MOLECULAR ANALYSIS OF SEVEN INTRODUCTIONS FODDER BEET

ABDEL –GHAWAD,M. F. [3], ZEINAB M. ABD EL-NABY [1], E. M.R. METWALI [2] AND E.M. ZAYED[1]*

1. *Forage Crops Research Section, FCRI, ARC, Giza, Egypt.*
2. *Botany Department, Faculty of Agric., Suez Canal University, Ismailia, Egypt.*
3. *Sugar Crops Research Institute, ARC, Giza , Egypt.*

Abstract

The fodder beet does not set flower in Egypt. Attempts have been done to induce flowering in fodder beet, but they have not been successful due to factors of infertility. So, seeds are importing from different countries such as France, Bulgaria and Hungary. The identity of germplasm resources of fodder beet would be a great value for breeding. Due to the floral biology of fodder beet varieties and hybrids, the expected genetic variability is high. Isozymes (Peroxidase and Esterase), seed protein and RAPD (five primers) markers were used in this study. It was observed that Betarose and Starmoon have the highest values of germination of the seven varieties at 4 days and at 10 days. Results revealed that the genetic similarity matrix ranged from 68.6% to 93.3% for total isozyme, 61.5% to 94.1% for seed protein, 60.4% to 80.4% for RAPDs. The overall similarity matrix revealed that the highest similarity was (82.8%), which occurred between Rota and Jary followed by Jamon and Starmoon (81.4%) and Rota and Jamon (80.0%), while the lowest similarity was (67.5%) between Vroushanger and Monro followed by (68.9%) Vroushanger and Rota. The relative information value of each marker can be evaluated on the basis of its polymorphic information content (PIC), which ranged from 3.6 to 29%, while general mean was equal 21.3% with 2 number of alleles in every system. Heterozygosity ranged from 0.04 in β-esterase to 0.48 in OP-A19 followed by OP-G05 0.47.

Key words: *Fodder beet, Introduced varieties and hybrids, RAPD, Molecular analysis*
*Corresponding author: *Ehab Zayed, ehabzayed31@hotmail.com*

INTRODUCTION

The fodder beet does not set flower in Egypt. There have been attempts to induce flowering in fodder beet, but they have not been successful due to factors of infertility. Fodder beet (*Beta vulgaris* L. var.) is grown as a major root crop, which requires similar husbandry to sugar beet. It can produce substantial yields of high quality forage and is an excellent supplement to grass silage. Sowing period is during March – May. Medium dry matter varieties and hybrids tend to have a higher top ratio root and have a relatively low dirt tare. These can be fed completely to stock. High dry matter varieties and hybrids tend to sit further in the ground and require a sugar beet harvester to lift them. Due to the higher dirt tare and hardness of the root,

these varieties and hybrids may need to chopped and washed before feeding. After wilting, the tops may be feded to livestock and produce further yields of 3-4 tones of protein per hectare (Alexander 1971 and Hadjichristodoulou 1976). Fodder beet has high biomass, and it is salt a tolerant crop. This crop is grown successfully where no other conventional winter fodder available on marginal land and where brackish water is available (Khan et al. 2002).

Sugar beet and fodder beet are different varieties and hybrids of the same species *Beta vulgaris* ssp. *vulgaris*. Sugar beet is used for sugar production, while fodder beet feeds animals. The new classification system for cultivated plants distinguishes four cultivar groups: Leaf Beet Group, Garden Beet Group, Fodder Beet Group and Sugar Beet Group.

DNA content (C-value) of *Beta vulgaris* L. is reported to be between 714 and 758 million base pairs per haploid genome (n = x = 9) with variation reported within subspecies, as well as other cultivated type was (2n=2x =18) (Bennett and Smith 1976 and Arumuganathan and Earle 1991).The tetraploidy families having twice (2n =4x= 36),the normal chromosomes, were obtained around 1940 (Schwanitz 1938). Plants from the two ploidy level were crossed, producing triploid hybrids (2n =3x =27) (Bosemark 1993). A number of molecular marker genetic maps in Sugar beet have been constructed (Barzen *et. al.*, 1992, Rae *et. al.*, 2000 and McGrath *et. al.*, 2007). Many marker systems have been used, most are anonymous, including restriction fragment length polymorphisms (RFLPs), randomly amplified DNA polymorphisms (RAPDs), AFLPs, and SSRs, as well as a few morphological (e.g., color, seed type) and isozyme markers. Some single nucleotide polymorphisms (SNPs) within protein-encoding genes are available for mapping in sugar beet (Mohring *et. al.*, 2004; Schneider *et. al.*, 2001), and Pillen *et. al.* (1996) determined linkage relationships among 12 nuclear genes encoding chloroplast thylakoid proteins. Most maps showed strong clustering of markers in one or two regions of each linkage group, suggesting restricted genetic recombination, and perhaps are influenced by the type of marker used (Nilsson *et. al.*, 1997).

In this study molecular analysis for seven introduced fodder beet varieties and hybrids, and genetic distance, allele frequency and genotype frequency were made that assess the relationship between introduced varieties and hybrids. This work may help breeders in the study of field performance.

MATERIALS AND METHODS

Plant Materials

Two varieties and five hybrids were used in this study; the botanical name of them is *Beta vulgaris* L. *var. altissima* and all are tetraploidy (Table1).

Germination test

Germination test was carried out in the laboratory, five seeds of 7 varieties and hybrids were germinated in Petri dishes. Number of germinated seeds was recorded every five days, length of seedling and number of leaflets were measured later.

Table 1. Common names, origin, case breeding, and chromosome number of the seven-fodder beet.

.No	Common name	origin	case of breeding	2n
1	Rota	Hungary	variety	18
2	Vroushanger	Hungary	variety	18
3	Beta rose	France	hybrid	18
4	Jamon	France	hybrid	18
5	Jary	France	hybrid	18
6	Monra	France	hybrid	18
7	Starmoon	France	hybrid	18

Isozymes analysis

For enzymes analysis, 10% Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducting to identify isozyme variations among studied varieties and hybrids using two isozyme systems according to (Stegemann *et. al.* 1985 and Brown 1978). Isozymes were extracted from the five leaves of seedlings leaf of the seven varieties and hybrids.

Protein analysis

Total seed soluble protein was extràcting by a modifieication of the procedures of Smith and Payne, (1984) 5 seeds from fodder beet varieties and hybrids were crushed and transferred to 350 µl of extraction buffer. Extracted soluble proteins were immigration in one-dimensional electrophoresis SDS-PAGE (Laemmli 1970). The gel was staining overnight in Coomassie brilliant blue-R250 followed by de-staining solution methanol and acetic acid for 30 min. Gel was further de-staining in acetic acid until background become clear for scoring. Absence and presence protein bands were referred to using (0) (1) symbol respectively.

