Original Research Article

The Phylogenetic Relationships among some Species of Vicia L. Based on Morphological Characteristics and Proteins of Seeds

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ABSTRACT:

Fabaceae species form an important economic base locally and regionally as food and fodder crops due to their high protein content.

Four species of Vicia L. (vetches) were chosen in this research to determine the phylogenetic relationships among them by combining the molecular characteristics, specifically the protein content, with the morphological ones. The species were *Vicia sativa* L. (Common Vetch), *Vicia galeata*Boiss. (Helmeted Vetch), *Vicia palaestina*Boiss. (Palestine vetch), *Vicia hybrida* L. (Hairy yellow vetch). They were collected from the gardens of Tishreen University in Latakia – Syria. Two dendrograms were constructed depending on 35 differences in morphological characteristics and 30 bands of seed protein content resulting from Sodium Dodecyl Sulfate-Polyacrylamide GEL Electrophoresis (SDS-PAGE) method, respectively.

The results showed that the dendrograms of both morphological and molecular characteristics were similar. Both of them consisted of three clusters. The first cluster included *V.palaestina*, that was the most distant genetically from the rest of the species, the second one contained *V.sativa*, whereas the third cluster showed a strong phylogeny between *V.galeata* and *V.hybrida*.

This type of studies is important to determine phylogenetic relationships among species and classifying them due to the lack of reported researches locally.

For our knowledge it was a first time that the total protein content of *V.palaestina* was studied.

Keywords: Fabaceae; electrophoresis; protein content; dendrogram; protein bands; globulin.

1. INTRODUCTION:

Vicia L. is one of the most important crops economically and its species are widely distributed all over the world [28]. Syrian Arab Republic is considered the tenth country globally for its production. In 2021, 17,392 million metric tons were produced on an area of 22,938 million hectares [11]. Vicia L. belongs to Fabaceae, and includes between 180 and 210 species according to [12]. There are approximately 38 species including several subspecies and varieties in Syria [24, 27].

Furthermore, some of them are considered among the most important candidate crops for increasing the sustainability and the global supply of plant protein. On a dry matter basis, its seeds contain about 29% protein, more than 80% of the previous percentage consists of globulins (the main storage proteins) that in turn are composed of vicilin (7S protein) and legumin (11S protein) [31].

Seeds are used as an important protein source for animal and human nutrition, not only for their high nutritional value (high in protein, lipids and dietary fiber), but also for their ability to adapt to different soils and climates [25]. In addition, many studies have also revealed that they contain potential health beneficial properties, in particular anti-Parkinson, antidepressant, anticancer, antimicrobial, antidiabetic, anticytotoxic, , antiinflammatory, anticoagulant, estrogenic, diuretic and antihypoxic activities [6, 29].

The researchers have relied on morphological characteristics to identify the species of Vicia L. according to a study by [1, 2], where number of Macro- and micro-morphological characteristics were used to determine the relationship among some species and subspecies of Vicia L. However, it is difficult to rely on only morphological characteristics to study the whole genetic diversity for this genus[13]. Therefore, other characters have recently been added, such as the study of the seed protein content. [3] indicated that the study of seed storage proteins in Fabaceae is very important, as it is considered one of the classification tools due to the stability of their components. [19] also used the seed morphology and seed proteins of 11 Vicia taxa as an effective tool for distinguishing between the inter- and intra-specific of the genus Vicia L.

[17] confirmed by studying six species of legumes that variation in their protein content could be useful in species/varietal identification.

Sodium Dodecyl Sulfate-Polyacrylamide GEL Electrophoresis (SDS-PAGE) is the most important and widespread technique for analyzing the protein profile of plant species. Therefore, electrophoresis of seed protein content was used for determining genetic diversity and evaluating taxonomic relationships at the level of genera, species, subspecies and varieties. [5, 10, 15, 20, 23]. Present study aimed to find the relationship among four species of the genus Vicia L. by using the morphological characteristics and molecular ones.

