Studying the molecular genetic variations for some plants belonging to family Compositae (Asteraceae) depending on RAPD markers

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Summary
This study is aimed at determining the genetic distance between 13 species of family Compositae: Asteraceae are Anthemis L., Launea, and J.Gay. Matricaria L. and Sonchus L. determination of genetic variation takes place by using a numerical rating of the DNA to possess genetic variation and demonstrate its differences and its similarities. The study was performed from the 1st of April 2015 to 12nd of March 2016. Estimating the genetic dimension have been conducted using RAPD results by using (NTsys-pc. Version 1.7) the lowest value of genetic dimension equaled 0.015 and was between Sonchus arvensis L. and Sonchus plastics L. which indicates the presence of high similarity between the two species depending on the used primers in this study. The highest genetic dimension was between Anthemis desert Boiss and Muschi. Launeamcronata (forssk) which equaled 0.284 which indicate the presence of lower percent of similarities between these two types as they got engaged by the lowest number of bands.

Key Words: Genetic variation, Compositae, RAPD, Genetic distances

How to cite this article: Khalaf SM, Mussa MO (2020): Studying the molecular genetic variations for some plants belonging to family compositae (Asteracene) depending on RAPD markers, Ann Trop Med & Public Health; 23(S19): SP232102. DOI: http://doi.org/10.36295/ASRO.2020.232102

Introduction
The Compositae family is one of the largest families in the plant kingdom, as it gathers about 950 genera and about 20,000 species, most of which are herbaceous and rarely dendritic. This family has been renamed according to the international rules of naming botanic names to become Asteraceae, in addition to the name of “Sunflower family”. Some of its plants are distinguished by producing the botanic milk juice that is known as latex, flowers are usually complete and mono or bisexual, the plant is usually monocious, rarely dioecious, the blossom is infinite, vertical (Capitulum head) embraced by involucrateenvelops consists of one or many rows, the flower consists of a modified scaly or fluffy calyxcalledpappus, sometimes the calyx is absent. The coronet consists of five gamopetalous which may be tubular or in the form of ligulate. Stamens are five settles on the calyx, united Syngenesious ,there is one pistil composed of two carpels. The ovary is lower and uni-chamber, the ovule is basal, and the style ends with two branches and two stigmas, the fruit is poor (Achene) called Cypsela, usually pressed, the seed is non-endospermic [1]. The medicinal plants that belong to the family Compositae are of high economic value included in the fields of medicinal drugs, nutrition oils, ornamentals, and cosmetics [2].

Various studies have been conducted to determine the genetic variation of environmental patterns on plants belong to family Compositae depending on the molecular markers, each of which showed their own advantages to identify valuable signs, Solouki and his group conducted a study on the genetic variation of some environmental patterns of Iranian chamomile depending on morphological characteristics and the molecular markers [3]. In 2010, Pirkhezri and his group also studied the environmental genotypes in southwestern Iran using morphological characteristics and molecular markers explaining the importance of this in giving very important positive results that cannot be overlooked [4]. Anthemis L. is a species belongs to subfamily Anthemideae that include more than 179 species, which is considered one of the aromatic plants rooted in the Mediterranean region, southwest Asia, east to Iran. In
addition to the spreading of other types of this species in the United Kingdom and other large parts of the world. As in Iraq, it has more than 25 species spread in the deserts and mountainous regions.

As for *Launea J. Gay*, it is considered one of the wide distributed genera too; most of its species are wild plants. There are about 51 species spread in most countries of the world while *Matricaria L.* genus includes 20 species distributed in the temperate regions of Europe, Asia, and America, this species is considered one of the most medically important species in the world for each genus, there are 7 other species distributed in Iraq.

