تقدير التنوع الوراثي في بعض أصناف الفول البلدي بإستخدام الصفات المورفولوجية والدلائل الجزيئية

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الملخص العربي

تم دراسة التنوع الوراثي لثمانية أصناف من الفول البلدي بإستخدام خمسة أنواع مختلفة من الدلائل الجزيئية (RAPD, ISSR, SSR, EST, STS) بالإضافة إلي الصفات المورفولوجية، وأجري كل من التحليل الأساسي المنسق (PCOORDA) والتحليل العنقودي من أجل دراسة التنوع الوراثي بإستخدام كل من الصفات المورفولوجية والدلائل الجزيئية. وأظهرت النتائج أنه يمكن إستخدام الدلائل الجزيئية في دراسة التنوع الوراثي للحصول علي نتائج أكثر واقعية من الصفات المورفولوجية. تم الحصول علي إرتباط ضعيف (r = 0.32) بين كل من الدلائل المورفولوجية والجزيئية ويمكن حل هذه المشكلة عن طريق إستخدام الكثير من الصفات المورفولوجية. كذلك يمكن إستنتاج أن كلا من التحليل العنقودي والتحليل الأساسي المنسق أظهرا تقريبا نفس النتائج ولكن نتائج التحليل الأساسي المنسق تعطي توضيح أفضل من التحليل العنقودي في دراسة التنوع الوراثي ويمكن الإستفادة من هذا البحث في تحديد العلاقات الوراثية بين التراكيب الوراثية لإدخال المتباعد منها في برامج التربية.

DETERMINATION OF GENETIC DIVERSITY OF SOME FABA BEAN (*Vicia faba* L.) VARIETIES USING MORPHOLOGICAL TRAITS AND MOLECULAR MARKERS

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ABSTRACT: *Genetic diversity of eight faba bean (Vicia faba L.) varieties was investigated using different types of molecular markers (RAPD, ISSR, SSR, EST, STS) and morphological traits. Both cluster analysis and principal coordinate analysis (PCOORDA) was conducted in order to study the genetic diversity. The results cleared that molecular markers could be used to obtain reliable determination of genetic diversity than morphological traits. Poor correlation (r = 0.32) was obtained between morphological and molecular markers. Both cluster analysis and principal coordinate analysis showed more or less the same results but the principal coordinate analysis give better resolution than cluster analysis in the study of genetic diversity. Such research is useful in determination of genetic relationships among genetic material to be used in new varietal development programs.*

Key words: *Genetic diversity, Vicia faba, RAPD, SSR, ISSR, EST, STS, morphological traits.*

INTRODUCTION

Faba bean (*Vicia faba L.*) is a diploid species with 2n=12chromosomes. It belongs to the family of Fabaceae (legumineacea), subfamily of Papilionoideae, tribe of Viceae (Fernandez *et al.*, 1996). Faba bean (*Vicia faba L.*) is one of the oldest crops grown by humans and is a valuable protein-rich food and animal feed (Link *et al.,* 1995; Duc, 1997; Zong *et al.,* 2009). Faba bean origin is mostly assigned to the Central Asian, Mediterranean, and South American centers of diversity. The wild progenitor and the exact origin of faba bean remain unknown. Several wild species (*V. narbonensis L.* and *V. galilaea* Plitmann and Zohary) are taxonomically closely related to the cultivated faba bean crop, but they contain 2n = 14 chromosomes, whereas cultivated faba bean has 2n = 12 chromosomes. Therefore, nmerous attempts to cross the wild species to cultivated faba bean have failed (Bond *et al.*, 1985).

Analysis of genetic relationships in crop species is an important component of crop improvement programs, as it provides information about genetic diversity (Mohammadi and Prasanna, 2003) to be used in plant breeding programs.

Knowledge of genetic variation and relationships between accessions or genotypes is important as it helps to (1) understand the genetic variability available and its potential use in breeding programs, (2) offer evidence of the evolutionary forces shaping the genotypic diversities, and (3) choose genotypes to be given priority for conservation (Thormann *et al.,* 1994). This knowledge is essential and of critical importance in establishing, managing and ensuring a long-term success of crop improvement programs (Gwak, 2008; Ma *et al.,* 2009).

