*CATRINA* (2008), 3 (1): 61 -70
PROCEEDINGS OF THE SECOND INTERNATIONAL CONFERENCE ON THE ROLE OF GENETICS AND BIOTECHNOLOGY IN CONSERVATION OF NATURAL RESOURCES, ISMAILIA, EGYPT, JULY 9-10, 2007


# Cytogenetics Comparison of Cultivated and Wild Relatives of Genus *Vigna* in Egypt

**Magda I. Soliman[1], Reda M. Rizk[2], and Noha M. El-Eraky[1]**
[1]Botany Department, Faculty of Science, Mansoura University, Egypt
[2]National Gene Bank, Ministry of Agriculture and Land Reclamation, Egypt

**ABSTRACT**

Cytogenetics and morphological studies have been conducted to compare between the cultivated and wild relatives of the genus *Vigna in* Egypt. Eleven germplasms, representing three species of the genus *Vigna*, were obtained from National Gene Bank (NGB), as well as collected taxa from natural habitats. In general, the plant hairness, flower color, pod wall thickness, cotyledon color, seed color, eye pattern and color, seed turgidity and seed crowding are the most important morphological attributes to distinguish between two the subgenera, *Vigna* and *Ceratotropis* of the genus *Vigna.* All the studied *Vigna* germplasms are diploid with twenty-two chromosomes in somatic cells. Germplasm of the subgenus *Ceratotropis* recorded the highest interchromosomal asymmetry index (A2, 0.21 - 0.28) and only two chromosome types (nsm(-) & nm) were recorded in the karyotype formula. Types and proportions of mitotic abnormalities were recorded. The electrophoretic protein analysis showed twenty-six bands of molecular weight ranging from 73 to 45.25 KD. The unique band of molecular weight 64.750 KD was found to be specific to *Vigna unguiculata* subspecies *unguiculata* cv-group *unguiculata* cv. Fodder. While the band of molecular weight 46.000 and 45.250 KD were specific to *Vigna unguiculata* subspecies *unguiculata* cv-group *unguiculata* cv. Kafer El-Sheikh. These bands could be taken as a positive marker for the two cultivars. Cluster analysis and PERMAP-Biplot between the studied eleven germplasms of genus *Vigna* revealed the importance of helim colour (attribute 39), eye length (attribute 37), length of space between cotyledons (attribute 41), standard petal width (attribute 8), pod curvature (attribute 18), polyploidy%, total chromosome volume (attribute 62), and chromosome radius (attribute 63) to split subgenus *Vigna* into two sections: *Catiang* and *Vigna*.

**Keywords:** Cytogenetics, electrophoresis, *Vigna luteola, Vigna radiate, Vigna unguiculata*

## INTRODUCTION

The genus *Vigna* family *Fabaceae* – Pea family is native to the warm regions of both the old and the new world (Richard, 2002). Genus *Vigna* comprise two species that are of considerable economic importance, Cowpeas [*V. unguiculata* (L.) Walp.] and mung beans [*V. radiata* (L.) Wilczek]. The *Vigna* is one of the most important legume vegetables grown in Egypt. Not only because of its high protein content that range from 22% to 28%, but value as forage, and green manure crops (Richard, 2002). The protein in cowpeas seed is rich in the amino acids, lysine and tryptophan, compared to cereal grains. However, it is deficient in methionine and cystine when compared to animal proteins (Small, 1999). Therefore, cowpeas seed is valued as a nutritional supplement to cereals and an extender of animal proteins.

The genus *Vigna* has been subjected to cytotaxonomical studied by Galasso *et al*. (1992 & 1993) and Zheng *et al.* (1991).

A sound breeding program is primarily based on evaluation and exploitation of the collected and preserved genetic diversity. The *Vigna* germplasm which are available at National Gene Bank of Egypt are subjected to morphological and cytogenetics characterization to be available for breeding program and to meet the need of the national strategies of sustainable uses of genetic resources for food and agriculture.

## MATERIALS AND METHODS

### (1) Seed collections

Viable seeds of the studied cultivated taxa (Plate 1) were obtained from National Gene Bank (NGB), Agricultural Research Center (ARC), Giza, Egypt. Seeds of wild taxa were collected from two different ecotypes from Rosetta in El-Behera Governorate and Basendela in Dakahlia Governorate (Table 1).

### (2) Morphological data

For morphological data, the collected seeds were cultivated in the greenhouse of Mansoura University, Dakahlia Governorate. The characterization and evaluation data of the studied taxa basically follows the standard format descriptor list for *Vigna* (IBPGR, 1983). The morphological measurements given are the mean of twenty-five healthy well-developed plant materials. Stearn (1973) was used for terminology of seed characters.

### (3) Cytological analysis

Actively growing root tips were pretreated for 2-4 h in 0.002 M 8-hydroxyquinoline (Tjio and Levan, 1950). Examination of roots was done in permanent root tip squash preparations by using different staining techniques: 4% alcoholic hydrochloric carmine (Snow, 1963), 2% aceto orcein after acid treatment (Chattopadhyay and Sharma, 1988) and modified carbol fuchsine (Koa, 1975 a and b). It was found that

* *Corresponding author: magdaisoliman@yahoo.com*

**Table (1):** List of investigated taxa.

| No. | Taxon |
|---|---|
| 1 | *Vigna unguiculata* (L.) Walp. subspecies *unguiculata* cv- group *unguiculata* E.Westphal cultivar Doki-126 |
| 2 | *Vigna unguiculata* (L.) Walp. subspecies *unguiculata* cv-group *unguiculata* E.Westphal cultivar Doki-331 |
| 3 | *Vigna unguiculata* (L.) Walp. subspecies *unguiculata* cv-group *unguiculata* E.Westphal cultivar Kafr El-Sheikh |
| 4 | *Vigna unguiculata* (L.) Walp. subspecies *unguiculata* cv-group *unguiculata* E.Westphal cultivar Kahha-1 |
| 5 | *Vigna unguiculata* (L.) Walp. subspecies *unguiculata* cv-group *unguiculata* E.Westphal cultivar Kream-7 |
| 6 | *Vigna unguiculata* (L.) Walp. subspecies *unguiculata* cv-group *unguiculata* E.Westphal cultivar Fodder |
| 7 | *Vigna luteola* (Jacq.) Benth. ecotype Rosetta |
| 8 | *Vigna luteola* (Jacq.) Benth. ecotype Basendela |
| 9 | *Vigna radiata* (L.) R. Wilcz. variey *radiata* cultivar Qumi 1 |
| 10 | *Vigna radiata* (L.) R. Wilcz. variey *radiata* genotype VC2719 |
| 11 | *Vigna radiata* (L.) R. Wilcz. variey *radiata* genotype L303 |

modified carbol fuchsine with aceto orcein after acidtreatment gave the best results. The chromosome types identified following Abraham and Prasad (1983). Karyotypes analysis was carried out using "Micro Measure" computer program (Reeves, 2001). The mean measurements of three cells for each taxon were used to construct the karyotype. Types and proportions of chromosomal abnormalities observed at mitotic division were recorded for all *Vigna* species examined.