Among the common genera, there is *Sonchus L.* genus that belongs to family Lactuceae which include 38 other species grow in Europe, Asia, and North Africa. Its members characterize by containing the botanic milk juice, taking into consideration that most of these species distribute as weeds in agricultural fields, inside cities, gardens and neglected parts; it has about 9 species in Iraq that grow as weeds. Although morphological characteristics are essential and useful signs in characterizing plant species, it has many negatives as it is affected by the environmental conditions that result in the appearance of DNA indicators, and polymorphisms to give a higher degree of individuality, so it becomes a valuable tool to determine the genetic relations and differences between genetic patterns. RAPD indicators are one of the PCR-based partial markers that would be useful for studying the genetic types of medicinal herbs because of the advantages of simplicity without the need to know the DNA sequence of the genome. This study aimed at knowing the genetic variation between 13 different species belonging to 4 genera of wild plants belonging to the family Compositae growing in Iraq, by using molecular markers RAPD.

**Materials and methods**

This study was conducted over the period from 1/4/2015 to 12/3/2016 in a private molecular studies laboratory (Al-Mussayb Bridge) in Baghdad - Iraq, as the samples of the four genera (*Anthemis, Launea, Matricaria, Sonchus*) were collected in Kirkuk provinces (FKI) and Arbel (FAR) (Table 1) Appendix (1). Two plant samples were collected from each location. The plant materials were transported from the field to a laboratory in the liquid nitrogen container and the young leaves were used to extract the DNA.

<table>
<thead>
<tr>
<th>Distribution Province</th>
<th>code</th>
<th>Nature and permanence</th>
<th>Common name</th>
<th>Kinds (scientific names)</th>
<th>Order</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAR*</td>
<td>S13</td>
<td>Annual herb</td>
<td>Nettle</td>
<td><em>Anthemis arvensis L.</em></td>
<td>1</td>
</tr>
<tr>
<td>FKI**</td>
<td>S12</td>
<td>Annual Herb</td>
<td>Moringa</td>
<td><em>Anthemis deserti Boiss.</em></td>
<td>2</td>
</tr>
<tr>
<td>FAR</td>
<td>S4</td>
<td>Two-year herb</td>
<td>Centaurea</td>
<td><em>Launea angustifolia</em> (Desf.) O.Kuntza</td>
<td>3</td>
</tr>
<tr>
<td>FKI</td>
<td>S3</td>
<td>Perennial herb</td>
<td>Taraxacum</td>
<td><em>Launea mucronata</em> (Forssk) Muschl</td>
<td>4</td>
</tr>
<tr>
<td>FAR</td>
<td>S9</td>
<td>Two-year herb or Perennial herb</td>
<td>Camomile</td>
<td><em>Launea nudicaulis</em> (L.) Hook</td>
<td>5</td>
</tr>
<tr>
<td>FAR</td>
<td>S11</td>
<td>Annual herb</td>
<td>Baboneg</td>
<td><em>Matricaria aurea</em> (Loefl.) Sch.-Bip.</td>
<td>6</td>
</tr>
<tr>
<td>FAR</td>
<td>S10</td>
<td>Annual herb</td>
<td>Field milk thistle</td>
<td><em>Matricaria chamomilla L.</em></td>
<td>7</td>
</tr>
<tr>
<td>FKI</td>
<td>S2</td>
<td>Perennial herb</td>
<td>Field sowthistle</td>
<td><em>Sonchus arvensis L.</em></td>
<td>8</td>
</tr>
<tr>
<td>FKI</td>
<td>S1</td>
<td>Annual herb</td>
<td>Rough milk thistle</td>
<td><em>Sonchus asper</em> (L.) Hill.</td>
<td>9</td>
</tr>
<tr>
<td>FAR</td>
<td>S7</td>
<td>Perennial herb</td>
<td>Sow thistle</td>
<td><em>Sonchus maritimus L.</em></td>
<td>10</td>
</tr>
<tr>
<td>FKI</td>
<td>S6</td>
<td>Annual herb</td>
<td>Smooth sow thistle</td>
<td><em>Sonchusoleraceus L.</em></td>
<td>11</td>
</tr>
<tr>
<td>FAR</td>
<td>S5</td>
<td>Perennial herb</td>
<td>Marsh sowthistle</td>
<td><em>Sonchus plaustris L.</em></td>
<td>12</td>
</tr>
<tr>
<td>FKI</td>
<td>S8</td>
<td>Annual herb or Perennial herb</td>
<td>Slender sow thistle</td>
<td><em>Sonchustenerrimus L.</em></td>
<td>13</td>
</tr>
</tbody>
</table>

