAATGGCTACCACTACC | 400-1300 | 4 | 4 | 100 |
| | SCoT9 | ACAATGGCTACCACTGCC | 800-1000 | 0 | 3 | 0 |

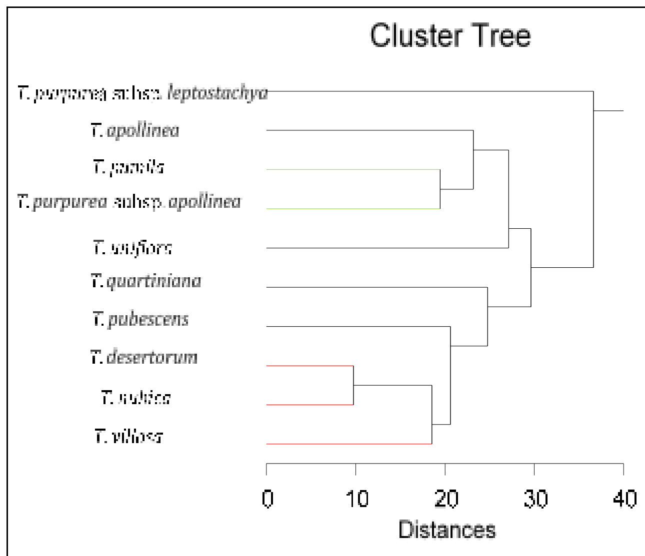


**Fig 7:** Cluster analysis among ten taxa of *Tephrosia* based all molecular markers (RAPD, ISSR &SCoT)

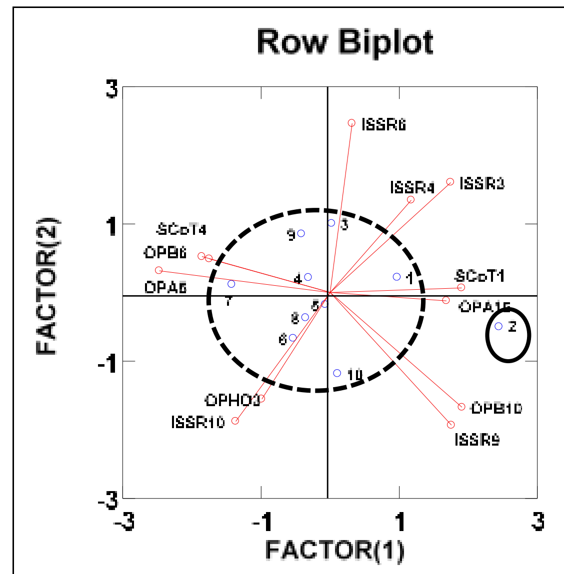


**Fig 8:** Biplot among ten accessions of *Tephrosia* based on molecular attributes (RAPD, ISSR &SCOT)

**Table 3:** Bands characteristics produced by molecular markers (RAPD, ISSR& SCoT) in ten taxa of *Tephrosia*.

<table>
<tr><th colspan="2">Parameter</th><th colspan="5">RAPD</th><th colspan="3">ISSR</th><th>SCoT</th></tr>
<tr><td colspan="2">Studied taxa</td><td colspan="5">10</td><td colspan="3">10</td><td>10</td></tr>
<tr><td colspan="2">No. of primers</td><td colspan="5">5</td><td colspan="3">5</td><td>5</td></tr>
<tr><td colspan="2">Marker range (bp)</td><td colspan="5">30-900</td><td colspan="3">60-1570</td><td>300-1300</td></tr>
<tr><td colspan="2">Total bands</td><td colspan="5">39</td><td colspan="3">37</td><td>23</td></tr>
<tr><td colspan="2">Monomorphic bands</td><td colspan="5">3</td><td colspan="3">7</td><td>10</td></tr>
<tr><td colspan="2">Polymorphic bands</td><td colspan="5">36</td><td colspan="3">30</td><td>13</td></tr>
<tr><td rowspan="3">Unique bands</td><td>No. of unique bands</td><td colspan="5">11</td><td colspan="3">5</td><td>1</td></tr>
<tr><td>Primers</td><td>OPA5</td><td>OPA15</td><td>OPB6</td><td>OPB10</td><td>OPHO3</td><td>ISSR3</td><td>ISSR4</td><td>ISSR6</td><td>SCoT1</td></tr>
<tr><td>Number of unique bands for each primer</td><td>1</td><td>6</td><td>1</td><td>2</td><td>2</td><td>2</td><td>1</td><td>2</td><td>1</td></tr>
<tr><td colspan="2">% Polymorphism</td><td colspan="5">92.31 %</td><td colspan="3">81.08 %</td><td>56.52%</td></tr>
</table>