2. MATERIAL AND METHODS :

2.1. Plant material:

A number of samples of Vicia L. were collected from the gardens of Tishreen University (Latakia - Syria), Figure (1). The period of collection limited from February until the end of May for the two years 2019 and 2020, with focusing on the flowering and fruiting stages, Figure (2).

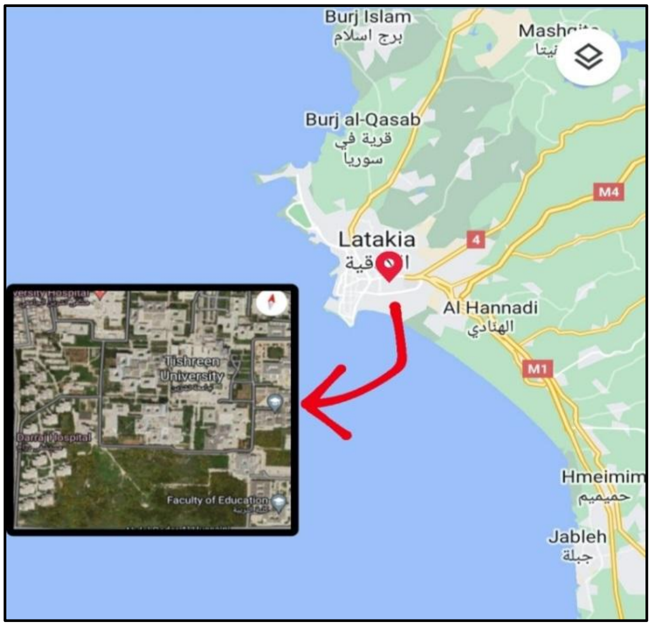


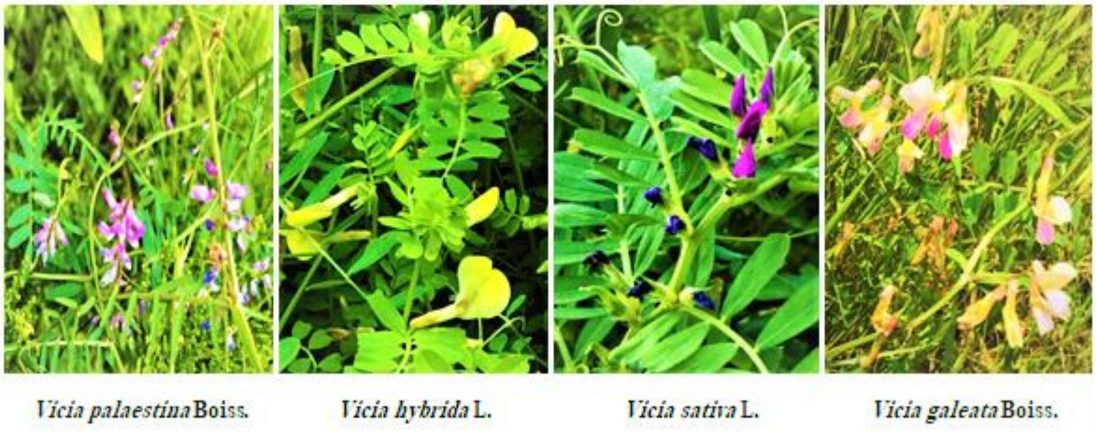
Figure 1: The collection sites of the studied species at Tishreen University (Syria).

The samples were identified according to the relevant references [7, 16, 24, 27] and they were, *Vicia galeata*Boiss., *Vicia sativa* L., *Vicia hybrida* L., and *Vicia palaestina*Boiss. . For each species, a number of morphological characteristics were studied, and they were (life form - stipules - leaf shape - inflorescence - number of flowers per inflorescence - corolla color - calyx - pod shape - pod texture - number of seeds per pods - seed shape – seed size - seed color - presence of dark spots on the seed coat - hilum shape - the presence of papillae and waxy ends).

The plant samples were preserved using two ways:

- the liquid way where F.A.A fixative is used.
- the dry way as herbarium.

whereas the seeds were dried for protein analysis.