**(4) Protein analysis**

According to Bradford (1976) total protein extracts of seeds were analyzed. For electrophoresis analysis, the method for discontinuous SDS-PAGE techniques was based on that of Laemmli (1970). The analysis percentages of the bands were carried out using BIO-RAD Video densitometer. The similarity coefficient between the species was based on comparisons of their SDS-PAGE profiles.

The gel was photographed and analyzed using BIO-RAD Video documentation system, Model Gel Doc 2000. The relationship between the cultivars was measured by calculating their average (genetic) taxonomic distance and presented as phenogram using SYSTAT version 7.0 (Wilkinson, 1997).

## RESULTS AND DISCUSSION

**(I) Morphological attributes**

Plate (1) and table (2) show the distinguished morphological attributes of the studied taxa of cultivated and wild relatives of genus *Vigna* in Egypt. These morphological attributes have been characterized as reported by IPGRI, 1983.

The comparison of the two subgenera, *Vigna* and *Ceratotropis*, revealed that, the Plant hairiness, flower color, pod wall thickness, cotyledon color; seed color, eye pattern and color, seed turgidity and seed crowding are the most important morphological attributes to distinguish between the two subgenera of the genus *Vigna.*

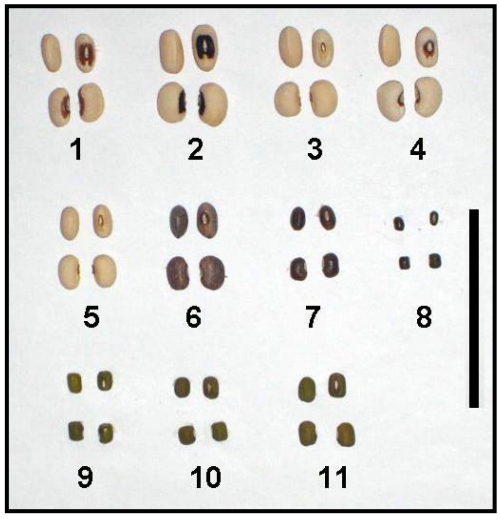


**Plate (1):** Seeds of studied taxa of *Vigna.* For taxa name see table (1). Bar = 10 cm.

Concerning the differences between the two species of subgenus *Vigna,* the plant hairiness, flower color and pod attributes are the most useful criteria.

**(II) Karyotype analysis:**

The taxa under study are diploid, twenty-two chromosomes are observed in somatic cells of the eleven studied taxa of *Vigna* (Plate 2) which coincides with previous cytological reports on the other species of the same genus (Venora *et. al*, 1995 and 1999).

Karyogram of each taxon are illustrated in Plate (3) and haploid idiogram in figure (1). The symmetric indices (Syi), resemblance between chromosomes (Rec), Total form percentage (TF%), intrachromosomal asymmetry index ($A_1$), interchromosomal asymmetry index ($A_2$), symmetry percent (S%), mean chromosome length (MCL),chromosome radius and Total chromosome volume (TCV) of all taxa are stated in table (3).

*Vigna radiata* variety, *radiata* Line, VC2719 recorded the smallest values of mean chromosome length (MCL, 1.177μ), total complement length (TCL,

**Table (2):** Morphological attributes of the studied taxa of genus *Vigna*, (**V.u.**: *Vigna unguiculata*, **V.l.** : *Vigna luteola*, **V.r.** : *Vigna radiata*).