RAPD-PCR analysis

DNA extraction

Genomic DNA was isolated by a modified cetyltrimethylammonium bromide (CTAB) method (Kidwell and Osborn, 1992) and Sajjanar (2002). and purified by phenol extractions from bulked leaf samples from five plants(two weeks old). DNA was checked for its quality and quantity by 1% agarose gel electrophoresis using a standard containing 100 ng per l μL genomic DNA. By grinding one g of leaf sample in liquid nitrogen using chilled pestle and mortar. CTAB extraction buffer[(N-lauryl sarcosine, cetyltrimethylammoniumbromide), O. 14 M sorbitol, O. 22 M Tris, :pH 8.0, 0.22 M EDTA, 0.8 M NaCL, which was pre-warmed to 65°C and added to each tube and placed on a water bath (65°C) for 15 minutes and cooled to room temperature. Equal volume of chloroform: 'isoamylalcohol (24: 1) was added and vortexed for few seconds and centrifuged at 13,000 rpm for 10-15 minutes. The supernatant was transfers to a new eppendorf tube. The DNA was precipated by adding equal volume of prechilled isopropanol and incubated overnight at -20°C. DNA was recovering by centrifugation and the pellet washed with 70 percent ethanol and dissolved in 10 TE (10mM Tris-HCl1mM EDTA, pH 8.0). DNA concentration was estimating with a spectrophotometer and by Gel analysis. RNase solution (10 mg ml^{-1}) treatment was give to remove RNA from the samples.

Polymerase chain reaction condition

PCR amplifications were performed in a Master Thermal Cycler-5331-Eppendorf version 2.30, 31-09, Germany, by loading 20 ul reaction [l x PCR assay buffer, 200 m dNTP mix (eppendorfl,20 ng primer (Operon Technologies Inc, Almeda, CA, USA), 2U Taq. The (DMA) DNA polymerase (Bangalore Genei, India) and approximately was 50 ng template DNAI. Five primers used for screening in the present study. The thermal profile used were 1 cycle of 95°C for 5 min, 40 cycles each of 94°C for 1 min, 36°C for 1 min and 72°C for 2 min and one cycle each of 72°C for 8 min for amplification. The PCR amplified products had resolved on 1.5 % agarose gel electrophoresis and visualized by ethidium bromide staining and Gel Documentation System (UVI Tech, Cambridge, England) photographed the Gel.

Table 2. Randome primers names and their sequences for RAPD-PCR analysis.

Primer name	Sequence
OP-A10	5´-GTG ATC GCA G-3´
OP-A19	5´-CAA TCG CCG T- 3´
OP-G05	´5´ -CTG AGA CGG A- 3
OP-L16	´5´ -AGG TTG CAG G-3
OP-M17	´5´ -TCA GTC CGG G- 3

Band Scoring and Data Analysis: The frequency of two isozymes, protein and RAPD-PCR polymorphism between 7 fodder beet genotypes were calculated based on the presence of band as '1' or absence of band as '0 (Ghosh *et. al*., 1997). The genetic associations between varieties and hybrids evaluated by calculating the Jaccard similarity coefficient for pair-wise comparisons based on proportion of shared bands produced by primers. Similarity matrices were generated using SPSS (Jaccard 1901). Similarity coefficients were used for cluster analysis of varieties, hybrids were used SPSS subprogram, and dendrogram, was built based on the UPGMA. Principal coordinate analysis SPSS was performed to generate a two-dimensional representation of genetic relationship among seven fodder beet genotypes.

Combined Data analysis.

Various genetic distances and identities can be calculated for any level of the hierarchy—Nei's and Nei's minimum (original and unbiased, Nei, 1972, 1978), Rogers' 1972) and modified Rogers' (Wright, 1978), and co ancestry (Reynolds et al. 1983). Clustering analysis is by UPGMA (Sokal and Michener, 1958); bootstrap values and consistency indices (the number of loci that support each node)

Exact tests for population differentiation (Raymond and Rousset 1995) in terms of allele frequencies are available, with the option to assign populations or other groups from the hierarchy to one of two test groups. All of them used as single and total data for two isozymes, protein and RAPD –PCR.

Data analysis: The SPSS statistical package version 17.0 was used for statistical evaluation (SPSS Inc., Chicago, IL, USA). Heterozygosity, a measure of allelic diversity, estimated by:

$$H = 1 - \sum_{i=1}^{k} P_i^2$$

where P_i is the frequency of the it allele and k is the number of alleles (Nei 1978).

RESULTS AND DISCUSSION

Germination test

Seed quality refers to genetic purity, germination percentage, vigour, mechanical integrity, disease and pest infection, size and appearance of seeds. The determinants of seed quality are purity and germination (FAO-ISTA 1961). From figure 1, it was observed that Betarose and Starmoon have the highest value of the germination of the seven varieties at 4 days and at 10 days, while, Jary and Jamon have the lowest values at 4 days and at 10 days (53.33% and 60% as well as 73% for the two varieties respectively). The color of seedling was red in Beta rose, Vroushanger, Jary, Rota and Starmoon, although the color seedling of Jamon was

yellow, while, the color seedling of Monra was Slash red to yellow. On the other side, Vroushanger have lowest value (5cm.) of length after 10 days, Monra and Starmoon have the highest value (11cm.). Moreover, the highest number of leaflets was in Monra and Starmoon (6 leaflets), while, the lowest number of leaflets was in Vroushanger, Jary and Jamon (two leaflets).

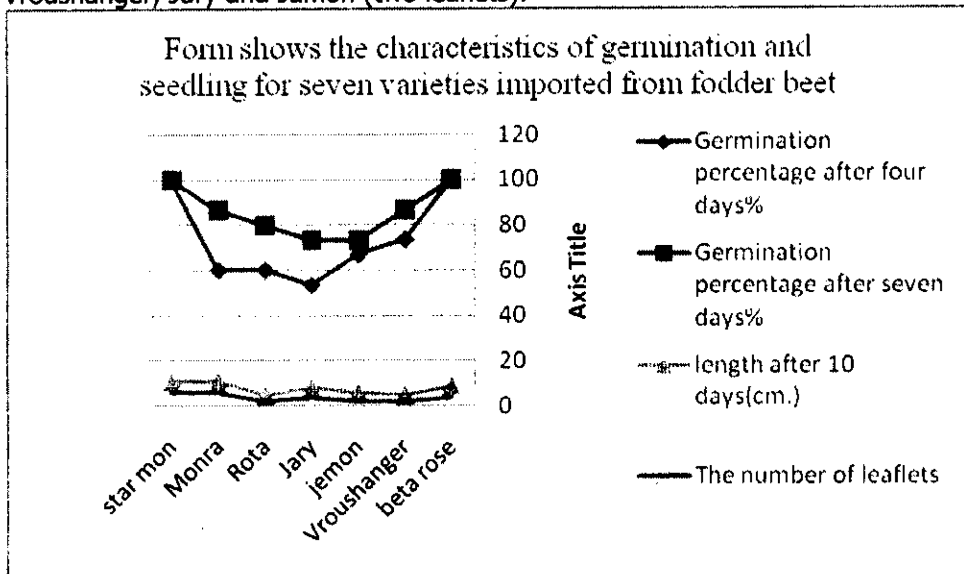

Figure 1. Shows the germination characteristics of different seedling of 7 cultivars and hybrids of fodder beet imported.