Vicia palaestina Boiss. *Vicia hybrida* L. *Vicia sativa* L. *Vicia galeata* Boiss.

Figure 2: Life form of the studied species (*Vicia galeata*Boiss., *Vicia sativa* L., *Vicia hybrida* L., and *Vicia palaestina*Boiss).

2.2. Extraction of protein content:

Seven seeds of each species were used for the extraction process that was carried out according to the following steps:

- The seeds were ground in a mortar until a very fine powder was obtained.
- 1 ml of extraction solution (3 ml Tris-Hcl pH 6.8 - 6 ml glycerol 25% - 0.24 ml bromophenol blue 1% - 4.8 ml SDS 10% - 8.76 ml distilled water - 1.2 ml of ß-mercaptoethanol[21] was added to the powder .
- each mixture was stirred by a heating magnetic stirrer for an hour, at room temperature, and was centrifuged with the set of 11,000 cycles/sec for 20 minutes.
- after that, the supernatant of each sample was taken and kept in containers at -20°C until electrophoresis.

2.3. Electrophoresis:

Only 5µl of supernatant of each sample was centrifuged. Electrophoresis was carried out on a 30% SDS-PAGE polyacrylamide gel by applying an electric current of 90 amperes until the samples entered the separating gel. Then it was raised to 120 amps for three hours, until the samples reached the end of the gel, in order to separate the proteins according to their molecular weights [21]. A standard protein label (BLUelfPrestained protein Ladder) was used, with weights ranging from 5-245 kDa. The gel was transferred to a solution of Coomassie Brilliant blue 0.2% to stain the resulting protein bands for one hour. Then immersed in the destaining solution, the last stage was repeated several times until the bands were clearly visible.

2.4. Data analysis:

A database of total of 65 attributes was developed. They distributed among 35 differences in morphological characteristics and 30 of protein bands resulting from electrophoresis in each studied species. They were treated as a binary characteristic in a data matrix by coding the presence or the absence of the character (1 or 0), respectively. The data was treated by IBM SPSS Statistics 23. The analysis was carried out to construct a morphological dendrogram and a molecular one by Hierarchical Cluster Analysis using Average Linkage (Between Groups) and Squared Euclidean Distance.

3. RESULTS AND DISCUSSION

3.1.The morphological features:

Table (1) shows a comparison of the distinctive morphological characteristics, that differed in each species according to the related references [7, 16, 24, 27], these characteristics were used as a database to determine the degree of relationship.

Table 1: The morphological characteristics used to identify the four studied species.

Species and morphological characteristics	*V. galeata*	*V. sativa*	*V. hybrida*	*V. palaestina*
Life form	creeping or climbing annual herbs			

Stipules	Ovate blunt	semi-toothed, lanceolate,	semi-toothed, lanceolate,	semi-toothed, lanceolate
Leaf shape	Inverted oval to rectangular	Inverted oval to rectangular	Inverted oval to rectangular	inverted lanceolate,
Flower	Panicle (raceme)	Panicle (raceme)	single flower	Panicle (raceme)
Number of flowers per inflorescence	2-5	1-3	1	2-10
Corolla color	pink	Purple	yellow	Bluish purple
Calyx	oblique-tubular	bell-tubular	oblique-tubular	oblique-tubular
Pods shape	longitudinal, broad,	longitudinal, broad	rhombic-rectangular,	rhombic-rectangular
Pods texture	Glabrous	Pubescent	Pubescent	Glabrous
Number of Seeds per pod	2-4	6-12	2-5	1-3
Seeds shape	spherical, sub-spherical,	spherical and sub-spherical, and sub-cushion	spherical and sub-spherical	spherical
Seeds color	Brown and brown-orange	Dark and light brown	Brown and brown-orange	brown
Presence of dark spots on the seed coat	present	Absent	present	absent
Hilum shape	rectangular	Wedged	rectangular	rectangular
Presence of papilla and waxy ends	Present			

3.2. Electrophoresis:

30 bands were obtained by exposing the total protein content of the four studied species to SDS-PAGE. The molecular weights of these bands ranged from 5 to 70 kDa. *V.hybrida* had the highest number of bands (23 bands) with (5-70) kDa, followed by *V.sativa* that showed 20 bands with (5-60) kDa.*V.galeata* gave 19 bands with (5-63) kDa, while *V.palaestina* showed the least number of bands (10 bands) whose weight ranged between 5 and 30 kDa (Fig. 3).