| No. | | (V.u.) Doki-126 | (V.u.) Doki-331 | (V.u.) Kafer El-Sheikh | (V.u.) Kahha 1 | (V.u.) Kream7 | (V.u.) Fodder | (V.l.) Rossita | (V.l.) Basendela | (V.r.) Qumi | (V.r.)Vc2719 | (V.r.) L303 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Plant hight (cm) | 32 | 50 | 60 | 45 | 70 | 95 | 105 | 98 | 100 | 98 | 95 |
| 2 | Number of branches | 3 | 3 | 4 | 4 | 5 | 12 | 4 | 5 | 7 | 6 | 7 |
| 3 | Plant hairness (1.glabrous, 2.slightly hairy, 3.hairy) | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 3 | 3 | 3 |
| 4 | Plant reach maturity (week) | 12 | 13 | 14 | 12 | 15 | 15 | 14 | 15 | 12 | 14 | 13 |
| 5 | Flower colour (1.white-purple, 2.white-yellow, 3.pale yelow, 4.yellow) | 1 | 1 | 1 | 2 | 2 | 2 | 4 | 4 | 3 | 3 | 3 |
| 6 | Flower length (mm) | 20 | 19 | 20 | 20 | 20 | 22 | 24 | 22 | 18 | 20 | 18 |
| 7 | Calyx lobe length (mm) | 4.2 | 4.2 | 3.9 | 4 | 4.3 | 4 | 4 | 4 | 2.9 | 2.7 | 2.9 |
| 8 | Standard petal width (mm) | 25 | 26 | 23 | 25 | 24 | 27 | 29 | 26 | 19 | 18 | 19 |
| 9 | Standard petal length (mm) | 18 | 18 | 19 | 20 | 19 | 21 | 23 | 20 | 17 | 19 | 17 |
| 10 | Appear of first flower after planting (week) | 7 | 7 | 8 | 7 | 8 | 10 | 7 | 8 | 7 | 8 | 7.5 |
| 11 | Pod length (cm) | 12 | 11 | 11.5 | 7.5 | 11.5 | 12.5 | 7.5 | 5.5 | 6 | 5.5 | 3 |
| 12 | Pod width (cm) | 0.8 | 0.8 | 0.8 | 0.85 | 0.85 | 0.9 | 0.75 | 0.65 | 0.65 | 0.65 | 0.65 |
| 13 | Pod weight (g) | 0.42 | 0.51 | 0.46 | 0.33 | 0.37 | 0.85 | 0.27 | 0.19 | 0.37 | 0.19 | 0.10 |
| 14 | Pod wall thickness (1.thin, 2.intermediate, 3.thick) | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 3 | 3 | 3 |
| 15 | Number of locules per pod | 15 | 10 | 12 | 7 | 12 | 16 | 9 | 11 | 7 | 8 | 4 |
| 16 | Number of seeds per pod | 11 | 9 | 12 | 5 | 11 | 16 | 9 | 10 | 7 | 7 | 4 |
| 17 | Seed/locule % | 73.3 | 90.0 | 100 | 71.4 | 91.7 | 100 | 100 | 90.9 | 100 | 87.5 | 100 |
| 18 | Pod curvature (1.Straight, 2.slightly curved) | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 1 | 1 |
| 19 | Pod color (1.pale tan or straw, 2.dark tan, 3.tan brown, 4.black or dark purple, 5.dark green) | 1 | 1 | 2 | 2 | 2 | 1 | 3 | 4 | 4 | 5 | 5 |
| 20 | Pod surface (1. glabrous, 2. Slightly hairy, 3. hairy) | 1 | 1 | 1 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 |
| 21 | Pod attachment to peduncle (1.pendant, 2.30-90 from erect) | 1 | 2 | 1 | 1 | 1 | 2 | 2 | 2 | 2 | 2 | 2 |
| 22 | Texture (1.hard, 2.semi hard, 3.not hard | 3 | 3 | 3 | 2 | 3 | 2 | 1 | 1 | 1 | 1 | 1 |
| 23 | Seed length (mm) | 14.3 | 14.8 | 15.1 | 13.4 | 14.4 | 12.3 | 11.4 | 12.7 | 13.2 | 14.8 | 15.5 |
| 24 | Seed width (mm) | 6.40 | 6.64 | 6.44 | 4.88 | 6.22 | 5.48 | 4.78 | 4.92 | 6.08 | 6.40 | 5.52 |
| 25 | Seeds weight/100 (g) | 15.0 | 20.3 | 16.5 | 18.3 | 12.9 | 11.9 | 3.9 | 1.3 | 4.3 | 5.2 | 6.2 |
| 26 | Seed length/Width ratio | 2.23 | 2.23 | 2.35 | 2.75 | 2.32 | 2.24 | 2.38 | 2.59 | 2.18 | 2.31 | 2.81 |
| 27 | Seed shape (1.Kidny, 2.ovoid, 3.rhomboid) | 1 | 1 | 1 | 2 | 1 | 1 | 2 | 3 | 3 | 3 | 3 |
| 28 | Seed volume (cm^3) | 0.14 | 0.20 | 0.14 | 0.17 | 0.10 | 0.10 | 0.05 | 0.01 | 0.03 | 0.04 | 0.05 |
| 29 | Seed colour (1.creamy, 2.mottled brown, 3.dark brown, 4.dark green, 5.black) | 1 | 1 | 1 | 1 | 1 | 2 | 3 | 5 | 4 | 4 | 4 |
| 30 | Testa texture (1.smooth, 2.rough, 3.rough to wrinkled , 4.wrinkled) | 4 | 3 | 3 | 2 | 3 | 1 | 1 | 1 | 1 | 1 | 1 |
| 31 | Seed surface nature (0.not shiny, 1.shiny) | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| 32 | Eye pattern (0.absent, 1.narrow eyes, 2.small eyes, 3.holstein group, 4.self coloured) | 3 | 3 | 0 | 1 | 0 | 2 | 2 | 2 | 4 | 4 | 4 |
| 33 | Eye colour (0.creamy, 1.brown, 2.tan brown, 3.green, 4.black) | 1 | 4 | 0 | 2 | 0 | 2 | 2 | 2 | 3 | 3 | 3 |
| 34 | Eye length (mm) | 3.00 | 2.70 | 2.25 | 3.50 | 2.25 | 2.50 | 2.30 | 1.80 | 1.38 | 1.72 | 1.69 |
| 35 | Eye width (mm) | 1.35 | 1.50 | 1.00 | 1.36 | 1.00 | 1.35 | 9.90 | 0.70 | 0.50 | 0.49 | 5.20 |
| 36 | Eye length/width ratio | 2.22 | 1.80 | 2.25 | 2.57 | 2.25 | 1.85 | 0.23 | 2.57 | 2.76 | 3.51 | 0.33 |
| 37 | Eye length / seed length (%) | 21.0 | 18.2 | 14.9 | 26.0 | 15.6 | 20.4 | 20.2 | 14.2 | 10.4 | 11.7 | 10.9 |
| 38 | Eye width / seed width (%) | 21.1 | 22.6 | 15.5 | 27.9 | 16.1 | 24.6 | 20.7 | 14.2 | 8.2 | 7.7 | 9.4 |
| 39 | Helim colour (0.absent, 1.white, 2.brown, 3.brown-black, 4.black) | 2 | 4 | 0 | 4 | 0 | 3 | 1 | 1 | 1 | 1 | 1 |
| 40 | Cotyledones colour (1.white, 2.pale creamy, 3.dark creamy, 4. Pale yellow, 5.faint green) | 1 | 1 | 2 | 4 | 3 | 2 | 1 | 1 | 5 | 5 | 5 |
| 41 | Lenth of space between cotyledons (mm) | 2.10 | 3.50 | 2.25 | 3.10 | 2.00 | 2.94 | 2.85 | 0.89 | 1.85 | 1.87 | 1.46 |
| 42 | Width of space between cotyledons (mm) | 0.50 | 1.30 | 0.90 | 1.25 | 0.90 | 0.65 | 1.60 | 0.86 | 0.40 | 0.38 | 0.49 |
| 43 | Lenth of space between cotyledons/seed length (%) | 14.7 | 23.7 | 14.9 | 23.1 | 13.9 | 24.0 | 25.1 | 7.0 | 14.0 | 12.7 | 9.4 |
| 44 | Width of space between cotyledons/seed width (%) | 7.8 | 19.6 | 14.0 | 25.1 | 14.5 | 11.9 | 33.5 | 17.5 | 6.6 | 5.9 | 8.9 |
| 45 | Area of space between cotyledons (mm3) | 1.05 | 4.55 | 2.03 | 3.88 | 1.80 | 1.91 | 4.56 | 0.77 | 0.74 | 0.71 | 0.72 |
| 46 | Space between cotyledons area / seed area (%) | 1.15 | 4.63 | 2.08 | 5.91 | 2.01 | 2.84 | 8.39 | 1.22 | 0.92 | 0.75 | 0.84 |
| 47 | Seed turgidity (1.less turged, 2.mediate terged, 3.terged, extremely terged) | 3 | 2 | 3 | 1 | 3 | 2 | 1 | 3 | 4 | 4 | 4 |
| 48 | Space between cotyledons orientation (1.dorsal, 2.central, 3.ventral) | 1 | 1 | 1 | 2 | 2 | 3 | 2 | 2 | 3 | 3 | 2 |
| 49 | Attachment of testa(1. testa not firmly attached to seed, 2.testa firmly attached to seed) | 2 | 2 | 2 | 2 | 2 | 2 | 1 | 2 | 2 | 2 | 2 |
| 50 | seed crowding (1.Crowd, 2.semi-crowd, 3.not crowd) | 3 | 3 | 3 | 3 | 3 | 3 | 2 | 2 | 1 | 1 | 1 |

