# *THE USING OF BIOCHEMICAL ANALYSIS OF NUCLEIC ACIDS AND PROTEIN IN TAXONOMICAL STUDIES OF SOME VICIA SPECIES*

*BY*

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*ABSTRACT*

The present investigation have been carried out to determine the possibility of using some wild species of Vicia section Faba as a source of protein by studying its total protein contents, free amino acids and the protein bands separated by electrophoretic technique. In addition, to show how much these biochemical specific characters could be used to suppert the morphological studies to distinguish and classify the different species of Vicia section Faba.

The results showed that V. faba as a cultivated species had distinctive characters varied from the wild ones. The wild species have many similar characters make them link together and also with V. faba. Also, the biochemical analyses could be used to support the morphological study.

From the biochemical analyses results, V. johannis and V. hyaeniscyamus could be recommended as a source of dietary for human consumption but after biological evaluation.

*INTRODUCTION*

Leguminous seeds are considered as important source of food protein and energy for large sector of the world population. The increasing interst in the food legumes has been demonstrated by several international symposia on this topic at the last decade (Miler, 1973; Wall, 1973). Therefore, the present study is looking for new source of protein from the wild types of Vicia distributed in different regions.

Section Faba has been chosen for this study because its importance. It included the cultivated species of V. faba with its varieties and the relative wild species. All these species have been shared in many features as mentioned by Kupicka (1976).

Many authors (Schafer, 1973; Cubero, 1984; Kupicka, 1976; Plitmann, 1967 and Khattab 1987) studied, each separately, the relationship between taxa within section Faba. They used wide range of different characters. However, there is no sharp line between these taxa or the relationship between them.

The questions which often arise when studying the relationship between taxa are how much morphological characteristics alone are of importance? and how much chemical traits can be helpful to determine this relation? So, the major aims of this study are to evaluate some wild species of faba bean as a source of protein supply by using some chemical analyses e.g. total crude protein, free amino acids, RNA and DNA and the protein bands of albumin and globulin fractions separated by electrophoretic technique. In addition, the possibility of using these chemical analyses to support the morphological data to distinguish and classify these taxa under consideration.

## *MATERIALS AND METHODS*

*Materials:*

Materials which were used in the present study are divided into: Three samples of ripe seeds which belong to V. faba (as cultivated species) from different locations, in addition to eleven samples of seeds belong to the wild species of section Faba and its varieties were used in this study. The list of the materials and its origin is given in Table (1).

- Fresh plants growing from the above seeds, in addition to the herbarium specimens loaned from different herbaria such as Royal Botanic Gardens, Kew, UK; British Museum (Natural History), UK; Southampton Univ., herbarium UK. and Vicia specimens which collected from Syria and Turkey (Khattab, 1987).

*Methods:*

*1) Morphological analyses:*

All the available vegetative, floral and reproductive characters of the herbarium specimens and fresh plants as well as the seed characters were scored in a score sheet contain as many characters as possible with character states. After scoring, the data were analysed using Cluster Analysis technique using the LINKAGE programe (Sneath and Sokal, 1973).

Table (1): List of the materials and its origin

| Gel. No. | Species | Varieties | Origin |
|---|---|---|---|
| 1 | V. faba | faba | UK |
| 2 | V. faba | faba | India |
| 3 | V. faba | faba | Crete |
| 18 | V. kalakhensis | ---- | Syria* |
| 19 | V. kalakhensis | ---- | Syria** |
| 15 | V. hyaeniscyamus | ---- | Syria1 |
| 16 | V. hyaeniscyamus | ---- | Syria2 |
| 17 | V. hyaeniscyamus | ---- | Syria3 |
| 25 | V. johannis | johannis | USSR |
| 26 | V. johannis | johannis | Turkey |
| 28 | V. johannis | procumbens | USSR |
| 29 | V. johannis | procumbens | Turkey1,a |
| 30 | V. johannis | procumbens | Turkey2,b |
| 51 | V. galilaea | ----- | Israel |

*,**: Samples of V. kalakensis from different regions of Syria country.

1,2,3,: Samples of V. hyaeniscyamus from different regions of Syria country.

1,a, 2,b,: Samples of V. johannis var procumbens from different regions of Turkey Country.

*2) Biochemical analyses:*

*A) Extraction and determination of free amino acids:*

The free amino acids were extracted by using ethanol (80%, v/v) according to the method of Bell et al., (1971), then the free amino acids were separated and determined by using apparatus of Amino Acids Analyzer (LKB Biochrom, 4151 ALPHA Plus Amino Acid Analyzer) according to the method of Solar et al., (1989).