Genotyping and Allele Frequency

The genetic makeup, as distinguished from the physical appearance, of an organism or a group of organisms; which is the combination of alleles located on homologous chromosomes that determines a specific characteristic or trait were means genotyping. Therefore, some unique bands appeared in all molecular markers in this study as shown in Tables (3 and 4). The relative information value of each marker can evaluate based on its polymorphic information content (PIC) Table (4). The imported variety Vroushanger revealed seven unique bands as follow: bands No.2 in peroxidase, band No. 6 and band no. 7 in α-esterase and no.5 in β- esterase all above were unique bands, which means the genotype was different from the other genotypes, as well as informs about the possibility of different behavior in field. Vroushanger gave also with primer RAPD-PCR-OP-A19 two positive unique bands in 200 bp and 1150 bp; primer OP-G05 gave negative unique bands with Vroushanger in 600 bp. The variety Starmoon exhibited two positive unique bands with peroxidase in band no.7, and with primer OP-M017 in 2500bp. On other hand, the Starmoon variety revealed four negative unique bands with primer-OPA19 in 150 bp,OP-G05 in 200 bp, and two band with OP-M17 in 900 and 700 bp. Moreover, the Rota variety gave four bands as unique bands, which were positive band with peroxidase in no.10, and OP-

A19 in 900 bp; whereas negative protein marker at 11Kda and with OP-A19 in 750 bp. Four absent unique bands in Jar variety produced with protein in 200,170,160,140 KDa.

Jamon variety produced three present unique bands with primer OP-A19 in 250 and 800 bp, and with OP-M17 in 200 bp; the negative unique bands produced by Jamon with OP-M17 in 150 bp. The Monro variety was give five bands two positive unique bands with OP-A19 and OP-M17 in2500 and 650 bp respectively. The same variety gave three negative bands with the same primers OP-A19 and OP-M17 1050,500 and 1450 bp respectively. The variety Betarose give one present unique bands with OP-M17 in 350bp.

The analysis of isozyme peroxidase was appeared 39, 32, 7 bands and 82.1% in total bands, polymorphic bands, monomorphic bands and polymorphism respectively as shown in Table (3) and plate (1). The data in Table (4) revealed the allele (a viable DNA coding that occupies a given locus on a chromosome) frequency in peroxidase analysis give 0.76 in major gene and 0.24 in minor gene for two alleles with gene diversity 33.5% and with efficient Polymorphic information content (PIC) 26.8 % Table (4) . The α-esterase analysis revealed 55, 20, 35 bands and 36.4% in total bands, polymorphic bands, monomorphic bands and polymorphism respectively, (Table 3 and plate1). The allele frequency in α-esterase analysis was give 0.89 and 0.11 for major and minor gene by one allele with gene diversity in all varieties and hybrids by ratio 15.4 in efficient marker (PIC) 12.5%. β- Esterase had 41,6,35 and 14.6% total bands, polymorphic bands, monomorphic bands and polymorphism respectively (Table 3 and plate 1). The value 0.98 and 0.02 were obtained for major and minor gene with one allele, 4.1 % gene diversity and (PIC) 3.6%. All above analysis were co dominant in heredity type. The accumulate enzymes were appeared, a major allele, minor allele, No. of allele, gene diversity and PIC were 0.86, 0.14, 2, 20% and 16% respectively (Table 4).

Table 3. Levels of polymorphism, unique varieties and hybrids-specific bands, status of it based on isozymes peroxidase and esterase (Alfa and beta), seed protein and RAPD- PCR from five primers OP-A19,OP-A10,OP-G05,OP-L16 and OP- M17 data analysis.

No.	analysis	Kind of test	total bands	polymorphic bands	monomorphic bands	% polymorphism	Unique bands		
							varieties and hybrids	status	MW
1	peroxidase	Co dominant	39	32	7	82.1	Starmoon	present	No.7
							Rota	present	No.10
							Vroushanger	absent	No.2
2	α-esterase	Co dominant	55	20	35	36.4	Vroushanger	absent	No.6
							Vroushanger	absent	No.7
3	β- esterase	Co dominant	41	6	35	14.6	Vroushanger	absent	No.5
4	protein	dominant	99	50	49	50.5	jary	absent	200 KDa.
							jary	absent	170 KDa.
							jary	absent	160 KDa.
							jary	absent	140 KDa.
							Rota	absent	11 KDa.
5	OP-A19	dominant	103	101	14	86.4	Starmoon	absent	150 bp.
							Vroushanger	present	200 bp.
							Vroushanger	present	1150 bp.
							Jamon	present	250 bp.
							Jamon	present	850 bp.
							Monro	present	2500 bp.
							Monro	absent	1050 bp.
							Monro	absent	500 bp.
							Rota	present	900 bp.
							Rota	absent	750 bp.
6	OP-A10	dominant	25	4	21	16	-	-	-
7	OP-G05		47	19	28	40.4	Vroushanger	absent	600 bp.
							Starmoon	absent	200 bp.
8	OP-L16	dominant	51	2	49	3.9	-	-	-
9	OP-M17	dominant	104	90	14	86.5	Starmoon	present	2500 bp.
							Starmoon	absent	900 bp.
							Starmoon	absent	700 bp.
							Monro	absent	1450 bp.
							Monro	present	650 bp.
							Jamon	present	200 bp.
							Jamon	absent	150 bp.
							Rota	absent	1500 bp.
							Betarose	present	350 bp.

The stored seed protein analysis give 99 total bands,50 polymorphic bands,49 monomorphic bands with polymorphism ratio 50.5 % (Table 3 and Figure 1) on the other hand, the protein analysis give 0.83, 0.17,2, 22.2% and 17.8 % for a major allele, minor allele, no. of allele, gene diversity % and PIC (Table 4); although protein is dominant heredity type.

In RAPD-PCR analysis, the primer OP-A19 gave values 103, 89, 14 and 86.4 for percentage total bands, polymorphic bands, monomorphic bands and polymorphism respectively (Table 3 and Plate 2). In primer, OP-M17 values 104, 90, 14 bands and 86.5% induced with the same traits, which were highest value in all marker analysis. However, OP-L16 gave the lowest value with the traits 51,2,49 bands and 3.9% (Table 3). In addition, the primer OP-A10 gave quite lowest of value 25, 4, 21 bands and 16% polymorphisms. In the primer OP-G05, 47 bands from total bands,

19 bands from polymorphic, 26 bands for monomorphic and 40.4 % polymorphism were resulted. The allele frequency was appeared in total data RAPD-PCR as following values: 80 for major allele,0.20 for minor allele in the seven varieties and hybrids as sample size have two allels, 30 % gene diversity with efficient of RAPD-PCR marker 20% PIC. These values changed at primers alone (Table 4 and Plate 2). The RAPD for the determination of genetic variation in out crossing species where were no prior genotypic knowledge is available (Arpaia *et. al.*, 2005), (Ashworth et al. 2004) and (Inoue and Takahashi, 1990).

The general mean of allele frequency were produced from all of markers slight different from RAPD-PCR, which were 0.80,0.20,26.1% and 21.3% for major allele, minor allele, gene diversity and PIC (Table 4). It presumed that alleles having positive effect on traits under selection should have increased their frequencies. In addition to, those were with positive effect on such traits. In addition to, the frequency of alleles with large effects should increase or decrease faster than the frequency of alleles with relatively small effects (Delaney and Bliss 1991). Further evaluation, adaptation and selection can expected to lead to genetic gain for fresh, dry and quality yield and related traits. Directional selection and selection environment had significant effects on shifts in marker allele frequencies. Nevertheless, genetic diversity maintained within the populations. It is noticeable from Table (1) that multi-country between the imports of seeds, which Egypt depends largely on the import. Rai *et. al.* (1999) demonstrated that genetic shifts occur when pearl millet populations grown in different selection environments, permitting this crop to be fine-tuned for its adaptation to the growing environment.