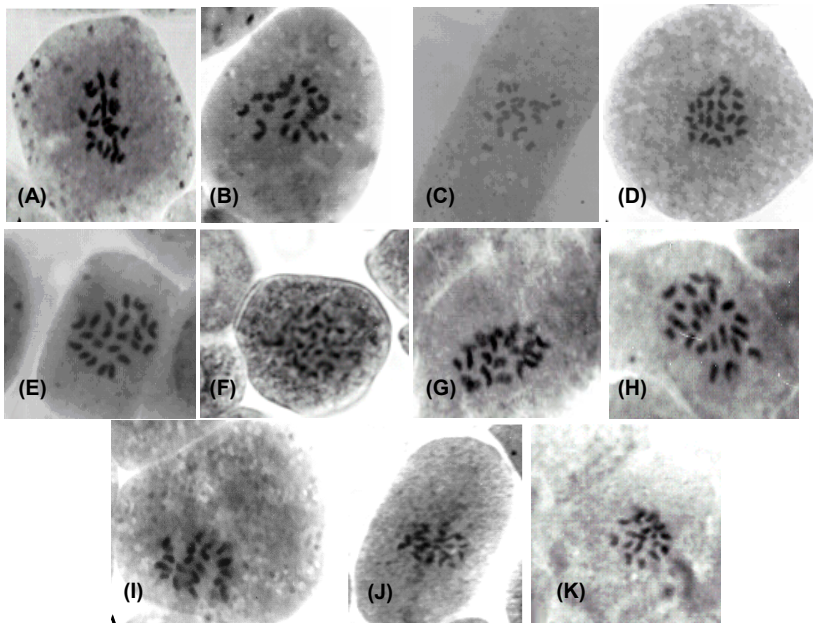


**Plate (2):** Somatic cell with chromosome number (2n=22).**(A)** (V.u.) Doki-126, **(B)** (V.u.) Doki- 31, **(C)** (V.u.) Kafr EL- Sheikh, **(D)** (V.u.) Kahha-1, **(E)** (V.u.) Kream-7, **(F)** (V.u.) Fodder, **(G)** (V.l.) Rosetta, **(H)** (V.l.)Basendela, **(I)** (V.r.) Qumi-1, **(J)** (V.r.) VC2719 and **(K)** (V.r.) L303, **(X=1000).**

**Table (3):** The karyotype formula and the average measurements of somatic chromosome morphology of genus *Vigna* in Egypt (V.u.: *Vigna unguiculata*, V.l.: *Vigna luteola*, V.r.: *Vigna radiata*).

| Taxon | Karyotype formula | Syi index | Rec index | TF% | A1 | A2 | S% | MCL | TCV |
|---|---|---|---|---|---|---|---|---|---|
| **(V.u.) Doki-126** | 3nsm(-)+6nm+2M | 0.42 | 0.71 | 72.56 | 0.29 | 0.14 | 0.36 | 1.40 | 11.80 |
| **(V.u.) Doki-331** | 1nsm(+)+6nsm(-)+4nm | 0.36 | 0.78 | 56.91 | 0.40 | 0.15 | 0.23 | 1.26 | 18.55 |
| **(V.u.) Kafer El-Sheikh** | 2nsm(-)+8nm+1M | 0.45 | 0.84 | 82.05 | 0.18 | 0.13 | 0.43 | 1.30 | 18.92 |
| **(V.u.) Kahha 1** | 4nsm(-)+6nm+1M | 0.40 | 0.66 | 67.94 | 0.31 | 0.15 | 0.37 | 1.21 | 11.04 |
| **(V.u.) Kream7** | 4nsm(-)+5nm+2M | 0.41 | 0.71 | 69.41 | 0.29 | 0.15 | 0.35 | 1.51 | 18.15 |
| **(V.u.) Fodder** | 2nsm(-)+7nm+2M | 0.43 | 0.77 | 74.74 | 0.23 | 0.19 | 0.31 | 1.50 | 11.71 |
| **(V.l.) Rosita** | 7nsm(-)+3nm+1M | 0.36 | 0.75 | 57.83 | 0.38 | 0.15 | 0.25 | 1.54 | 16.55 |
| **(V.l.) Basendela** | nsm(+)+5nsm(-)+4nm+1M | 0.38 | 0.74 | 61.48 | 0.38 | 0.16 | 0.23 | 1.31 | 19.84 |
| **(V.r.) Qumi** | 4nsm(-)+7nm | 0.41 | 0.65 | 68.22 | 0.28 | 0.21 | 0.29 | 1.35 | 12.00 |
| **(V.r.)Vc2719** | 3nsm(-)+8nm | 0.42 | 0.75 | 71.46 | 0.27 | 0.21 | 0.34 | 1.18 | 7.32 |
| **(V.r.) L303** | 5nsm(-)+6nm | 0.38 | 0.64 | 59.49 | 0.36 | 0.28 | 0.21 | 1.34 | 12.96 |

25.90 µ) and total chromosome volume (TCV, 7.321 $\mu^3$). *Vigna luteola* collected from Rosetta recorded the highest values of mean chromosome length (MCL, 1.54µ) and total complement length (TCL, 33.95 µ), While *Vigna luteola* collected from Basendela recorded the highest value of total chromosome volume (TCV, 19.842 $\mu^3$).

Samples in the subgenus*: Ceratotropis* recorded the highest interchromosomal asymmetry index (A2, 0.21 - 0.28) and only two chromosome types (nsm(-) & nm) were recorded in the karyotype formula.

*Vigna unguiculata* subspecies *unguiculata* cv-group *unguiculata* cv. Dokki-331, *Vigna luteola* collected from both Basendela and Rosetta and *Vigna radiata* variety *radiata* form L303 showed the lower values of the symmetric indices (Syi), total form percentage (TF%) and symmetry percent (S%). It is considered to be high evolved karyotype than the other studied taxa. The types and proportions of abnormalities observed at mitotic division are summarized in table (4). The highest percentage of total abnormalities (2.35) was shown in *Vigna unguiculata subspecies unguiculata cv-group unguiculata cv.* Kafer El-Sheikh, while the lowest percentage of total abnormalities (1.42) was shown in *Vigna luteola* collected from Basendela.