In faba bean, genetic diversity among collected germplasm accessions has been delineated by various kinds of genetic marker systems, including isozyme (Kaser and Steiner, 1983; Mancini *et al.*, 1989), random amplified polymorphic DNA (RAPD) (Torres *et al.*, 1993; Link *et al.,* 1995), restriction fragment length polymorphism (Torres *et al.,* 1993), inter-simple sequence repeat (Terzopoulos and Bebeli, 2008) and amplified fragment length polymorphism (AFLP; Zong *et al.*, 2009). Terzopoulos and Bebeli (2008) studied the genetic diversity of Greek faba bean local populations, using

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inter-simple sequence repeat (ISSRs). They classified the local populations and compare

the classification results with those derived from morphological/agronomic data.

The main objective of the present study was to determine the genetic diversity of eight Egyptian cultivars from Faba bean *(Vicia faba L.)* using cluster analysis and principal coordinate analysis (PCOORDA) based on different types of molecular markers (e.g. RAPD, SSR, ISSR, ESTand STS markers) and morphological traits.

MATERIALS AND METHODS
Plant material and DNA isolation:

Eight Egyptian Faba bean *(Vicia faba L.)* varieties were kindly obtained from the Agricultural Research Center, Field Crop Research Institute, (FCRI), Giza, Egypt. The varieties and their pedigrees are presented in Table (1). They are 'Giza 3', 'Sakha1', 'Giza 716', 'Giza 843', 'Nubaria 1', 'Misr 1', 'Sakha 2' and 'Sakha 3' varieties. Genomic DNA was isolated from leaves of 10–days seedlings using CTAB modified method according to Dellaporta *et al.,* (1983).

Morphological experiment:

The morphological experiments were carried out at the farm of Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minoufyia University, Egypt in the growing season of 2010\2011. Seeds of each variety were planted in four rows. The rows were planted with an additional empty row between the varieties. Ten morphological traits were measured during all the period of plant growth. These traits included number of leaves, number of leaflets, the percentage between the previous two traits, plant height (cm), number of legume pods, number of days to flowering, plant weight (g), number of seeds per plant, 100 seeds weight (g) and volumetric weight (g/l). Five measurements had been taken (from five different plants) for each trait and then the average of each trait was calculated to be used for statistical analysis.

RAPD analysis:

Ten random primers were tested for RAPD analysis (Table 2). The PCR amplification reactions were performed in a 25 µl volume using 50 ng DNA and 0.5 µmoles of the primer, 100 µM of dNTPs, 5 µl (1X) of *Taq* polymerase buffer, 1.5 mM MgCl$_2$ and 0.75 U *Taq* DNA polymerase (Promega). The PCR program included a denaturation step at 94°C for 4 min. followed by 39 cycles of 94°C for 1min., 30°C for 50 sec. and 72°C for 2 min., and a final extension step at 72°C for 7 min. The PCR products were separated on 1.5% agarose gel electrophoresis.

Table 1: Tested faba bean varieties, origin, and pedigree used for molecular marker analyses.

NO	Varity	Origin	Pedigree
1	Giza 3	EGYPT	G.1*NA 29
2	Sakha 1	EGYPT	(85/283/620x88/724/716)
3	Giza 716	EGYPT	83/453/503x83/824/461)
4	Giza 843	FCRI[1]	Cross 461 x cross561
5	Nobaria1	ESPAIN	By individual selection from Rina Blanka
6	Misr 1	FCRI[1]	(123A/45/76XG.3)×(62/1570/66×G.2)×(Romi×Habashi)
7	Sakha 2	FCRI[1]	Reina Blanka x461/845/83
8	Sakha 3	EGYPT	By individual selection from G.716

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Table 2: Primers names, sequences, total number and polymorphic number of bands of RAPD. ISSR. SSR. EST. STS markers for tested *Vicia faba* varieties.

Marker	Primer	Forward sequence ('5--3')	Reverse sequence ('5--3')	Total	Polymorphic	PIC