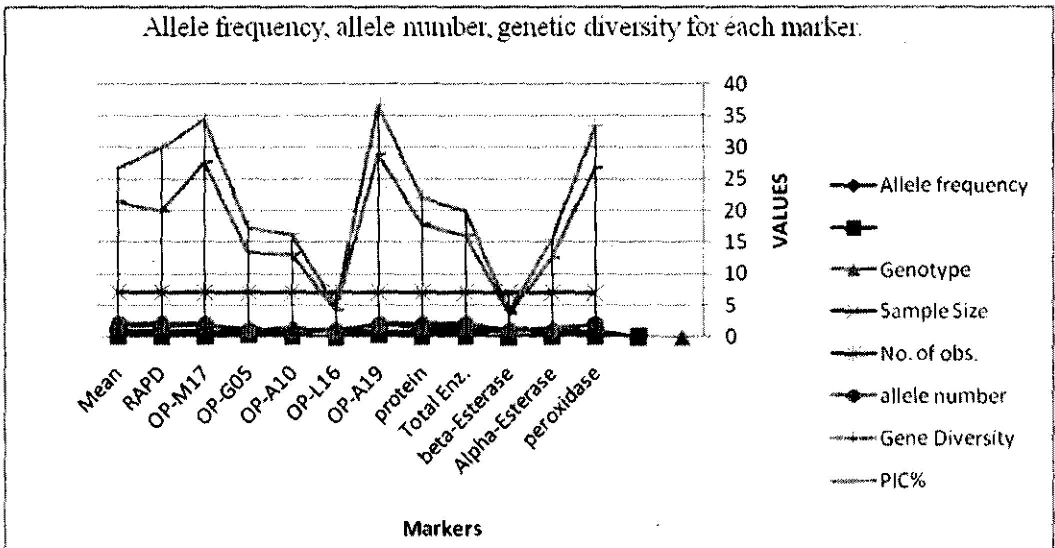

Figure 2. illustration of allele frequency, allele number, genetic diversity for each marker.

Clustering analysis and similarity matrix

Isozymes: The isozymes data developed by all isozymes of this study used to estimate the genetic similarities among the imported seven varieties and hybrids. The genetic similarity matrix based on all possible pairs of varieties and hybrids ranged from 68.6 % to 93.3 % (Table 5). The lowest genetic similarity value was between Jamon and Vroushanger (68.6%), while the highest genetic similarity was noted between Rota and Jamon. This value was repeat between Jamon and Starmoon (93.3%) followed by Betarose and Starmoon, Betarose and Rota (92.7%).

Table 4. estimation of Allele frequency, allele number, genetic diversity for each marker.

Marker	Allele frequency		Genotype no.	Sample size	No. of obs.	Allele number	Gene diversity %	PIC%
	Major	Minor						
peroxidase	0.76	0.24	1.9	7	7	2	33.5	26.8
Alpha-Esterase	0.89	0.11	1.4	7	7	1	15.4	12.5
beta-Esterase	0.98	0.02	1.2	7	7	1	4.1	3.6
Total Enz.	0.86	0.14	1.5	7	7	2	20	16
protein	0.83	0.17	1.6	7	7	2	22.2	17.8
OP-A19	0.72	0.28	1.9	7	7	2	36.6	29.0
OP-L16	0.96	0.04	1.1	7	7	1	5.1	4.1
OP-A10	0.89	0.11	1.4	7	7	1	16.3	13.0
OP-G05	0.73	0.27	1.3	7	7	1	17.3	13.3
OP-M17	0.75	0.25	1.9	7	7	2	34.4	27.7
RAPD	0.80	0.20	1.8	7	7	2	30	20
Mean	0.80	0.20	1.7	7	7	2	26.7	21.3

PIC = Polymorphic information content. No. of obs.= number of observation.

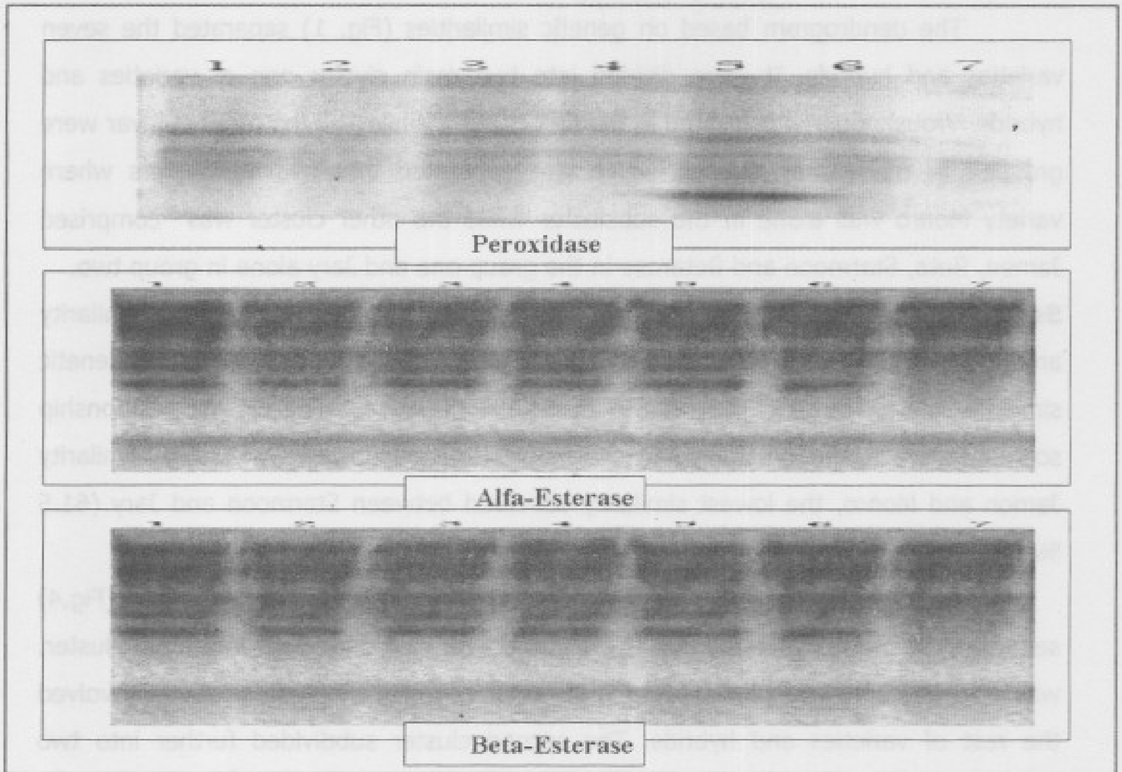

Plate 1. Isozymes analysis of seven imported fodder beet varieties and hybrids with peroxidase, Alfa and beta esterase Lane 1: Starmoon; 2-7:, Monro, Jamon, Jary, Rota, Betarose, Vroushanger