**Table 4:** Sperman correlation among ten accessions of *Tephrosia* based on molecular attributes

| Code | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | T10 |
|---|---|---|---|---|---|---|---|---|---|---|
| T1 | 1.00 | | | | | | | | | |
| T2 | 0.384 | 1.00 | | | | | | | | |
| T3 | 0.437 | 0.213 | 1.00 | | | | | | | |
| T4 | 0.518 | 0.124 | 0.291 | 1.00 | | | | | | |
| T5 | 0.545 | 0.229 | 0.299 | 0.943 | 1.00 | | | | | |
| T6 | 0.198 | 0.165 | 0.123 | 0.84 | 0.779 | 1.00 | | | | |
| T7 | 0.374 | 0.076 | 0.690 | 0.602 | 0.594 | 0.607 | 1.00 | | | |
| T8 | 0.465 | 0.347 | 0.553 | 0.500 | 0.657 | 0.547 | 0.757 | 1.00 | | |
| T9 | 0.512 | 0.347 | 0.515 | 0.737 | 0.749 | 0.662 | 0.642 | 0.577 | 1.00 | |
| T10 | 0.573 | 0.168 | 0.110 | 0.875 | 0.874 | 0.758 | 0.46 | 0.476 | 0.537 | 1.00 |

T1- *Tephrosia uniflora* Pers.
T2- *Tephrosia purpurea* subsp. *leptostachya* (DC.) Brummitt
T3- *Tephrosia apollinea* (Delile) DC.
T4- *Tephrosia desertorum* Scheele
T5- *Tephrosia nubica* (Boiss.) Baker
T6- *Tephrosia pubescens* Ewart & Morrison
T7- *Tephrosia pumila* (Lam.) Pers.
T8- *Tephrosia purpurea* subsp. *apollinea* (Delile) Hosni & Z.A.R.El-Karemy
T9- *Tephrosia quartiniana* Cufod. ex Greuter & Burdet
T10- *Tephrosia villosa* (L.) Pers.

**Discussion**

Molecular markers are widely used to evaluate both cultivated and wild species (Joshi-Saha *et al*., 2007) [13]. Different molecular markers used for genetic fingerprinting and deriving phylogenetic relationship include random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) in genetic variability assessment and breeding programmes (Smith *et al*., 1997). Studying the genetic diversity using information of molecular markers is important in the conservation, management, genetic improvement, identification of unique accessions or landraces and utilization of germplasm (Nevo *et al*., 2012; Rashid *et al*., 2012) [16, 23]. In this study, RAPD, ISSR and SCoT markers were used to estimate the genetic diversity and relationship among the ten accessions of *Tephrosia* in KSA. Comparative study using different molecular markers for genetic diversity assessment can provide a more informative classification than a single method alone (Souframanien and Gopalakrishna, 2004) [27].

In the present study, ten taxa from *Tephrosia* were fingerprinted using RAPD, ISSR and SCoT markers. Three RAPD primers (OPA5, OPA15 & OPHO3) can differentiate between ten studied species giving polymorphism 100 %, this result was higher in comparison to the polymorphism percentage of *Tephrosia* species in India by Lakshmi *et al*. (2008) [14]. In this investigation, both RAPD and ISSR molecular marker were generated high number of total bands as 39 and 37 respectively.in comparison with SCoT marker produced 23 total bands. The RAPD, ISSR and SCoT markers used in this work generated high percentage of polymorphism as 92.31 %, 81.08 and 56.52 % respectively in the studied taxa. This result was in agreement with result of Zhang *et al*. (2016) [31] who reported (ISSR: 82.35–96%; SCoT: 81.25–100%) in Switchgrass species. RAPD and ISSR markers in this investigation have strong power to indicate the genetic diversity among accessions of *Tephrosia* as indicated by the high values of the genetic diversity indices including number of total bands and high polymorphism. This showed that RAPD markers may be more efficient in studying genetic diversity and relationship of *Tephrosia* than ISSR and SCoT markers with regards to polymorphism detection. This result could be explained as RAPD marker cover the whole genome for amplification, but ISSR amplifies the region between two microsatellites.

The markers differentiated the accessions into two major groups or clusters which is in agreement with the previous work by Lakshmi *et al*. (2008) [14] using RAPD marker to study the genetic variability among 12 *Tephrosia* accessions. The farther away accessions are from each other indicate possibility of having more genetic diversity which also reflects their locations on clusters dendrogram (Skroch and Nienhuis, 1995) [26].

It was concluded that genetic diversity among ten studied taxa of *Tephrosia* using RAPD, ISSR and SCoT molecular markers. The polymorphism percentage showed that RAPD have the highest high efficiencies to distinguished among studied taxa than ISSR and SCoT markers. This genus need additional analysis using different molecular markers such as ITS (Internal Transcribed Spacers) or plastid/ mitochondrial DNA (RbCL, trn K, mat K etc.,) sequences will improve the accuracy of resolution of genetic relationships for sustainable conservation as medicinal plant by such as the construction of gene banks of the genus *Tephrosia*.

**Acknowledgement**

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