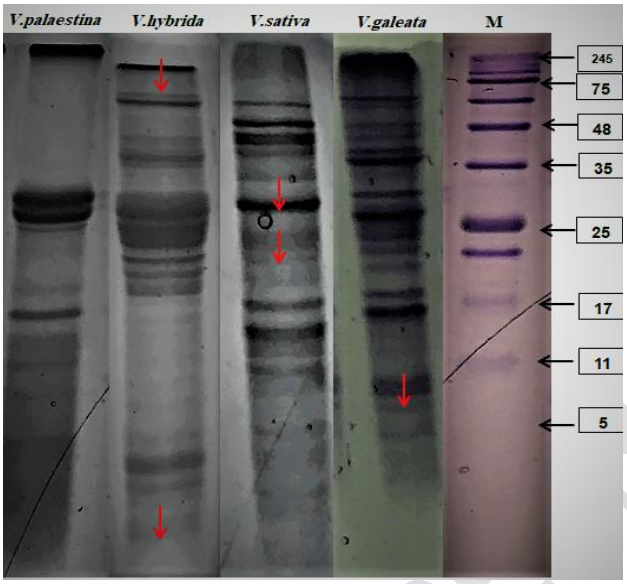


Figure 3: Protein profile of the four study species (*V.galeata, V.sativa, V.hybrida, V.palaestina*). M. refers to the marker (BLUelfPrestained protein Ladder).

Two common bands were found among the four studied species (5, 23) kDa, besides 28 different bands were identified. The 9 kDa band identified *V.galeata,* while both *V.sativa* and *V.hybrida* were distinguished by two bands (19, 29 - 3, 70) kDa, respectively.

3.3. Dendrogram:

The two Dendrograms formed from the analysis of morphological characteristics (Fig. 4, a) and the analysis of bands using electrophoresis (Fig. 4, b) were similar. Both of them showed a match in terms of the formed groups. Each dendrogram consisted of three clusters. The first cluster included *V.palaestina*, that was the most genetically distant. The second one contained *V.sativa*. While the third cluster grouped *V.galeata* and *V.hybrida* together that means they are closely related.