HIERARCHICAL CLUSTER ANALYSIS

Dendrogram using Average Linkage (Between Groups)

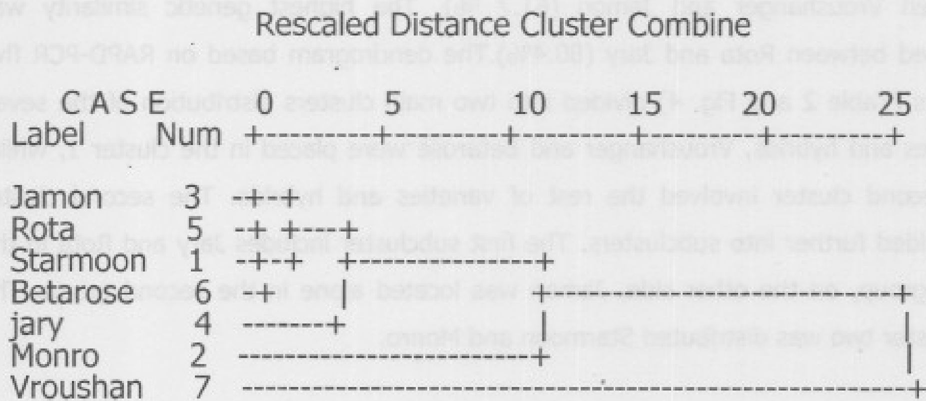

Figure 3. Cluster analysis of the isozymes peroxidase and esterase (Alfa and beta) data in case of the introduced fodder beet varieties and hybrids.

The dendrogram based on genetic similarities (Fig. 1) separated the seven varieties and hybrids. It were divided into two main cluster one of varieties and hybrids, Vroushanger was located in the first cluster. While, all the other cultivar were grouped in the second cluster, which was separated into two subclusters where variety Monro was alone in the subcluster while the other cluster was comprised Jamon, Rota, Starmoon and Betarose in the group one and Jary alone in group two.

Seed Storage Protein: The protein data used to estimate the genetic similarity among seven imported varieties and hybrids and hybrids fodder beet. The genetic similarity matrix ranged from 61.5% to 94.1% (Table 6). The closest relationship scored between Vroushanger and Starmoon (94.1) followed by 93.8% similarity Jamon and Monro, the lowest similarity observed between Starmoon and Jary (61.5 %).

The dendrogram based on seed storage protein similarity matrix (Fig.4) separated the seven varieties and hybrids and hybrids fodder beet into main cluster, where variety Jary was placed in separate cluster, while, the second cluster involved the rest of varieties and hybrids. The second cluster subdivided further into two subclusters. The first subcluster includes only Rota variety, while the rest of the cultivars occurred in the second subcluster.

RAPD-PCR Analysis: The RAPD data collected by all primers of this study used to estimate the genetic similarities among seven varieties and hybrids and hybrids (Table 2). Also, the genetic similarity matrix based on all possible pairs of varieties and hybrids ranged from 60.4% to 80.4% Table (7). The lowest genetic similarity was between Vroushanger and Monro (60.4%) followed by genetic similarity value between Vroushanger and Jamon (61.7 %). The highest genetic similarity was achieved between Rota and Jary (80.4%).The dendrogram based on RAPD-PCR five primers (Table 2 and Fig. 4) divided into two main clusters distribution of the seven varieties and hybrids, Vroushanger and Betarose were placed in the cluster 1, while, the second cluster involved the rest of varieties and hybrids. The second cluster subdivided further into subclusters. The first subcluster includes Jary and Rota in the same group, on the other side, Jamon was located alone in the second group. The subcluster two was distributed Starmoon and Monro.

Table 5. Similarity matrix among seven introduced fodder beet varieties and hybrids based on isozymes peroxidase and esterase(Alfa and beta) analysis.

Proximity Matrix

Case	Matrix File Input					
	Monro	Jamon	jary	Rota	Betarose	Vroushanger
Starmoon	.850	.933	.878	.909	.927	.706
Monro		.878	.865	.800	.811	.733
Jamon			.905	.933	.905	.686
Jary				.927	.895	.710
Rota					.927	.706
Betarose						.774
Vroushanger						

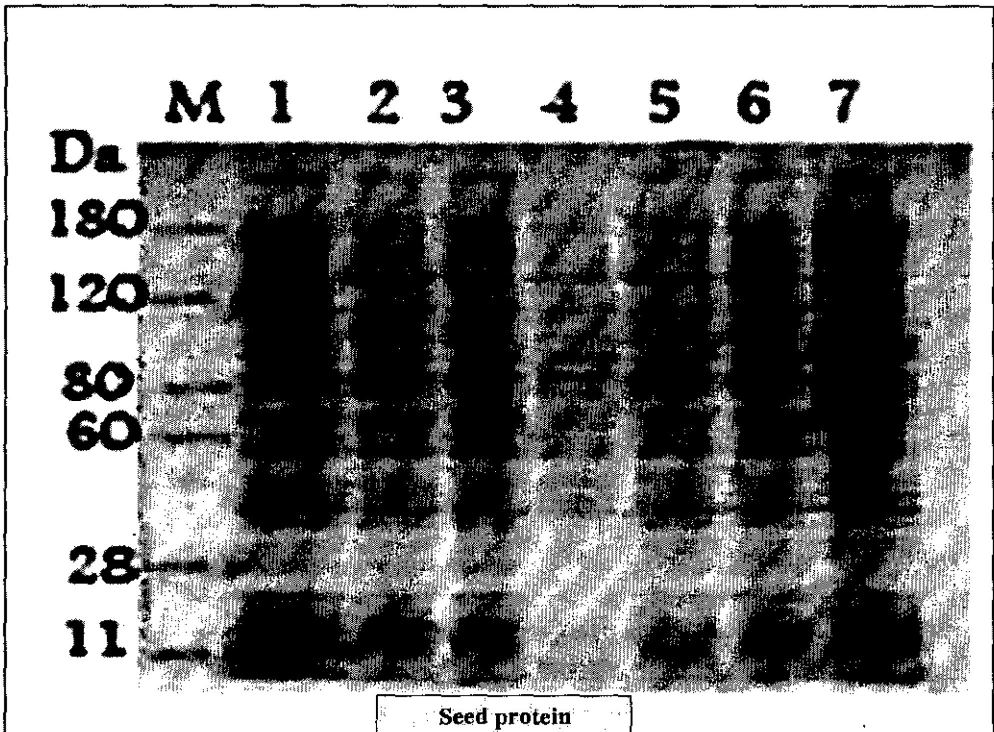

Figure 4. Seed protein analysis of seven imported fodder beet varieties and hybrids Lane 1: protein Marker; 2-8: Starmoon, Monro, Jamon, Jary, Rota, Betarose, and Vroushanger.

Compared with Isozymes, Protein and RAPDs data showed a relatively slight separation of the varieties and hybrids These results agree with (Abe et al. 1987) in isozymes and Reamon-Buttner et al. (1996) in RAPD-PCR. They suggests that the isozymes and RAPD data may provide a more accurate picture of relationships at the species level.