**(III) Seed proteins:**

The protein profile of the investigated taxa of Genus *Vigna* is given in table (5). Twenty-six bands of molecular weight ranging from 73 to 45.25 KD was

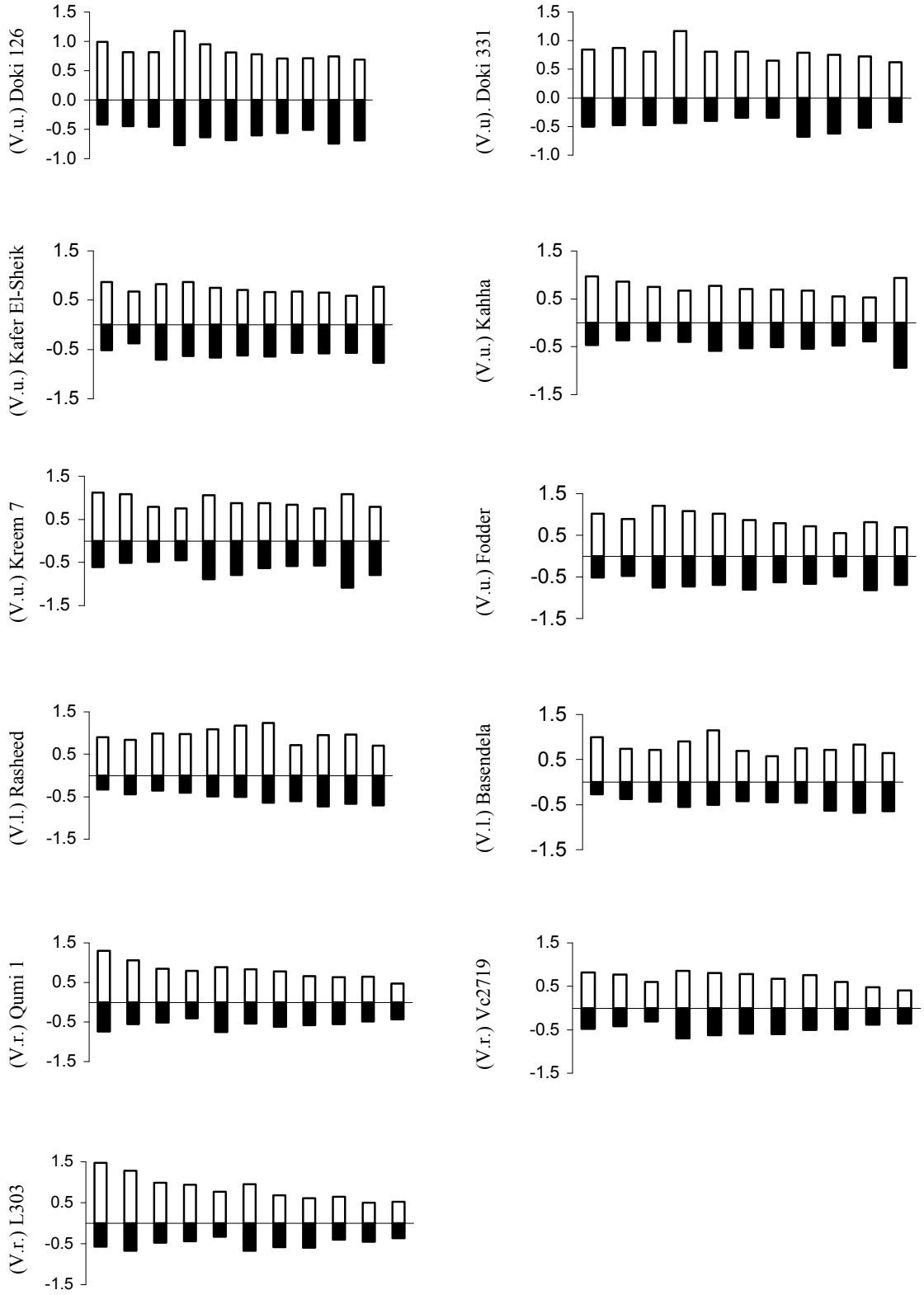


**Figure (1):** Idiogrammatic representation of the haploid karyotype of genus *Vigna* in Egypt.

□ long arm, ■ short arm.(V.u.: *Vigna unguiculata*, V.l. : *Vigna luteola*, V.r. : *Vigna radiata*).