* FAR Erbil Province = ** FKI Kirkuk Province

**Table 1:** Plant species, some of their characteristics and propagation sites.
DNA Extraction
DNA was extracted from the tissues of the young leaves where four leaves (two of each type) were filtered using the CTAB method. The purity and quantity of DNA samples were validated using electrophoresis, after which Use 10 RAPD indicators to apply PCR technology to the extracted samples. The resulting data was analyzed using Matrix 0-1 found in (Table 2) and entered in the Dendrogram NTSYS program for drawing relationships between species and measuring the amount of Convergence with the UPGMA method (Figure 1).

Table 2: Sequences of prefixes and related data in study samples.

<table>
<thead>
<tr>
<th>Primer code</th>
<th>Sequence 5’-3’</th>
<th>Total bands</th>
<th>Polymorphic bands</th>
<th>Polymorphic (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-1</td>
<td>AGTCAGCCAC</td>
<td>10.0/13.0</td>
<td>1.0/13.0</td>
<td>10.0%</td>
</tr>
<tr>
<td>A-3</td>
<td>GGGTAACGC</td>
<td>13.0/13.0</td>
<td>6.0/13.0</td>
<td>46.1%</td>
</tr>
<tr>
<td>B-1</td>
<td>GTTGCGATCC</td>
<td>0.0/13.0</td>
<td>0.0/13.0</td>
<td>0.00%</td>
</tr>
<tr>
<td>B-20</td>
<td>GGACCCCTAC</td>
<td>4.0/13.0</td>
<td>2.0/13.0</td>
<td>50.0%</td>
</tr>
<tr>
<td>C-2</td>
<td>GTGAGGGCTC</td>
<td>13.0/13.0</td>
<td>8.0/13.0</td>
<td>61.5%</td>
</tr>
<tr>
<td>C-8</td>
<td>TGGACCGGTTG</td>
<td>0.0/13.0</td>
<td>0.0/13.0</td>
<td>0.00%</td>
</tr>
<tr>
<td>OPA-03</td>
<td>AGTCAGCCAC</td>
<td>13.0/13.0</td>
<td>9.0/13.0</td>
<td>69.2%</td>
</tr>
<tr>
<td>OPA-17</td>
<td>GACCCGTTGT</td>
<td>10.0/13.0</td>
<td>9.0/13.0</td>
<td>90.0%</td>
</tr>
<tr>
<td>UBC-157</td>
<td>CGTGGGCAAGC</td>
<td>13.0/13.0</td>
<td>9.0/13.0</td>
<td>69.2%</td>
</tr>
<tr>
<td>UBC-104</td>
<td>GGGCAATGAT</td>
<td>13.0/13.0</td>
<td>10.0/13.0</td>
<td>76.9%</td>
</tr>
</tbody>
</table>

Results and discussion
The current study included studying the genetic markers based on the DNA of some species of family Asteraceae (Compositae) and conducting a numerical classification for it in an attempt to find the genetic variation of the studied samples and knowing the similarities and differences between them. RAPD indicators characterized by its sensitivity for any change in the reaction components so it is considered one of the reactions that can’t be repeated to obtain the same result so, to obtain good results, several experiments must be carried out to reach the optimization of the reaction. RAPD indicators were used in this study to determine the genetic variation of 13 species of the Compositae family divided as shown in table (1) depending on the analysis of the RAPD results by finding the genetic fingerprint and the genetic dimension between the species depending on the appearance or non-appearance of multiplication bands, as well as the difference in the partial weights of those bands that vary by the.

number of complementary sites of the initiator sequences on the DNA strand and also vary according to variation between the distance between one location and the other.  