Table 6. Similarity matrix among seven introduced fodder beet varieties and hybrids based on seed protein analysis

Proximity Matrix

Case	Matrix File Input					
	Monro	Jamon	jary	Rota	Betarose	Vroushanger
Starmoon	.909	.971	.615	.759	.875	.941
Monro		.938	.696	.846	.897	.839
Jamon			.640	.786	.903	.909
Jary				.737	.727	.667
Rota					.880	.815
Betarose						.933

HIERARCHICAL CLUSTER ANALYSIS

Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine

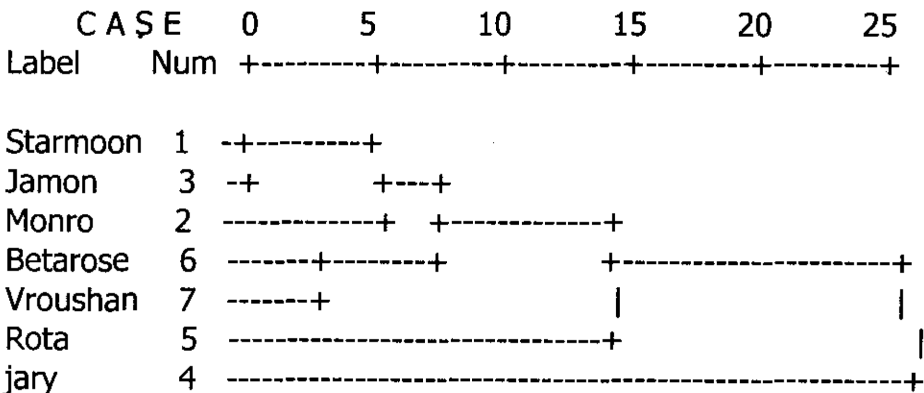

Figure 5. Cluster analysis of the seed protein data in case of the introduced fodder beet varieties and hybrids.

It is also clear the different composition of genetic varieties and hybrids of class tetraploid as well as hybrid genetic composition triploid shows how the difference between the data in the three techniques (Schwanitz 1938 and Arumuganathan and Earle 1991). Moreover, both methods provided good results in fingerprinting and identified all cultivars tested. Genetic similarity analysis (UPGMA) gave different results from RAPD and isozyme data. This could reflect different properties of both marker types (Samec *et. al.* 1998).

Table 7. Similarity matrix among seven introduced fodder beet varieties and hybrids based on RAPD- PCR data from five primers OP-A19, OP-A10, OP-G05, OP-L16 and OP- M17 analysis.

Proximity Matrix

Case	Matrix File Input					
	Monro	Jamon	jary	Rota	Betarose	Vroushanger
Starmoon	.723	.696	.723	.701	.696	.625
Monro		.674	.745	.660	.630	.604
Jamon			.783	.758	.711	.617
Jary				.804	.696	.688
Rota					.737	.687
Betarose						.787

HIERARCHICAL CLUSTER ANALYSIS

Dendrogram using Average Linkage (Between Groups)
Rescaled Distance Cluster Combine

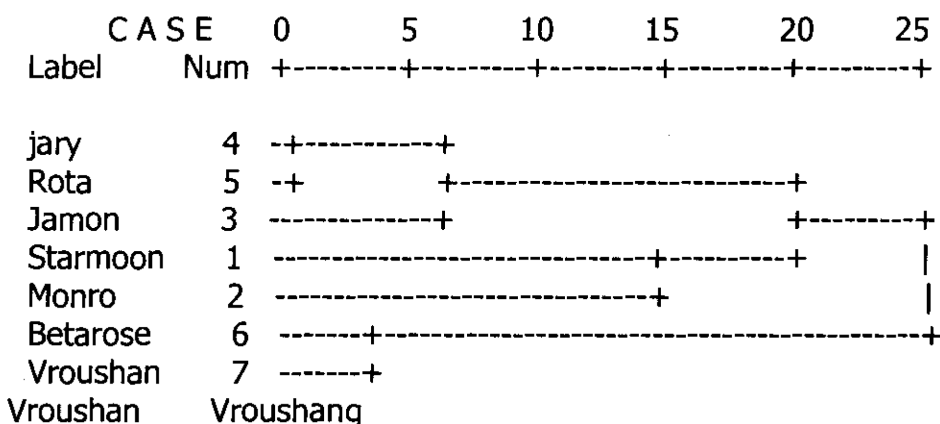

Figure 6. Cluster analysis of RAPD- PCR data from five primers OP-A19,OP-A10,OP-G05,OP-L16 and OP- M17 in case of the introduced fodder beet varieties and hybrids.

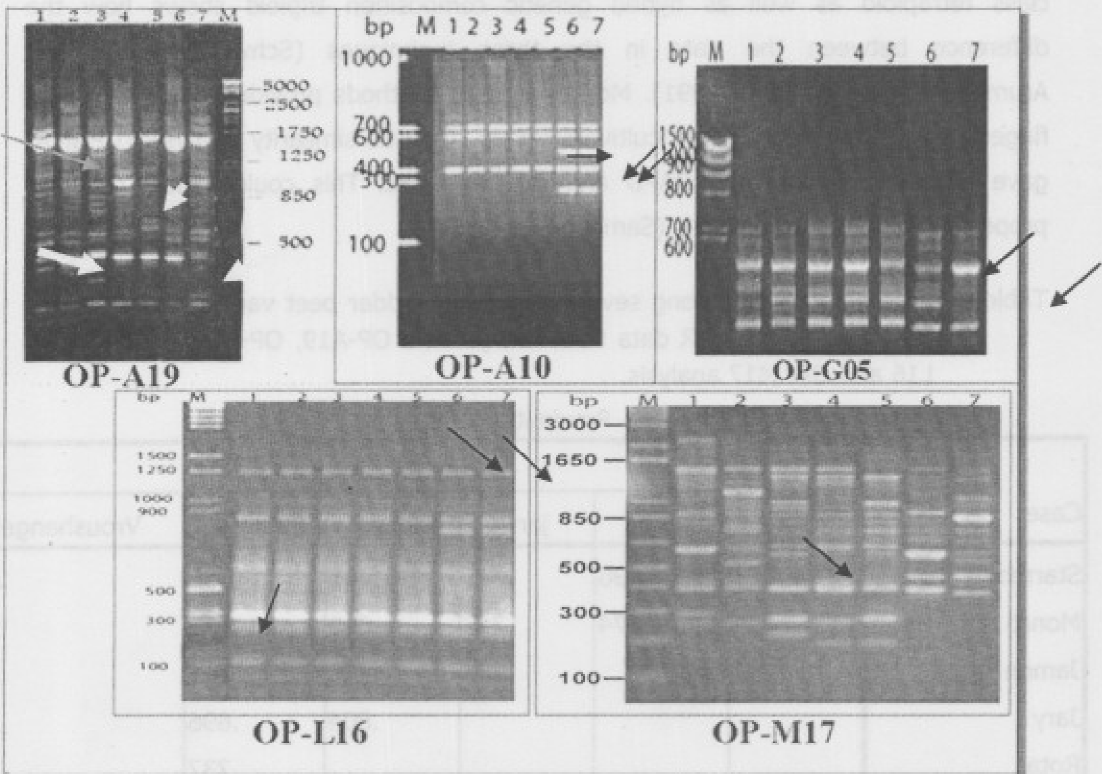

Plate 2. RAPD analysis of seven imported fodder beet varieties and hybrids with five primers (OP-A19, OP-A10,OP-G05,OP-L16 and OP- M17), Lane 1: DNA Marker;2-8: Starmoon, Monro, Jamon, Jary, Rota, Betarose, Vroushanger (the arrows were indicated to the unique bands).