type	name			Bands	Bands	
RAPD	SBSAa	GTGATCGCAG		9	9	0.73
	SBSPb	TCCCGCCTAC		16	15	0.95
	SBSPc	TGACCCGCCT		17	14	0.79
	SBSQd	AGGCTGGGTG		10	6	0.76
	SBSQe	AGTGCGCTGA		16	15	0.69
	SBSQ5	CCGCGTCTTG		16	14	0.92
	SBSQ15	GGGTAACGTG		11	10	0.76
	OPKO4	CCGCCCAAAC		14	10	0.66
	OPKO6	CACCTTTCCC		16	11	0.80
	OPLO9	TGCGAGAGTC		12	11	0.91
ISSR	ISSR1	CACACACACACACACAG		16	15	0.95
	ISSR2	GAGAGAGAGAGAGAGAYC		17	16	0.91
	ISSR3	AGAGAGAGAGAGAGAGT		13	12	0.88
	ISSR4	GACAGACAGACAGACA		12	11	0.83
	ISSR5	GAGAGAGAGAGAGAGAT		10	9	0.90
	ISSR6	ACACACACACACACACT		8	4	0.50
	ISSR7	GAGAGAGAGAGAGAGAC		9	8	0.84
SSR	SSR1	AGCGATGGTGCTCATGCTTA	TCTCTCACGGAATCACATCTT	8	6	0.75
	SSR2	TTTCAGCAAACTAGAACCAA	GGCATTCAGTTTTTACCTTGT	7	6	0.82
	SSR3	GCACTCGAAGGAATTAATTTT	GAACAGTTGTTTCGTGTCGTA	7	3	0.87
	SSR4	GATGTTGTTGGTGTTGTTTA	CAATTAGGAGCAAAATCAGA	9	8	0.84
	SSR5	GGTTTTGAATAGAAATGCAA	AAGATGTGTCAATATTGTTTT	8	5	0.69
	SSR6	GGCTATTGTCACGAACAAAT	GATTCAGACCCGGATACATT	7	4	0.74
	SSR7	AGAGTCCCAAAGAGTGGGTT	CCAAAGGCAAAAATGAGGGCTT	7	6	0.86
EST	EST1	TCCTCTGCAAACAAAACCCTA	TTCGCCAAATTCGACAATT	9	6	0.82
	EST2	AGCTGGCAGAAACTTGGAAA	TTGAAACCCAGGTCTATTCTCTTC	7	5	0.78
	EST3	TGGGGGAATTGAGTGAACAT	TCTTTCTTGCGCTTTTCTCTTC	6	4	0.73
	EST4	TTCACATTCTTCACTCACA	ATCCCTTGGACGATGAC	10	9	0.80
	EST5	GAAAAAGCTATGGGCCACAC	CGGTAGAGAATCCAGCGAGA	17	13	0.88
	EST6	CCATTACGTGTCCAAGGTCTC	CAAAAAGGAATCGGACTCTAGG	8	5	0.79
STS	STS1	AAAACTTGAAATTGGCCGCG	CACAGGCAATCAGTGGGAAGG	10	9	0.99
	STS2	CCGATCGCCAGGATGTCATA	CGAGATACCTGATGTTGGCAGT	7	4	0.77
	STS3	CCTATGTACTTCTTGAGGGAGAC	AGAAGCCCAGGGACTTGGAT	9	8	0.84
	STS4	GACAGTTAGTCAACCAAGTC	CACAACATAATCGATTG	13	10	0.85
	STS5	CACTTGCCACAGCTTCAAGA	ACACCACTGAGGAACTTTGG	11	9	0.89

ISSR analysis:

Seven ISSR anchored primers specific for *Vicia faba* (Table 2) were selected in order to carry out the ISSR analysis according to literature (Terzopoulos and Bebeli, 2008 and Han and Wang, 2009).

The PCR amplification reactions were performed in a 17 µl volume using 50 ng of DNA, 12.5 µl of Dream *Taq* master mix (fermentas) and 0.5 µmoles of the primer. The PCR program included a denaturation step at 94°C for 4 min followed by 28 cycles of 94°C for 1min., 40°C for 50 sec. and 72°C for 2 min., and a final extension step at 72°C for 7 min. The PCR products were separated on 1.5% agarose gel electrophoresis.

SSR analysis:

Seven *Vicia faba* specific primer pairs were selected for SSR analysis according to the literature (Zeid *et al*, 2009). The PCR amplification reactions were performed in a 17 µl reaction volume using 50 ng of DNA, 12.5 µl of Dream Taq master mix (fermentas) and 0.5 µmoles of each primer. The SSR reactions were carried out using Touchdown PCR program. The PCR program was carried out for six cycles at 94°C for 1 min., 45°C for 50 sec. decreasing 1°C in every cycle, and 72°C for 1 min. and then the last cycle was repeated 28 times. The previous program were preceded by a denaturation step at 94°C for 4 minutes and followed by an extension step at 72°C for 7 minutes. The PCR products were separated on 1.5% agarose gel electrophoresis.