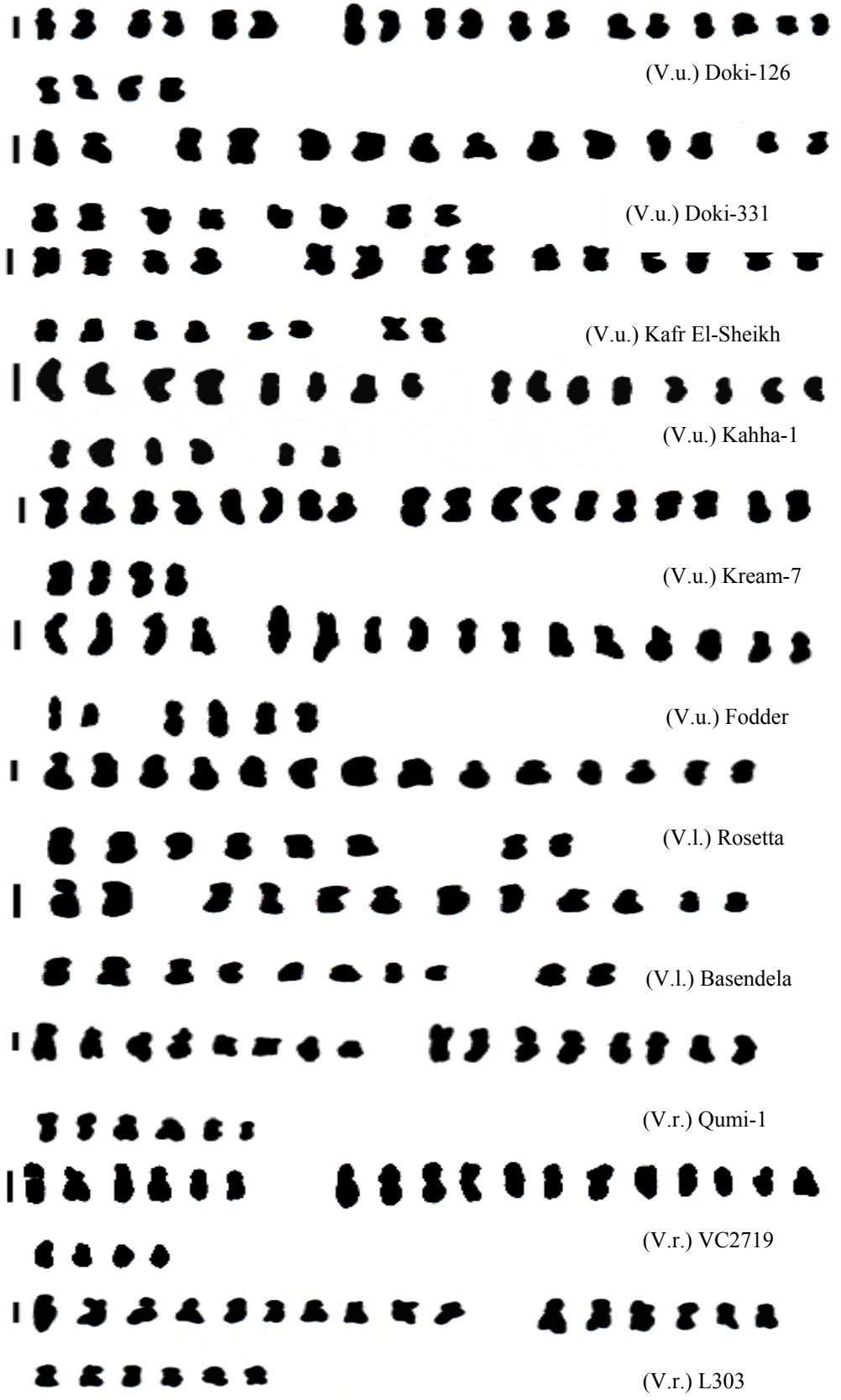


**Plate (3):** Karyogram of the studied taxa.

**Table (4):** Mitotic abnormalities percentage for all *Vigna* species examined. Total number of cells examined equal 10,000 (V.u.: *Vigna unguiculata*, V.l.: *Vigna luteola*, V.r.: *Vigna radiata*).

| Taxon | % of Normal cells | % of abnormal cells | | | | |
|---|---|---|---|---|---|---|
| | | Bridge | Laggards | Polyploidy | Non-congression | Total abnormalities |
| **(V.u.) Doki-126** | 97.667 | 0.000 | 0.333 | 1.333 | 0.667 | 2.333 |
| **(V.u.) Doki-331** | 98.324 | 0.186 | 0.000 | 0.466 | 1.024 | 1.676 |
| **(V.u.) Kafer El-Sheikh** | 97.649 | 0.000 | 0.313 | 0.784 | 1.254 | 2.351 |
| **(V.u.) Kahha 1** | 97.870 | 0.000 | 0.000 | 0.932 | 1.198 | 2.130 |
| **(V.u.) Kream7** | 97.797 | 0.000 | 0.000 | 0.826 | 1.377 | 2.203 |
| **(V.u.) Fodder** | 98.105 | 1.115 | 0.000 | 0.000 | 0.780 | 1.895 |
| **(V.l.) Rosita** | 97.512 | 0.000 | 0.000 | 1.493 | 0.995 | 2.488 |
| **(V.l.) Basendela** | 98.585 | 0.000 | 0.000 | 0.849 | 0.566 | 1.415 |
| **(V.r.) Qumi** | 97.945 | 0.000 | 0.000 | 0.514 | 1.541 | 2.055 |
| **(V.r.)Vc2719** | 98.145 | 1.082 | 0.000 | 0.464 | 0.309 | 1.855 |
| **(V.r.) L303** | 97.873 | 0.532 | 0.000 | 0.399 | 1.196 | 2.127 |

**Table (5):** Protein profile of the studied taxa of genus *Vigna*, (V.u.: *Vigna unguiculata*, V.l.: *Vigna luteola*, V.r.: *Vigna radiata*) 0: absent, 1 present.

| No. | Dalton | (V.u.) Doki-126 | (V.u.) Doki-331 | (V.u.) Kafer El-Sheikh | (V.u.) Kahha 1 | (V.u.) Kream7 | (V.u.) Fodder | (V.l.) Rossita | (V.l.) Basendela | (V.r.) Qumi | (V.r.)Vc2719 | (V.r.) L303 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **1** | 73000 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **2** | 72500 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| **3** | 72000 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 |
| **4** | 69500 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 |
| **5** | 69000 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| **6** | 67000 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| **7** | 65500 | 1 | 1 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| **8** | 64750 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| **9** | 64000 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 1 |
| **10** | 63000 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 1 | 1 | 1 |
| **11** | 62500 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **12** | 62000 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 |
| **13** | 61750 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| **14** | 61500 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **15** | 61000 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **16** | 60500 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 |
| **17** | 59500 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 |
| **18** | 58500 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 |
| **19** | 55500 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **20** | 54500 | 1 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **21** | 53500 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 |
| **22** | 53000 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| **23** | 50500 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 |
| **24** | 49500 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 1 | 0 |
| **25** | 46000 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| **26** | 45250 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Polymorphic | | 8.84 | 5.44 | 10.2 | 2.72 | 4.76 | 6.80 | 8.84 | 9.52 | 9.52 | 10.2 | 8.16 |
| Total seed protein | | 22.1 | 23 | 21.6 | 23.2 | 18.2 | 27.1 | 24.2 | 15.4 | 25.4 | 24.3 | 24.3 |
| ∑ no. of bands | | 15 | 10 | 17 | 6 | 9 | 12 | 15 | 16 | 16 | 17 | 14 |

detected from the studied taxa of genus *Vigna* in Egypt. The highest protein profile (17 bands, polymorphic bands % is 10.20) was recorded in both *Vigna unguiculata* subspecies *unguiculata* cv-group *unguiculata* cv. Kafer El-Sheikh and *Vigna radiata* variety radiata form VC2719, while the lowest protein profile (6 bands, polymorphic bands % is 2.72) was recorded in *Vigna unguiculata* subspecies *unguiculata* cv-group *unguiculata* cv. Kahha-1.

The unique band of molecular weight 64.750 KD was found to be specific to *Vigna unguiculata* subspecies *unguiculata* cv-group *unguiculata cv.* Fodder, while the band of molecular weight 46.000 and 45.250 KD were specific to *Vigna unguiculata* subspecies

**Table (6):** The Pearson correlation matrix between 11 taxa of genus *Vigna* in Egypt. (V.u.: *Vigna unguiculata*, V.l.: *Vigna luteola*, V.r.: *Vigna radiata*)