Combined Data Set

Based on isozymes, seed protein and RAPD-PCR combined analysis, the overall Similarity matrix (Table 8 and Fig.5) revealed that the highest similarity was (82.8%) and occurred between Rota and Jary followed by Jamon and Starmoon (81.4%) and Rota and Jamon (80.0%) , while, the lowest similarity was (67.5%) between Vroushanger and Monro followed by(68.9%) Vroushanger and Rota. The dendrogram resulting from the combination of the three systems, isozymes, protein and RAPDs (Figure 6) separated the seven imported varieties and hybrids into two main clusters. Betarose and Vroushanger were separated in the first main cluster, while, the rest of varieties and hybrids were located in the second main cluster, which were separated further into two subclusters. Jary and Rota grouped together in the first subcluster, while, the second subcluster were consists of two groups ,however, the first group was included Monro only, while the second group was separation between Starmoon and Jamon.

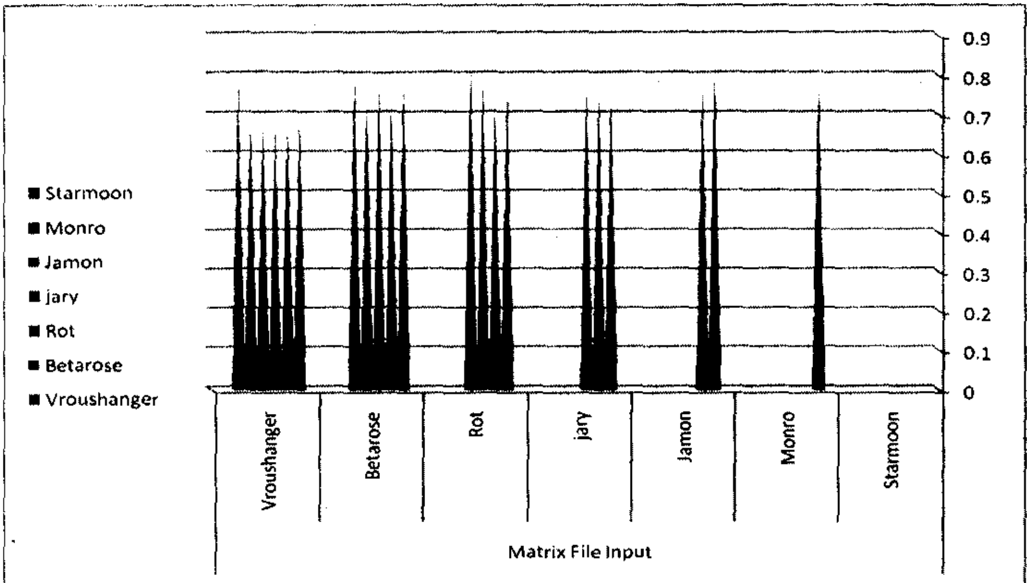

Figure 7. Illustration overall similarity matrix among seven introduced fodder beet varieties and hybrids based on isozymes peroxidase and esterase (Alfa and beta), seed protein and RAPD- PCR from five primers OP-A19,OP-A10,OP-G05,OP-L16 and OP- M17 data analysis.

Table 8. Similarity matrix among seven introduced fodder beet varieties and hybrids based on isozymes peroxidase and esterase (Alfa and beta), seed protein and RAPD- PCR from five primers OP-A19,OP-A10,OP-G05,OP-L16 and OP- M17 data analysis.

Proximity Matrix

Case	Matrix File Input					
	Monro	Jamon	jary	Rota	Betarose	Vroushanger
Starmoon	.790	.814	.745	.765	.788	.707
Monro		.776	.766	.724	.722	.675
Jamon			.792	.810	.798	.691
jary				.828	.750	.689
Rota					.807	.713
Betarose						.813

The results indicated that the combined-based dendrogram, as well as, the RAPD-based dendrogram were agreement with Williams et al. (1990), Arumuganathan and Earle 1991, Virk et al. l(1995), and Shen et al. (1996). Sangeeta Srivastava et al. (2007), who found that the much lower values of average genetic similarity based on

RAPD (0.27) and ISSR (0.35) indicated the ability of DNA based markers to detect high degree of polymorphism among these populations suggesting thereby, the possibility of screening a higher number of anonymous loci in sugar beet using these molecular markers as compared to isozymes.

HIERARCHICAL CLUSTER ANALYSIS

Dendrogram using Average Linkage (Between Groups)

Rescaled Distance Cluster Combine

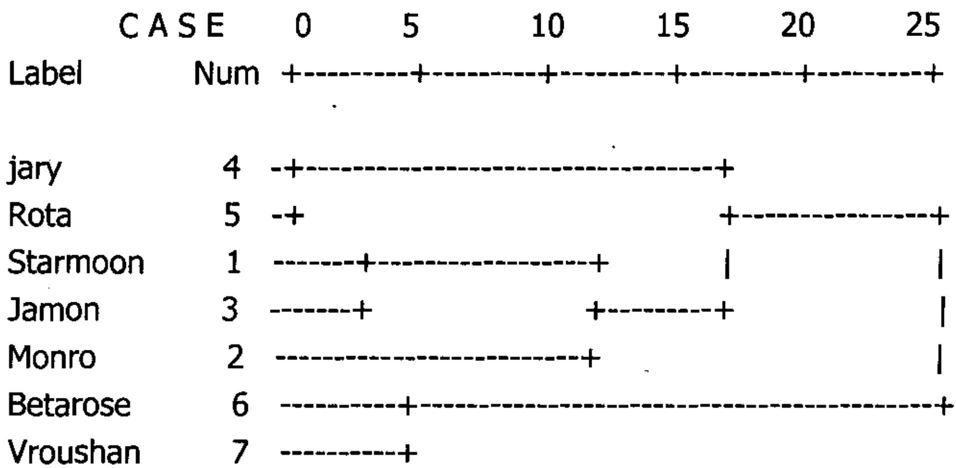

Figure 8. Cluster analysis of the isozymes peroxidase and esterase (Alfa and beta), seed protein and RAPD- PCR from five primers OP-A19, OP-A10, OP-G05, OP-L16 and OP- M17 data in case of the introduced fodder beet varieties and hybrids.