STS analysis:

Five STS common bean (*Phaseolus vulgaris.L*) specific primer pairs were selected in order to carry out the STS analysis for (*Vicia faba*) according to the literature (Liu *et al.*, 2008). The PCR amplification reactions were performed in a 17 µl reaction volume containing 50 ng of DNA, 12.5 µl of Dream Taq master mix (fermentas co.) and 0.5 µmoles of each primer. The STS-PCR was carried out using Touchdown PCR program. The primary program was carried out for seven cycles at 94°C for 1 min., 51°C for 50 sec. decreasing 1°C in every cycle, and 72°C for 1 min. this program was followed by 28 cycles at 94°C for 1 min., 45°C for 1 min. and 72°C for 1 min. The previous programs were preceded by a denaturation step at 94°C for 4 minutes followed by an extension step at 72°C for 7 minutes. The PCR products were separated on 1.5% agarose gel electrophoresis.

EST analysis:

Six EST common bean (*Phaseolus vulgaris.L*) specific primer pairs were selected in order to carry out the EST analysis for (*Vicia faba*) varieties according to the literature (Hanai *et al.*, 2009). The PCR amplification reactions were performed in a 17 µl reaction volume containing 50 ng of DNA, 12.5 µl of Dream Taq master mix (fermentas) and 0.5 µmoles of the primer. The EST reactions were carried out using Touchdown PCR program. The primary program was carried out for seven cycles at 94°C for 1 min., 47°C for 50 sec. decreasing 1°C in every cycle and 72°C for 1 min. This program was followed by 28 cycles at 94°C for 1 min., 42°C for 1 min. and 72°C for 1 min. The previous programs were preceded by a denaturation step at 94°C for 4 minutes followed by an extension step at 72°C for 7 minutes was added at the end of the program. The PCR products were separated on 1.5% agarose gel electrophoresis.

Data analysis:

The gels were scored for band presence or absence as (1) or (0), respectively. The total number of bands and polymorphic bands were calculated as well as the polymorphic information content (PIC) which was calculated according to Anderson *et al.* (1993) using the following simplified formula: $PIC_i = 1 - \Sigma p^2_{ij}$

Where p_{ij} is the frequency of the $_j$th allele for marker $_i$th summed across all alleles for the locus. Similarity coefficient matrices were calculated using the simple matching algorithm for all the molecular markers to obtain one general matrix representing all molecular markers. Simple matching algorithm was used also for similarity matrix calculation from the standardized morphological data. One dendrogram was constructed for all the molecular markers as well as for the morphological traits using the UPGMA method. Correlations among all obtained similarity matrices (molecular and morphological) were performed using the Mantel's test (Mantel, 1967). The scored morphological and molecular data were standardized, decantered for the interval

data then the Eigenvalues and the Eigen vectors were calculated. The principal coordinate analysis (PCOORDA) was performed for both molecular and morphological data on the basis of the distance matrices. All the above mentioned analyses were carried out using the NTSYS PC 2.0 (Rohlf, 1998).

RESULTS AND DISCUSSION:
Polymorphism and polymorphic information content (PIC):

The total number of bands and the number of polymorphic bands of *Vicia faba* varieties were calculated for each marker (e.g. RAPD, ISSR, SSR, EST, STS; Table 2).

The ten RAPD primers produced in total 137 bands, 115 from which were polymorphic (84% polymorphism, Table 2). The total number of bands for each primer ranged from nine for the primer SBSAa to 17 for the primer SBSPc while the number of polymorphic bands ranged from six for the primer SBSQd to 15 for the primer SBSQe. The polymorphic information content (PIC) for RAPD primers ranged from 0.66 for the primer OPKO4 to 0.95 for the primer SBSPb (Table 2).

The total number of bands generated from the seven ISSR primers was in total 85 bands, 75 from which were polymorphic (88% polymorphism). The total number of bands for each primer ranged from eight for the primer ISSR6 to 1٧ for the primer ISSR2 while the number of polymorphic band ranged from four to 16 for the same primers, respectively (Table 2). The polymorphic information content (PIC) for ISSR primers ranged from 0.50 for the primer ISSR6 to 0.95 for the primer ISSR1 (Table 2).

The seven SSR primers pairs produced in total 53 bands, 38 from which were polymorphic (71% polymorphism, Table 2). The total number of bands for each primer pair ranged from seven for the primer pairs SSR2, SSR3, SSR6 and SSR7 to nine bands for the primer pair SSR4 while the number of polymorphic bands ranged from four for the primer pair SSR6 to eight for the primer pair SSR4. The polymorphic

information content (PIC) for SSR primer pairs ranged from ٠.٦٩ for the primer pair SSR5 to 0.87 for the primer pair SSR3 (Table 2).