10 initiators were used in this study which showed different bands according to the used initiators as some of which didn’t give any bands despite conducting the experiment several times (B1, B20, C8) shown in figures (3, 4, and 6). The reason may not be due to the lack of an appropriate pending site for the initiator on the DNA strand, this feature is not important in the classification of species but its importance comes from being a distinguishing feature for a specific genus than the others. The absence of results for some initiators is considered one of the basics on which the variations between the individuals and groups are built the other 7 initiators gave clear and variant bands in terms of its numbers, its molecular weight from one initiator to another, so these initiators have been divided as follow:

**Monomorphic bands**

Among the total initiators that have been used in this study that show bands on the agarose gel, 3 of them showed similar bands in terms of number and location which are (A1, A3, UBC 157) figure (1, 2, and 10). These initiators are having the diagnostic capability for distinguishing the species, the matter that goes with what has mentioned before as the presence of the common band between the studied species indicates that there is a common relation with specific features, and their presence makes RAPD markers more suitable than other DNA markers to study the genetic relationships that are depending on these bands.

**Polymorphic bands**

4 initiators (UBC104, OPA17, OPA3, C2) shown in (figures 5, 7, 8 and 9) produced mixed variant bands in terms of the number and molecular weight between the studied samples after being electrophoresed on agarose gel represented by (54) different bands of (143) bands, its molecular weights ranged from 240 and 1300 base pairs. It is possible to find the genetic variation through this set of initiators and adopt it as a basis in finding the genetic relationship after finding the ratio between the numbers of variant bands to the total number of bands.

The results of these initiators had three patterns of bands, some of which showed unique bands that appear in one species and do not appear in other species, so we can depend on them to determine the DNA fingerprinting of that type, the second is through the absence of the general bands of a specific type (referred to as an absent band) from the rest of the species. The third one is what produced variant bands in terms of numbers, and molecular weights in contrast with the studied models.

**Genetic distance estimation:**

The RAPD results were used in estimating the genetic distance between study samples using the genetic program (pc-NTsys version 7.1), which rely on the presence of co-bundles between each of the two species under study, and whose analyzes are based on the formula of Li &Nei (1979) as it is possible to use 10 initiators to find the genetic distances and the similarity percent between the studied models as the genetic distances ranged from (015.0- to 284.0) between the study samples.

In the case of the presence of identical genetic materials between any of the studied models, this indicates that the genetic distance between them equals zero and the percent of genetic similarities is 100% (17). The least value of the genetic distance was (015.0) between Sonchusarvensis and Sonchus plastics, this indicates that they have the highest percent of similarities in genetic material based on the used initiators in this study, from this point, it is concluded that the presence of similar morphological features participates in increasing the genetic similarity between the studied models in case of using these markers.

The highest genetic distance was between Anthemis desert Boiss and Launeamucronata, it equaled (0.284) which indicates the presence of less percent of similarity between these two species, as the shared lowest numbers of bands relative to the other species. As a result of all this, we can’t depend only on morphological and anatomical features in the classification of all species that makes the relay on the genetic markers based on DNA the basic tool to identify and classify the species, as they don’t get affected by any of the previous factors and not restricted to specific age and it is steady in all cells, therefore it has been adopted in molecular taxonomy studies, evolutionary studies and building genetic maps and it is considered as one of the important tools in studying genetic diversity which makes it the choice that has no alternative in developing suitable plans to preserve species. The values

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of genetic distance between the 10 species have been invested to find the genetic relation that gathers them all in the form of clusters represented by the figure (1)

**Figure (1):** for the result of PCR reaction with the initiator A1/

**Figure 2:** for the result of PCR reaction with the initiator A3.

**Figure (6):** for the result of PCR reaction with the initiator C2/

**Figure 5:** for the result of PCR reaction with the initiator C8

**Figure (8):** for the result of PCR reaction with the initiator OPA03 /

**Figure 7:** for the result of PCR reaction with the initiator OPA17
Figure 9: for the result of PCR reaction with the initiator UBC104. Figure 10: for the result of PCR reaction with the initiator UBC157

References