| | (V.u.) Doki-126 | (V.u.) Doki-331 | (V.u.) Kafer El-Sheikh | (V.u.) Kahha 1 | (V.u.) Kream7 | (V.u.) Fodder | (V.l.) Rossita | (V.l.) Basendela | (V.r.) Qumi | (V.r.)Vc2719 | (V.r.) L303 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| (V.u.) Doki-126 | 1.000 | | | | | | | | | | |
| (V.u.) Doki-331 | 0.959 | 1.000 | | | | | | | | | |
| (V.u.) Kafer El-Sheikh | 0.973 | 0.976 | 1.000 | | | | | | | | |
| (V.u.) Kahha 1 | 0.968 | 0.975 | 0.962 | 1.000 | | | | | | | |
| (V.u.) Kream7 | 0.955 | 0.978 | 0.989 | 0.959 | 1.000 | | | | | | |
| (V.u.) Fodder | 0.926 | 0.958 | 0.969 | 0.943 | 0.987 | 1.000 | | | | | |
| (V.l.) Rosita | 0.641 | 0.690 | 0.632 | 0.713 | 0.660 | 0.694 | 1.000 | | | | |
| (V.l.) Basendela | 0.876 | 0.929 | 0.944 | 0.901 | 0.973 | 0.979 | 0.660 | 1.000 | | | |
| (V.r.) Qumi | 0.879 | 0.926 | 0.951 | 0.893 | 0.972 | 0.981 | 0.622 | 0.988 | 1.000 | | |
| (V.r.)Vc2719 | 0.879 | 0.913 | 0.947 | 0.895 | 0.966 | 0.979 | **0.615** | 0.984 | **0.996** | 1.000 | |
| (V.r.) L303 | 0.817 | 0.866 | 0.848 | 0.859 | 0.872 | 0.894 | 0.922 | 0.879 | 0.867 | 0.859 | 1.000 |

*unguiculata* cv-group *unguiculata cv.* Kafer El-Sheikh. These bands could be taken as a positive marker for the two cultivars**.**

The highest total seed protein (27.1) was recorded in *Vigna unguiculata* subspecies *unguiculata* cv-group *unguiculata* cv. Fodder, while the lowest (15.4) was recorded in *Vigna luteola* ecotype Basendela**.**

**(IV) Data analysis:**

Cluster analysis was conducted to generate a dendrogram (Fig.2) illustrating possible relationships among the eleven taxa of genus *Vigna* in Egypt based on morphological, cytogenetical attributes as well as protein analysis.

All taxa are divided into two groups at a distance of 7.65. The first group is including taxa of subgenus *Vigna* and the second group is including taxa of subgenus *Ceratotropis.* The first group was further divided into two subgroups at a distance of 6.78. The Pearson correlation matrix between the eleven taxa ofgenus *Vigna* was shown in table (6). The lowest similarity (0.615) was recorded between *Vigna radiata* variety *radiata* form VC2719 and *Vigna luteola* collected from Rosetta. The highest similarity (0.996) was recorded between *Vigna radiata* variety *radiata* forma L303 and cv. Qumi-1.

To get the linkage between the studied taxa of genus *Vigna* in Egypt and the most important useful, morphological and cytogenetics attributes, data matrix were standardized and the coordinates were computed for plotting Biplot mapping by using perceptual mapping (PERMAP). Perceptual mapping (PERMAP) using combination of taxa and attributes was shown in figure (3).

PERMAP-Biplot shows the importance of helim colour (attribute 39), eye length (attribute 37), length of space between cotyledons (attribute 41), standard petal width (attribute 8), pod curvature (attribute 18),

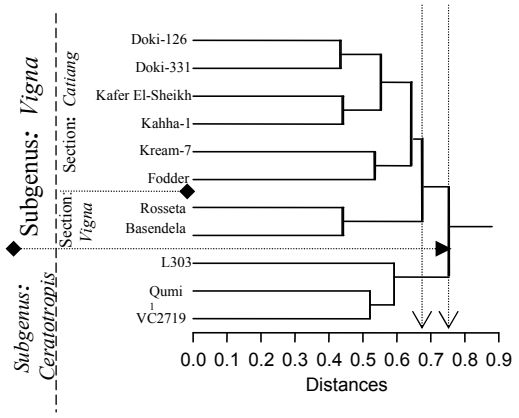


**Figure (2):** Phenogram showing the relationships between 11 taxa of genus *Vigna* in Egypt using Distance metric is normalized percent disagreement Complete linkage method (farthest neighbor).

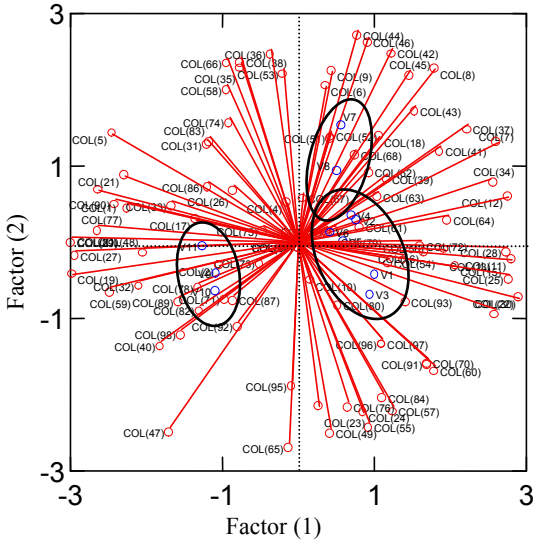


**Figure (3):** Perceptual mapping (Biplot) of the studied taxa of genus *Vigna* for combination of taxa and attributes, Configuration has been standardized. For Taxa name and attributes see table (1).

polyploidy %, total chromosome volume (attribute 62), chromosome radius (attribute 63), to splitting subgenus *Vigna* into two sections, *Catiang* and *Vigna* along the Factor 2 axis.

## REFERENCES


ABRAHAM, Z. AND P.N. PRASAD 1983. A system of chromosome classification and nomenclature. Cytologia **48**:95-101.

BRADFORD, M.M. 1976. A rapid and sensitive method for the quantitaion of microgram quantities of protein utilizing the principle of protein –dye binding. Anal. Biochem. **72**:248.

CHATTOPADHYAY, D. AND A.K. SHARMA 1988**.** A new technique for orcein banding with acid treatment. Stain Technology Vol.**63.**No.**5**: 283-287.

GALASSO I., D.PIGNONE AND P. PERRINO 1992**.** Cytotaxonomic studies in *Vigna.* I. General technique and *Vigna unguiculata* C-banding. Caryologia **45**: 155-161.

GALASSO I., D. PIGNONE AND P. PERRINO 1993**.** Cytotaxonomic Studies in *Vigna* .2. Heterochromatin Characterization in *Vigna unguiculata* and three Related Wild Species. Caryologia **46:** 275-282.

INTERNATIONAL BOARD FOR PLANT GENETIC RESOURCES (IBPGR) 1983. Descriptor for Cowpea. IBPGR SECRETARIAT Rome, 1983.

KOA, K.N 1975a. A nuclear staining method for plant protoplasts .In plant tissue culture methods Ch.10, O.L. Gamborg and L.R. Wetter (eds.), National Research Council of Canada, Ottawa, Ontaria.

KOA, K.N 1975b**.** A chromosomal staining method for cultured cells .In plant tissue culture methods Ch.10, O.L.Gamborg and L.R. Wetter (eds.), National Research Council of Canada, Ottawa, Ontaria.