*B) Determination of total protein:*

The crude protein was determined by using micro-kjeldahl method as described in A.O.A.C. (1980).

*C) Extraction and determination of RNA and DNA contents:*

The RNA and DNA were extracted according to the method of Ogur and Rosen (1950), then its contents were determined by using the method of Astawrov (1974).

*D) Electrophoretic separation:*

*1) Extraction of protein:*

*Water extract:*

Protein of the defatted meal was extracted with distilled water containing 0.01% sodium azide ($NaN_3$) according to Stegemann et al., (1980).

*Buffer extract:*

The residue after the water extraction was re-extracted with 0.125M tris-borate buffer at pH 8.9 containing 0.01% $NaN_3$ according to Stegemann et al., (1980).

*2) Separation of protein by electrophoresis:*

SDS-PAGE 5 and 15% in polyacrylamide was used in glycine buffer at pH 8.3 with 0.1% sodium dodecylsulphate according to Laemmli (1970).

## *RESULTS AND DISCUSSION*

*1) Morphological results:*

The results of linkage analysis of many herbarium and fresh specimens representing the cultivated and the wild species under investigation are shown in the dendrogram (Fig. 1) and can be summarized as follows:

- all the specimens of the cultivated species V. faba from different locations are isolated and separately distincted from the test of specimens examined. V. faba specimens were linked together at the first level of similarity and still linked as one cluster till the end and then start to link first with the specimens of V. galilaea.
- the specimens of V. hyaeniscyamus has many characters differed from the rest of the species, thus it is exist as a separate species and start to link with V. galilaea. This finding is in agreement with that of Ladizinsky (1975) who reported that V. hyaeniscyamus and V. galilaea are taxonomically more similar to V. faba than the rest of species in section Faba.
- specimens defined as V. kalakhensis are distinguishable and has some characters differed from the rest of taxa. This species start to link with V. hyaeniscyamus and finally with V. johannis with link quite late with the rest of species.

Although, the specimens examined have many characters similar to rank them in this section, they have some other varied characters. This variation in the characteristics

could be used to distinguish between the species as shown in Table (2). Therefore, the biochemical analyses were done to support if there is any similarity or dissimilarity between taxa of section Faba.

***2) Biochemical results:***

The biochemical analyses used in this study includes RNA, DNA, amino acids and total crude protein as well as the electrophoretic patterns for separation of protein fractions of the cultivated and wild species of Vicia. Because of the shortage in samples of some seeds only the taxa presented in Table (3) was used.

*Free amino acids:*

The free amino acid content of the wild varieties are listed in Table (3). Data showed that the major amino acids of V. johannis var. johannis and var. procumbens from Turkey and V. galilaea are as follows: Arg. 38.53, 47.88 and 87.17; His. 11.04, 12.26 and 8.90; Lys. 7.23, 5.00 and 10.14 mg/100g, dry weight, respectively. The last amino acids (except His.) are found comparatively with high amounts in the cultivated V. faba var. faba of UK.

In addition, Meth. was found as a remarkable amino acid (6.28) in V. johannis var. johannis, while Ala. and Phe. Ala of V. galilaea were found in high amount (8.57 and 10.75, respectively). On the other hand, the variety of V. johannis var. johannis had no Ser., Glu., Pro., Ala., Val., Isoleuc., Leuc., Tyr. and Phen. Ala, while the other variety of V. johannis had a minor amount of Asp., Threo., Pro., Val. and Isoleuc. V. galilaea also had a minor amount or nearly had not Glu., Gly., Val. and Threo., Pro., Meth. Isoleuc., Leuc., Tyr., respectively. Moreover, V. johannis var. procumbens and V. faba var faba of UK. were almost the same in total free amino acids. In contrary, V. johannis var. johannis, V. galilaea and V. faba are varied in their total free amino acids.

*Total protein:*

The results of total protein of both varieties of V. johannis from Turkey and V. galilaea are presented in Table (3). The obtained results indicate that the amounts of total protein of V. johannis var. johannis, var. procumbens and V. galilaea were 32.50, 29.26 and 26.62, respectively. These amounts are relatively far from that of V. faba var. faba of UK. (22.54%). The obtained results are in agreement with the findings of De Simone et al., (1983).