The six EST primer pairs produced in total 57 bands, 42 from which were polymorphic (73 % polymorphism, Table 2). The total number of bands for each primer pair ranged from six for the primer pair EST3 to 17 bands for the primer pair EST5 while the number of polymorphic bands ranged from four to 13 for the same primer pairs, respectively. The polymorphic information content (PIC) for EST primer pairs ranged from 0.73 for the primer pair EST3 to 0.88 for the primer pair EST5 (Table 2).

The five STS primer pairs produced in total 50 bands, 40 from which were polymorphic (80% polymorphism). The total number of bands for each primer pair ranged from nine for the primer pair STS3 to 13 bands for the primer pair STS4 while the number of polymorphic bands ranged from four for the primer pair STS2 to ten for the primer pair STS4, Table 2. The polymorphic information content (PIC) for STS primer pairs ranged from 0.77 for the prime pair STS2 to 0.99 for the primer pair STS1 (Table 2).

The polymorphic information content (PIC) for the different molecular markers types ranged in general from 0.5 for the primer ISSR6 to 0.99 for the primer pair STS1 (Table 2). The average of PIC for each marker type ranged from 79.6% for the SSR marker type to 86.8% for the STS marker type and thus it can be said that STS marker is more informative than the other marker types. It can be inferred also that the PIC percentage that obtained in this study is better than those obtained by Zeid *et al.,* 2009. The PIC in their study ranged from 0.16 to 0.72 when they used the SSR markers to discriminate the Orobanche-resistance in faba bean.

Cluster Analysis:

According to cluster analysis of all molecular markers (RAPD, ISSR, SSR, EST and STS), *Vicia faba* varieties were divided into two clusters; the first cluster consists of 'Giza 3', 'Giza 716', 'Nubaria1' and 'Sakha 1'

varieties (Figure 1). The second cluster consists of 'Misr1', 'Sakha 2' and 'Sakha 3' varieties while 'Giza 843' variety was separated alone apart from both clusters (Figure1). As it appears from the cluster analysis 'Sakha 2' and 'Sakha 3' varieties are the closest two genotypes according to the molecular markers data which conflict their common origin hence both varieties were originated from the Sakha station, Agriculture Research Center, Sakha Station, Kafr Elsheikh, Egypt. It can be inferred also that 'Giza 843' variety was separated apart from all other varieties and may refer to its different origin, whereas its pedigree is different from the other varieties (originated from the hybrid 'Cross 461 x cross561', Table 1).

For morphological traits, cluster analysis divided the *Vicia faba* varieties into three clusters; the first cluster consists of 'Giza3' and 'Misr1' varieties (Figure 2). The second cluster consists of 'Sakha 1', 'Sakha 2' and 'Giza 716' varieties while the third cluster consists of 'Nubaria1', 'Sakha 3' and 'Giza 843' varieties (Figure 2). The notable result is falling of 'Sakha 1' and 'Giza 716' varieties in the same cluster, where according to the pedigree 'Giza 716' variety is ancestor for 'Sakha 1' variety. The other varieties did not distribute according to their pedigrees.

Duc *et al.*, (2010) reported that large genetic variability has already been identified in *V. faba* in terms of floral biology, seed size and composition. Our results are in agreement with their results hence they reported that the use of amplified fragment length polymorphism (AFLP) or simple sequence repeat (SSR) markers has allowed genetic resources to be distinguished according to their geographic origin and the structuring of collections.

Correlation between morphological and molecular markers:

Correlations among all obtained similarity matrices (molecular and morphological) were performed using the Mantel's test (Mantel, 1967) of the NTSYS PC2.0 software. The correlation among morphological and molecular markers was moderately low (r = 0.32). This poor correlation between morphological and molecular markers may refers to the low number of morphological traits that was used to determine the genetic diversity and the habit of the morphological traits that affected by the environmental stresses. One another reason may affect the poor correlation is the low number of the primers used for the molecular markers, but the numerous markers that were used may cover this point. These results are in agreement with those obtained by Black-Samouelsson *et al.*, 1997. They carried out survey on the amount and pattern of genetic variation in two Swedish and three Czech populations of the plant "*Vicia pisiformis*" Their results showed that Mantel test based on comparisons between Mahalonobis and Jaccard distance for morphological and RAPD data, respectively, did not reveal any significant correlation between the two matrices.