LAEMMLI, U.K 1970. Cleavage of structural proteins during the heat of bacteriophage T4.Nature **227**:680-685.

REEVES, A. 2001. MicoMeasure: A new computer program for the collection and analysis of cytogenetic data. Genome **44**:439-443.

RICHARD, L. F. 2002**.** New Opportunities in *Vigna.* In: J. Janick and A. Whipkey (eds.) Trends in new crops and new uses. ASHS Press, Alexandria, VA.

SMALL, E. 1999. New crops for Canadian agriculture. In: J. Janick (ed.), Perspectives on new crops and new uses. p. 15–52. ASHS Press, Alexandria, VA.

SNOW, R. 1963. Alcoholic hydrochloric acid-carmine as a stain for chromosomes in squash preparation. Stain Tech.**38**: 9-13.

STEARN, W.T. 1973**.** Botanical Latin. Newton Abbott: David and Charles.

TJIO, J.H AND A. LEVAN 1950**.** The use of oxyquinoline in chromosome analysis. Anales de La Estac.Exper.De Aula dei **2**:21-64.

VENORA, G., I. GALASSO AND D. PIGNONE 1995 .Retrospects and Perspectives of Cytogenetical Studies in *Vigna.* Biologischen Zentralblatt **114:**231-241

VENORA, G., S. BLANGIFORTI AND R. CREMONINI 1999. Karyotype analysis of twelve species belonging to genus *Vigna. Cytologia,* **64**: 117-127.

WILKINSON, L. 1997. SYSTAT: The system analysis for statistics. SYSTAT, Evanston III.

ZHENG, J., M. NAKATA , H. UCHIYAMA, , H. MORIKAWA AND, R. TANAKA 1991. Giemsa C-banding patterns in serveral species in *Phaseolus* L. and *Vigna Savi*, Fabaceae. Cytologia, **56:** 459-466


Received July 10, 2007
Accepted February 10, 2008

# دراسات خلوية- وراثية مقارنة على جنس اللوبيا المنزرعة ونظائرها البرية في مصر

**ماجدة إبراهيم سليمان[1]، رضا محمد رزق[2]، نهى محمد العراقى1**
[1]قسم النبات، كلية العلوم، جامعة المنصورة، مصر
[2]البنك القومي للجينات، مركز البحوث الزراعية، وزارة الزراعة وإستصلاح الأراضى، مصر

## الملخص العربـــى

تهدف الدراسة الحالية إلى توضيح درجة القرابة الو راثية بين إحدى عشر وحدة تصنيفية من جنس اللوبيا المنزرعة وأقاربها البرية والمنتمية إلى ثلاث أنواع وشملت الأصناف المنزرعة ( *Vigna unguiculata, Vigna radiata, Vigna luteola*) (1)- *Vigna unguiculata* وفيها تم دراسة 6 أصناف زراعية وهى (كريم 7و دقي 126 ودقي 331 وكفر الشيخ و قها 1و لوبيا العلف). (2)-*Vigna radiata* (Mung bean) وفيها تم دراسة الصنف الزراعي قومي 1 بالإضافة إلى السلالتان (L303&VC2719)

وتم الحصول على هذه البذور من البنك القومي للجينات، مركز البحوث الزراعية بالقاهرة. كما تم جمع البذور البرية من موقعين بيئيين مختلفين: رشيد- محافظة البحيرة وبسنديلة – محافظة الدقهلية.

وقد أجريت دراسات على الشكل الظاهري وتشمل صفات البذور والأزهار والثمار والمجموع الخضري على النباتات قيد الدراسة . كما أجريت دراسات سيتولوجية واشتملت على دراسة الهيئة الكروموسومية (الكاريوتايب) وكذلك دراسة تماثل الكاريوتايب وذلك من خلال المعايير الآتية: (DCL, MCL, TCV, S%, TF%, A1, A2, Syi index, Rec Index)

وأيضا أجريت دراسة أنواع البروتينات للبذور باستخدام (SDS-PAGE) للأصناف المنزرعة والبرية وكذلك تعيين النسبة المئوية للبروتين الكلى في بذور جنس اللوبيا قيد الدراسة. وقد خلصت دراسة الشكل الظاهري على أن أهم الصفات المستخدمة للتمييز بين تحت الجنسين(*Vigna* & Ceratotropis) هي لون البذرة ولون الفلقة ولون الزهرة وازدحام البذور داخل القرن وسمك جدار القرن وتواجد الشعيرات على النبات ومنطقة السرة من حيث لونها ومساحتها.

وقد أظهرت الدراسة السيتولوجية أن العدد الكروموسومى لكل الوحدات التصنيفية قيد الدراسة كانت (2ن=22) ودلت النتائج على أنه لا يوجد مجموعة كروموسومية متماثلة في أي من أصناف اللوبيا قيد الدراسة وقد أظهرت نتائج تحليل الكاريوتايب أن الصنف الزراعي دقي 331 والسلالةL303 يعتبرا أكثر الأصناف تطورا بينما الصنف الزراعي كفر الشيخ أقل تطورا. كما أظهرت النتائج أنواعا من الشذوذات الكروموسومية ( مثل : التعدد الكروموسومى و القناطر الكروموسومية والكر وموسومات المتلكئة وكر وموسومات غير منتظمة الانقسام) في الأصناف المختلفة.

وتم فحص التباين في أنماط التفريد الكهربي للبروتين وذلك باستخدام تقنية الفصل الكهربي SDS-PAGE وجد أن العدد الكلى لأشرطة البروتين 26 شريطا ذات وزن جزئي يتروح بين 250.45 إلى 73.000 كيلو دالتون وكان عدد الأشرطة لأصناف اللوبيا المختلفة بين 6 أشرطة (قها 1) و 17 شريطا (كفرالشيخ & VC2719 ). وقد أوضحت الدراسة وجود اختلافات واضحة في أنماط البروتين ووجود بعض الأشرطة المميزة لبعض الأصناف.

وتم تحليل النتائج باستخدام برنامج (SYSTAT version 7.0) لتقدير علاقة القرابة بين الأنواع من خلال بناء شجرة قياس المسافة (Dendrogram) وقد تم عمل تحليل إحصائي لهذه البيانات التي تم الحصول عليها باستخدام طرق التحليل. وقد استطاعت هذه الطرق في التفريق بين الأصناف المختلفة حسب درجة قرابتها. كما أوضحت دراسة التصنيف العددي تجميع الوحدات قيد الدراسة إلى تحت جنسي (*Vigna & Ceratotropis*). كما تم تقسيم ال (*Vigna* ) إلى قسمين (*Vigna & Catiang*). وتم مناقشة انتماء الأصناف إلى المجموعات السابقة في ضوء وصفها التصنيفي طبقا لتصنيف جنس اللوبيا في دراسات سابقة.