**Table (2):** Morphological characters varied between taxa in section Faba.

| Character | V. faba | V. johannis | V. galilaea | V. hyaeniscyamus | V. kalakhensis |
|---|---|---|---|---|---|
| Tip of leaf | mucronate | tendrillous | tendrillous | tendrillous | mucronate or simple tendril |
| Stipule shape | semisagittate or entire | semisagittate or semihastate | semisagittate | semisagittate dentate | semisagittate dentate |
| Stipule colour | green | green | green | purple | green with dark violet base |
| Stipule nectary colour | green | green | dark purple | dark purple | green |
| Edge of stipule | translucent | not translucent | not translucent | not translucent | not translucent |
| Calyx colour | green | green | green | purple | green with dark violet base |
| Flower No. | 1-12 | 1-3 | 1-2 | 5-6 | 3-5 |
| Standard colour | white | whitish violet | whitish violet | yellow cream | white with violet back |
| Colour of wings and spot | white with black spot | yellowish with brown spot | yellowish with violet brown spot | yellowish with brown spot | white without spot |
| Legume No. | 1-6 | 1-2 | 1-2 | 3-4 | 3-4 |
| Legume pubscence | absent | moderatley hairy | densely hairy | densely hairy | moderately hairy |
| Seed No. | 1-10 | 2-7 | 2-4 | 3-5 | 3-5 |
| Seed shape | oblong or compressed | spherical | spherical | spherical | spherical |
| Hilum shape | linear or | oval | oval | oval | wedge shaped |
| Hilum colour | black | dark brown | dark brown | black | |
| Colour of centre strip of hilum | white | beige | beige | beige | beige |

Table (3) : Individual free amino acids , total free amino acids and total protein of different specimens of Vicia ( V. faba , and wild varieties ) from different locations .

| Free amino acids | V. faba var faba ( U.K. ) | | V. johannis var.johannis ( Turkey ) | | V. johannis var.procumbens ( Turkey ) | | V. gelilaea ( Israel ) | |
|---|---|---|---|---|---|---|---|---|
| | mg/100g | % | mg/100g | % | mg/100g | % | mg/100g | % |
| Asp. | 2.61 | 2.65 | 2.97 | 4.05 | 0.92 | 0.93 | 3.65 | 2.59 |
| Threo. | 0.00 | 0.00 | 0.57 | 0.78 | 1.67 | 1.69 | 0.00 | 0.00 |
| Ser. | 3.41 | 3.46 | 0.00 | 0.00 | 3.97 | 4.02 | 3.45 | 2.45 |
| Glu. | 0.00 | 0.00 | 0.00 | 0.00 | 3.05 | 3.09 | 1.69 | 1.20 |
| Pro. | 0.00 | 0.00 | 0.00 | 0.00 | 0.33 | 0.33 | 0.00 | 0.00 |
| Gly. | 1.50 | 1.52 | 1.65 | 2.25 | 1.98 | 2.00 | 1.13 | 0.80 |
| Ala. | 1.78 | 1.81 | 0.00 | 0.00 | 2.81 | 2.85 | 8.57 | 6.08 |
| Val. | 1.89 | 1.92 | 0.00 | 0.00 | 1.21 | 1.23 | 2.60 | 1.85 |
| Meth. | 0.00 | 0.00 | 6.28 | 8.56 | 1.99 | 2.02 | 0.00 | 0.00 |
| Iso leuc. | 0.00 | 0.00 | 0.00 | 0.00 | 1.25 | 1.26 | 0.00 | 0.00 |
| Leuc. | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| Tyr. | 0.00 | 0.00 | 0.00 | 0.00 | 2.80 | 2.84 | 0.00 | 0.00 |
| Phenylala. | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 10.75 | 7.63 |
| Hist. | 0.00 | 0.00 | 11.04 | 15.06 | 16.26 | 16.46 | 8.90 | 6.31 |
| Lys. | 10.79 | 10.95 | 7.23 | 9.86 | 5.00 | 5.06 | 10.14 | 7.31 |
| Ammonia | 6.10 | 6.19 | 5.05 | 6.89 | 7.64 | 7.74 | 2.90 | 2.06 |
| Arg. | 70.45 | 71.50 | 38.53 | 52.55 | 47.88 | 48.48 | 87.17 | 61.84 |
| Total free amino acids ( TFAA ) | 98.53 | 100.0 | 73.32 | 100.0 | 98.76 | 100.0 | 140.95 | 100.0 |
| Total protein g / 100g | 22.54 | | 32.50 | | 29.26 | | 26.62 | |

* These values were calculated on dry weight basis .

***RNA and DNA:***

As shown in Table (4) of RNA and DNA contents, it is observed that the high contents of both were found in V. hyaeniscyamus located at Syria (i.e. in case of $Syria_1$ (5.58, 2.92) and (4.92, 2.05) in $Syria_2$, respectively). The other wild species and varieties had moderate amounts related or closed to the amount of V. faba var. faba of UK. (2.96 mg/1g dry weight).