Principal Coordinate Analysis (PCOORDA):

Principal coordinate analysis separated all *Vicia faba* variety by using the first three principal coordinates PC1, PC2 and PC3, accounting for ٣3.97%, 21.47%and 15.22% of the total genetic variance, respectively (Figure 3). According to the first principal coordinate (PC1), 'Giza 843' variety represented at low level of that principal coordinate while all the other varieties were represented at high level of that PC. According to the second principal coordinate (PC2), both 'Sakha 1', 'Giza 716', 'Nubaria 1', and 'Giza 3' varieties represented at high level of the same PC (Figure 3). The varieties 'Giza 843' and 'Misr1' were located at medium level of this PC. The varieties, 'Sakha3' and 'sakah 2' were separated at the lowest level of that principal coordinate (Figure 3). At the PC3, 'Nubaria1' variety was represented at the highest level while 'Sakha 1'variety was represented at the lowest level of that PC. The rest varieties were represented at medium level of the same PC (Figure 3).
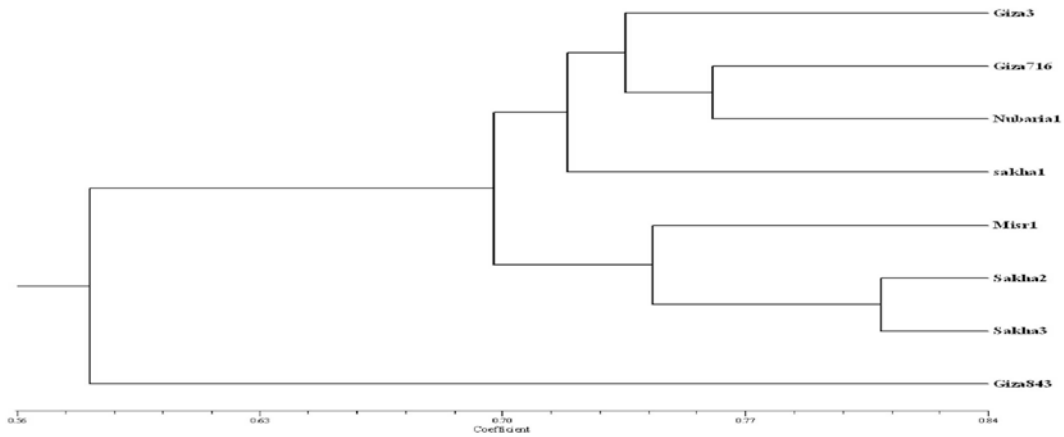
Figure 1: Cluster analysis of tested *Vicia faba* varieties using the molecular markers and UPGMA clustering method.
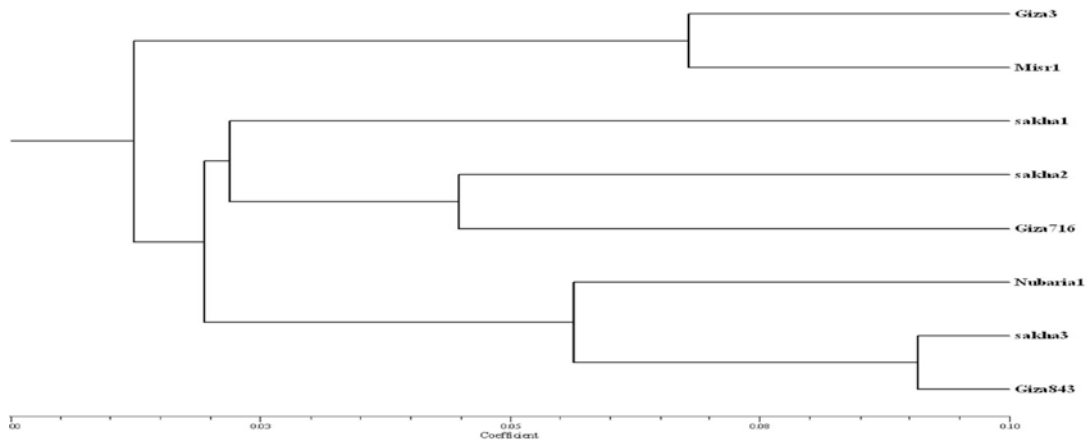