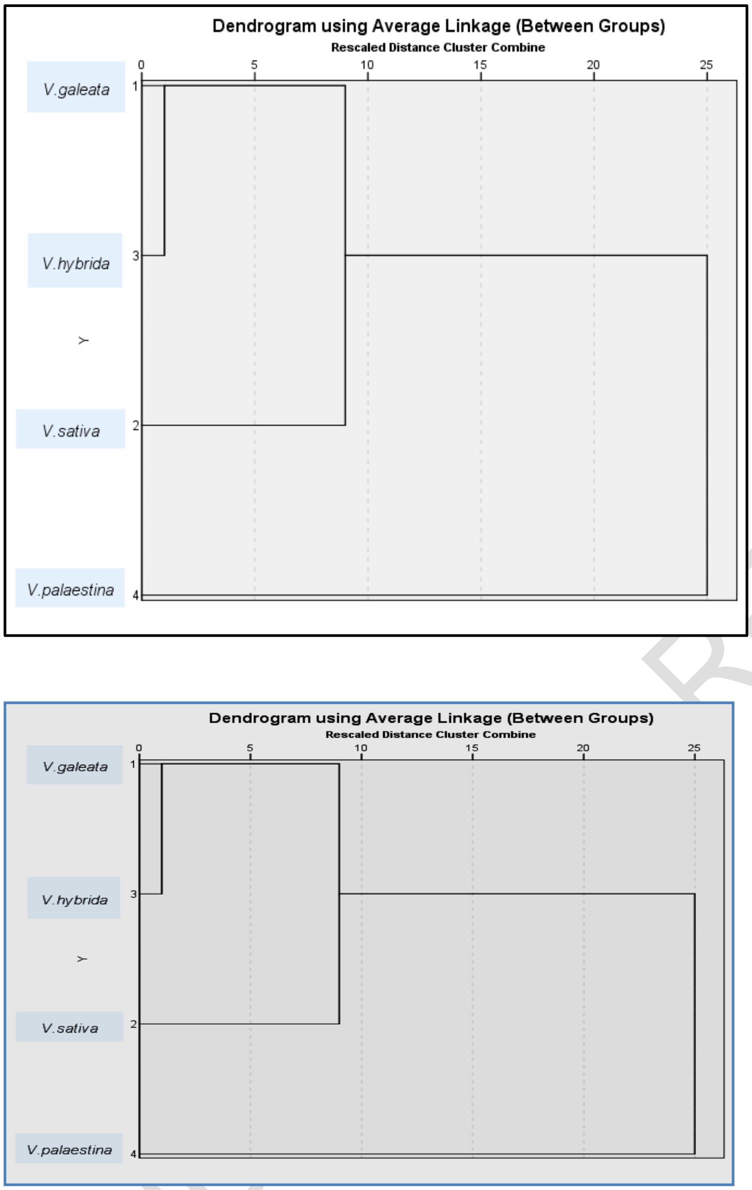


Figure 4. dendrogram constructed using UPMGA analysis formed from a. 35 differences of morphological characteristics – b. 30 protein bands.

4. Discussion:

The study of morphological features is still the basis for distinguishing the different species. Many recent researches relied on morphological classification solely to determine the degree of relationships between species and establishing taxonomic criteria to identify them. As in the studies of, [1, 2] who analyzed 48 differences in the total morphological and partial characteristics of the leaf composition in some Vicia L. species. On the other hand, [14] concluded that the use of macro- and micro-morphological characteristics of the seeds surface are taxonomically useful in distinguishing the studied species belonging to the genus Vicia L.. Whereas [19], by studying species of the genus Vicia L., found that the morphological characteristics of the seeds could not be used as a separately

classificationevidence. But it could be reliably combined with the properties of seed proteins, and this agrees with the current research.
It is noteworthy that the high molecular weight bands were absent in *V.palaestina*, that is consistent with [8]. On the contrary, *V.hybrida*, had the highest weight among the studied species, that was confirmed by [9], where the high weight of seed proteins in *V.hybrida* (18.4-116) kDa were studied. On the other hand, the separation of *V.palaestina* in the most distant cluster of dendrogramcorresponds to studies like [4] that showed that *V.palaestina* was related to the unknown wild ancestor of the Faba bean.

In this study, the total protein content of *V.palaestina* was studied for the first time. These specie grows in low rainfall regions, so it could be cultivated in dry areas, especially that it represents an important source of high nutritional value seeds. In addition, its protein content is estimated at 25.2%, and this is a good percentage considering that *V.sativa*contains 28-32% protein (the highest percentage among the studied species) [30]. Modern agricultural programs also seek to select it as fodder and green fertilizer for cereals [22, 26]. Previous studies on the aforementioned four species and other species of genus Vicia L., used Transfer Ribonucleic acid leucine DNA markers, also agreed with the results of the current study [18].

5. Conclusion:

The current research presented a new step at the local level, as molecular studies of these species are rare in Syria. Therefore, it is considered as a necessary step for subsequent studies that could be concerned with economically important crops at the local level. It is worth noting that there are few studies on *V.palaestina* worldwide, despite its importance as fodder and fertilizer, and its ability to coexist in low rainfall environments. The precise study of morphological characteristics could be adopted taxonomically. However, the molecular study is more accurate and deeper in identifying species, subspecies, and varieties. It is important to shed light on the seed protein content in future studies concerned with biodiversity at the genetic level, in addition to adopting it as an established taxonomic criterion.

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