It is also observed that, the highest ratio of RNA/DNA was found in V. galilaea (3.14), then V. hyaeniscyamus from Syria (1.91-2.40) and in V. johannis var. johannis from Turkey and USSR (2.14-1.71). The cultivated V. faba of UK. had a ratio of 1.73; from these results, the RNA/DNA ratio in the wild and the cultivated species is quite similar or varied in a narrow range, this indicates the similarity between taxa.

It could be concluded that, the biochemical analyses may be suitable to differentiate between taxa even in species level or above that, in addition to the electrophoretic separation of protein (Afify, 1987 and Abdalla and Gunzel, 1979). Also it could be stated that some wild types can be used as a good source of dietary for human or animal consumption, but after biological evaluation.

***Electrophoretic separation of protein:***

Albumin and globulin in the different wild species of Vicia under investigation were detected by the electrophoretic separation of polyacrylamide gel electrophoresis using SDS----PAGE technique and compared with those of the cultivated species, Fig. (2). The results of globulin fractions of the different species and varieties showed identical major protein bands, and therefore, could not be used as a finger printing to differentiate between these taxa.

Contrary, the albumin protein fraction showed a different profile bands for each taxa. The results indicate that V. kalakhensis was identical in its albumin protein bands except that of 55 K.D. which showed strong band in one compared to the other.

The same observation have been recorded in the two samples of V. johannis var. johannis, while, var. procumbens showed different protein bands when compared with the other wild species. V. hyaeniscyamus showed identical profile of its albumin protein bands, except the band of 50 K.D. which

Table ( 4 ) : RNA , DNA contents and RNA / DNA ratio of different specimens of Vicia ( faba and wild ) varieties from different locations .

| Nucleic acids contents | V.faba var.faba (U.K.) | | V. hyaeniscyamus Syria[1] | | V. hyaeniscyamus Syria[2] | | V. johannis var. johannis USSR | | V. johannis var. johannis Turkey | | V. johannis var. procumbens USSR | | V. johannis var. procumbens Turkey | | V. galilaea ( Israel ) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | mg / g | % | mg/g | % | mg/g | % | mg/g | % | mg /g | % | mg/g | % | mg/g | % | mg/g | % |
| RNA | 2.96 | 63.38 | 5.58 | 65.65 | 4.92 | 70.59 | 2.53 | 63.09 | 3.0 | 68.18 | 3.41 | 68.20 | 2.98 | 66.97 | 3.11 | 75.85 |
| DNA | 1.71 | 36.62 | 2.92 | 34.35 | 2.05 | 29.41 | 1.48 | 36.91 | 1.40 | 31.82 | 1.59 | 31.80 | 1.47 | 33.03 | 0.99 | 24.15 |
| Total nucleic acids | 4.67 | 100.0 | 8.50 | 100.0 | 6.97 | 100.0 | 4.01 | 100.0 | 4.40 | 100.0 | 5.00 | 100.0 | 4.45 | 100.0 | 4.10 | 100.0 |
| RNA/DNA ratio | 1.73 | | 1.91 | | 2.4o | | 1.71 | | 2.14 | | 2.14 | | 2.03 | | 3.14 | |

* These values were calculated on dry weight basis .

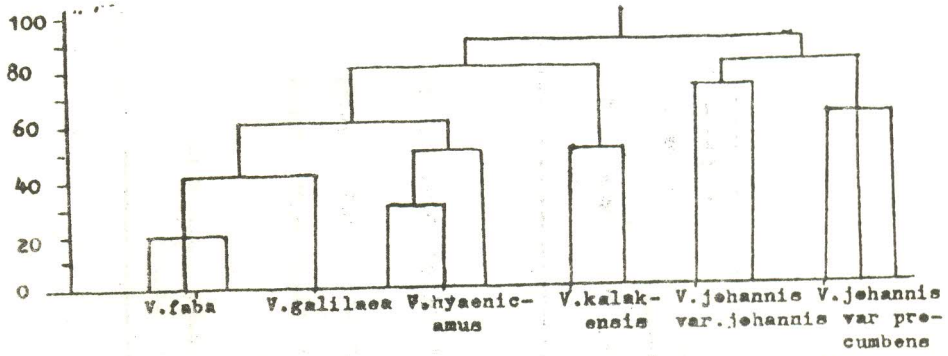


Fig.(1):Dendrogram of similarity between some taxa of section faba .