Figure 2: Cluster analysis of tested *Vicia faba* varieties using the morphological traits and UPGMA clustering method
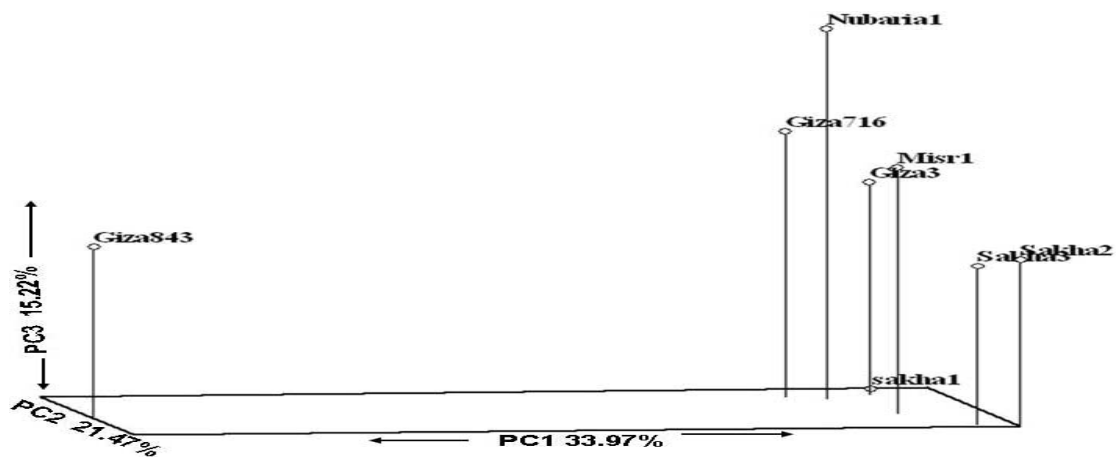

Figure 3: 3-Dimensional PCOORDA analysis for tested *Vicia faba* varieties obtained by the molecular analysis

For the morphological data, PCOORDA analysis was constructed for the standardized de-centered data and the 3-D diagram is presented in Figure 4. The first three principal coordinates accounted for 65% of the total genetic variations that found in all genotypes. The first three PCs accounted for 27.84%, 21.83%and 15.35% of the total genetic variance for PC1, PC2 and PC3, respectively (Figure 4). According to the morphological data, 'Giza 3' variety was represented at the highest level of the first principal coordinate (PC1) while the variety 'Sakha 3' was represented at the lowest level of the same PC. The varieties 'Giza 716', 'Sakha 1', and 'Misr 1' were located at high level of that PC whereas both 'Giza 843', 'Nubaria1' and 'Sakha 2' varieties were represented at medium level of that PC (Figure 4). At PC2, 'Misr 1'variety was represented at the highest level of f the second principal coordinate (PC2) while 'Sakha 2' and 'Giza 716' varieties were represented at the lowest level of the same PC. The varieties 'Sakha 3', 'Giza 843' and 'Giza 3' was located at high level of that PC whereas the varieties 'Nubaria 1' and 'Sakha 1' and were represented at medium level of that PC (Figure 4). At PC3, 'Giza 3' variety was represented at the highest level of f the third principal coordinate (PC3) while 'Sakha 1' variety was represented at the lowest level of the same PC. The varieties 'Giza 716', 'Misr 1'and 'Nubaria 1' were represented at low level of the same PC whereas 'Sakha 3', 'Giza 843' and 'Sakha 2' varieties were represented at medium level of that PC (Figure 4).

In our study, both cluster analysis and PCOORDA separated the faba bean varieties similarly. For example, at the molecular level 'Giza 843' variety was separated apart from all other varieties in both cluster analysis and PCOORDA (Figures 1 and 3) and 'Giza 3' and 'Misr 1' varieties being the most closest two varieties according to morphological data in both analyses. These results are in agreement with those obtained by Terzopoulos and Bebeli (2008) when they studied the genetic diversity of Mediterranean faba bean (*Vicia*

faba L.) using ISSR markers. They reported that both cluster analysis using unweighted pair-group method with arithmetic mean (UPGMA) method and principal coordinate analysis separated four minor-type populations from the Mediterranean-type populations.