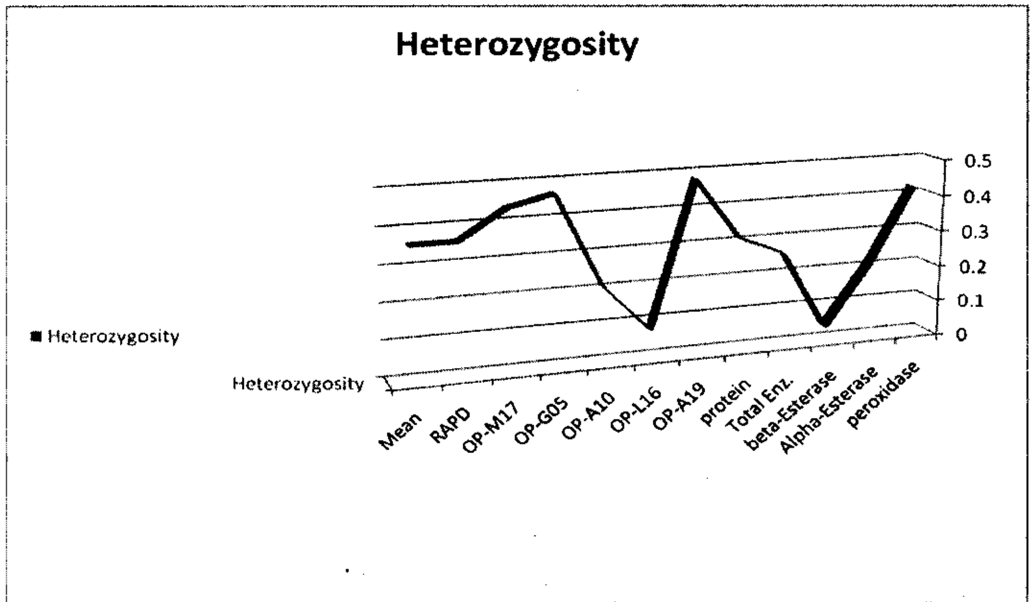

Figure 9. illustration different level from allele diversity with molecular marker used in this study with seven imported varieties and hybrids.

Generally, combining isozymes, seed protein and RAPD-PCR systems offered better resolution between seven imported varieties and hybrids Yu et al. (2005) studied Genetic distances (GDs) based on morphological characters, isozymes and storage proteins, and random amplified polymorphic DNAs (RAPD) were used to predict the performance and heterosis of crosses in oilseed rape (*Brassica napus* L.). In addition, Jung (2004) were used combined data in work of genetic map and genetic diversity. Moreover, Sangeeta Srivastava et al. (2007) used GS coefficients of isozyme, RAPD and ISSR were to cluster the genotypes based on the UPGMA method and the dendrograms obtained were compared to develop consensus phylogenetic trees.

Heterozygosity

The observed heterozygosity ranged from 0.04 in β-esterase to 0.48 in OP-A19 followed by OP-G05 0.47. The total enzymes, seed protein, RAPD-PCR and the general mean gave 0.26, 0.31, 0.36 and 0.36, respectively, (Fig. 8 and Table 3). The level of polymorphism ranged from 3.9 to 89.4,the lowest polymorphism 3.9% with OP-L16, which reflects the ability of the few disperse between varieties, while, the highest polymorphism is 89.4 with OP-M17 followed by 89.3 with OP-A19. Low values of heterozygosity are expected in populations of a recently introduced were in agreement with Martins *et. al.* (2007) who found The analysis of isozymes showed a polymorphism and a Heterozygosity ranging from 25 to 100% and 0.174 to 0.277,

respectively. This illustrates the extent of high capacity for these primers to differentiate between items. Moreover, Brown and Langley (1979) determine the level of Heterozygosity of the most abundant proteins in *Drosophila melanogaster* adults from a natural population. These results support the use of the simpler techniques to estimate relative levels of genetic divergence between imported varieties.

Recommendations

It could be recommended that the genotypes Vroushanger, Jamon and Jary grown with each other was select the best for different environments. Selecting one genotype of genetic makeup to cultivate the following in the same place for the convergence of the genetic material (Jamon and Starmoon), (Rota and Betarose) and (Rota and Jary).

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التحليل الجزيئي لسبعة مستوردات من بنجر العلف

مصطفي فرج عيد الجواد[٣]،زينب محمد عبد النبي[١]،إيهاب محمد ربيع متولي [٢] ,إيهاب محمد زايد[١]

١. *قسم بحوث محاصيل العلف، معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية, جيزة –مصر*
٢. *قسم النبات، كلية الزراعة، جامعة قناة السويس،إسماعيلية – مصر.*
٣. *معهد بحوث المحاصيل السكرية – مركز البحوث الزراعية – جيزة – مصر .*

لا ينتج بنجر العلف أزهارا في ظروف الزراعة المصرية. وقد تمت العديد من المحاولات العشوائية لإزهار بنجر العلف التي لم تستكمل مراحلها لإنتاج بذرة خصبة ويرجع ذلك لعوامل عدم الخصوبة التي تتعلق بالظروف المناخية. لذلك تعتمد زراعة بنجر العلف في مصر علي استيراد التقاوي من عديد من البلدان الخارجية مثل فرنسا، وبلغاريا والمجر.

واستيراد المصادر الوراثية وتعريفها من أهم المحددات ذات القيمة العليا لمربي النبات،يرجع ذلك إلي التباينات الوراثية العالية في تركيب الزهرة والتي تتعلق بإنتاج البذرة في بنجر العلف.

وقد إستخدم في هذة الدراسة تقنيات المشابهات الإنزيمية (لإنزيم البيروكسيد وإنزيم استريز إلفا وبيتا) ، البروتين في البذرة وRAPD (خمسة بادئات جزيئية) تقنيات لمعرفة المادة الوراثية لبنجر العلف المستورد في مصر.

في اختبار الإنبات كان أعلى قيمة للإنبات في بيتاروز و ستارمون في ٤ أيام وحتى ١٠ يوما، في حين حقق الهجين جامون أدنى قيمة في ٤ أيام وحتى ١٠ يوما (٥٣,٣٣ ٪ و ٦٠ ٪ وكذلك و٧٣ ٪ للنوعين على التوالي).

كما أظهرت النتائج أن نسبة التشابه الوراثي تراوحت بين ٦٨,٦ ٪ إلى ٩٣,٣ ٪ لكل المشابهات الإنزيمية، 61.5 ٪ إلى ٩٤,١ ٪ للبروتين في البذرة ، ٪ كما كان في المعلم الجزيئي لRAPDs ٦٠,٤ ٪ إلى ٨٠,٤.

و أوضح التحليل الجزيئي المشترك لجميع البيانات في كل الأنظمة الجزيئية و التشابه الوراثي العام ما بين الأصناف أن التشابه الوراثي كان أعلى بنسبة (٨٢,٨ ٪) بين روتا وجاري يليها جامون وستارمون بنسبة (81.4 ٪) ، وروتا و جامون بنسبة (80.0%) ، بينما كان أدنى تشابه بنسبة (٦٧,٥ ٪) بين فورشنجر و مونرو ويليها نسبة (٦٨,٩ ٪) بين فورشنجر و روتا.

ويمكن تحديد القيمة النسبية لكل معلم جزيئي على أساس مضمونها و بما أعطته من معلومات متعددة الأشكال المظهرية (PIC) والتي تراوحت من 3.6 إلى ٢٩ ٪ في حين كان علي المستوي العام = ٢١,٣ ٪ مع عدد من الأ ليلات ٢ في كل نظام تقنية مستخدم. وتراوحت نسبة الخلط Heterozygosity من ٠,٠٤ في استريز – β إلى ٠,٤٨ في RAPD ل البادئ الجزيئي A19 يليها ٠,٤٧ ل البادئ الجزيئي G05 .