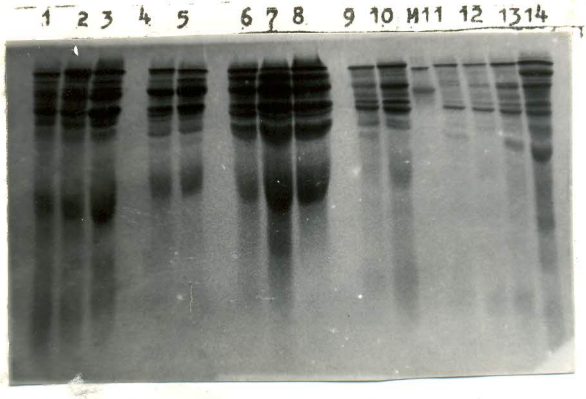


Fig. ( 2 , A ) : Electrophoretic separation pattern of albumin fraction .

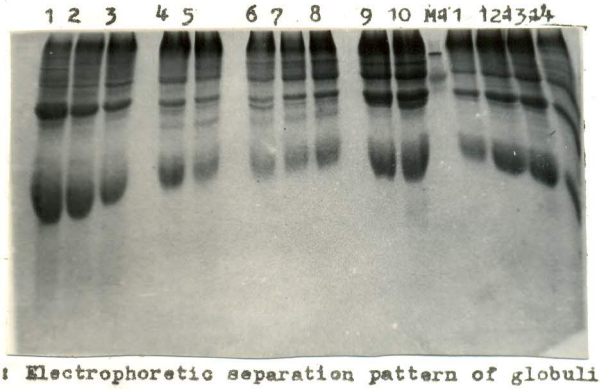


Fig. ( 2 , B ) : Electrophoretic separation pattern of globulin fraction.

Fig. ( 2 , A , B ) : Electophoretic separation patterns of some Vicia species by SDS - PAGE according to Laemmeli ( 1970 ) in Tris-glycine buffer at pH 8.3 .

(1) V. faba var.faba (UK) (2) V. faba var.faba ( India )
(3) V. faba var.faba (Crete ) (4) V. kalakhensis ( Syria* 1 )
(5) V. kalakhensis ( Syria ** 2 ) (6) V. hyaeniscyamus ( Syria 1 )
(7) V. hyaeniscyamus ( Syria 2 ) (8) V. hyaeniscyamus ( Syria 3 )
(9) V. johannis var.johannis ( USSR) (10)V. johannis var.johannis (Turkey)
(M) Pretein marker (Bovin albumin 67 K.D., Ovalbumin 43 K.D., and Lysozyme 14.3 K.D. ) .
(11)V. johannis var. procumbens (USSR) (12)V. johannis var. procumbens (Turkey 1 )
(13)V. johannis var. procumbens (Turkey 2 ) (14)V. galilaea ( Israel )

showed strong spot in one sample. The major bands of 55, 50, 20, 15 were identified in most wild species except in V. johannis var. procumbens which the bands could not or fant identified.

Finally, it could be concluded that, V. galilaea have identical protein bands to those of V. hyaeniscyamus, V. kalkhensis and V. johannis var. johannis as well as to that of the cultivated V. faba. These results are in agreement with those obtained in the present study by analysing the morphgological characters (Fig. 1).

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# استخدام التحاليل الكيماويه الحيويه

## للاحماض النوويه والبروتين فى الدراسات التقسيميه لبعض اصناف جنس الفـــــول

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اجرى هذا البحث لتحديد مدى امكانية استخدام بعض أصناف الفول البريـــة كمصدر للبروتين وذلك بدراسة محتواها الكلى من البروتين والاحماض النوويـــة والامينيه وتفريد وحدات البروتين المفصوله للتفريد الكهربائى . بالاضـــافة امكانية استخدام التحليلات الكيماويه السابقه لتدعيم الدراسات التقسيميـــة المورفولوجيه المستخدمه فى تصنيف وتقسيم هذه الاصناف المزروعه والبريـــة .

أوضحت النتائج البيوكيميائيه والمورفولوجيه أن الصنف المزروع V.faba مميز فى معظم صفاته المورفولوجيه والكيميائيه عن بقية الاصناف البريـــة داخل نفس القسم التابعين له section Faba كما أن الاصناف البريـــة لها العديد من الصفات المتشابهه والتى تتميز بها عن الصنف المزروع وهذا بالاضافة الى وجود درجة تشابه بين تلك الاصناف مما يجعلها تصنف داخل نفس القسم .

وقد أوصى البحث بامكانية استخدام بعض الاصنـــاف البرية مثـــل V.johannis و V.hyaeniscyamus كمصدر للبروتين وذلك بعد اجراء الدراسات البيولوجية اللازمـــة . كذلك بأهمية استخدام التحليلات البيوكميائية لتدعيـــم وتأكيد الدراسات التقسيميـــة .