In this study, principal coordinate analysis gave better resolution of genetic diversity than cluster analysis. For example, 'Misr 1' variety was the closest variety to 'Giza 3' variety according to morphological data using both cluster analysis and PCOORDA, but in PCOORDA analysis 'Giza 3' variety was represented at the highest level of PC3 while 'Misr 1' was represented at very low level of the same PC (Figure 4). The same varieties were aggregated almost together according to molecular markers data using the PCOORDA analysis (Figure 3) while the cluster analysis separated them apart (Figure 1). The same results were also obtained from previous studies in different crops (Maize, Abdellatif and Khidr, (2010); Wheat, Zhang *et al.*, (2011) and Faba bean, Han and Wang 2009). Han and Wang (2009) studied genetic diversity and relationships among 11 populations of two closely related northeast China *Vicia* species, *Vicia ramuliflora* and *V. unijuga* using RAPD and ISSR analyses. They reported that the UPGMA dendrogram and principal coordinates analysis showed that *V. ramuliflora* and *V. unijuga* were more closely related than either of them was to the out-group species, *V. cracca* with better resolution accompanied with principal coordinate analysis.

It can be concluded that molecular markers can be used to obtain reliable determination of genetic diversity than morphological traits. The correlation between morphological and molecular markers is poor and this problem could be solved by using a lot of morphological characteristics. It can be inferred also that principal coordinate analysis gives better resolution than cluster analysis in the study of genetic diversity.
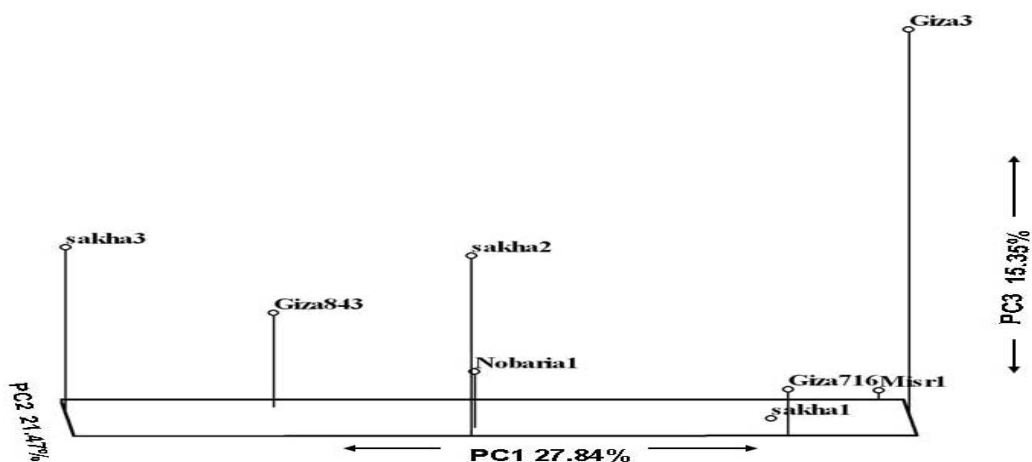
Figure 4: 3-Dimensional PCOORDA analysis for tested *Vicia faba* varieties obtained by the morphological analysis.

Acknowledgment:

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تقدير التنوع الوراثي في بعض أصناف الفول البلدي بإستخدام الصفات المورفولوجية والدلائل الجزيئية

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الملخص العربي

تم دراسة التنوع الوراثي لثمانية أصناف من الفول البلدي بإستخدام خمسة أنواع مختلفة من الدلائل الجزيئية (RAPD, ISSR, SSR, EST, STS) بالإضافة إلي الصفات المورفولوجية، وأجري كل من التحليل الأساسي المنسق (PCOORDA) والتحليل العنقودي من أجل دراسة التنوع الوراثي بإستخدام كل من الصفات المورفولوجية والدلائل الجزيئية. وأظهرت النتائج أنه يمكن إستخدام الدلائل الجزيئية في دراسة التنوع الوراثي للحصول علي نتائج أكثر واقعية من الصفات المورفولوجية. تم الحصول علي إرتباط ضعيف (r = 0.32) بين كل من الدلائل المورفولوجية والجزيئية ويمكن حل هذه المشكلة عن طريق إستخدام الكثير من الصفات المورفولوجية. كذلك يمكن إستنتاج أن كلا من التحليل العنقودي والتحليل الأساسي المنسق أظهرا تقريبا نفس النتائج ولكن نتائج التحليل الأساسي المنسق تعطي توضيح أفضل من التحليل العنقودي في دراسة التنوع الوراثي ويمكن الإستفادة من هذا البحث في تحديد العلاقات الوراثية بين التراكيب الوراثية لإدخال المتباعد منها في برامج التربية.