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Estimation of Genetic Diversity in Wild and Cultivated Forms of Beet Using RAPD and AFLP Markers

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Abstract: Two PCR-based molecular marker techniques, i.e., RAPDs and AFLPs were used to assess the genetic relationships and genetic diversity within and among five types of beet (wild, sugar, table, fodder and chard). A total of 383 polymorphic bands (11.97 polymorphic markers per primer) out of 420 reproducible fragments were generated from the thirty-two RAPD primers. Whereas, five AFLP primer combinations produced a total of 341 scorable fragments among beet genotypes of which 275 bands were polymorphic with an average of 55 polymorphic bands per primer combination. Results indicated that the percentage of polymorphic markers was lower for AFLP than for RAPD (80.61 and 89.64%, respectively). Genetic similarity was measured on the basis of Dice's similarity coefficient (DSC's) and ranged from 49.3 to 86.9 with an average of 68.1 and from 58.7 to89.8 with an average of 74.25 for RAPD and AFLP markers, respectively. The phylogenetic trees generated using UPGMA cluster analysis for RAPD and AFLP data were highly similar with a few discrepancies in the values of similarity coefficients. Beet genotypes were divided into three major groups, according to the geographic distributions and traditional botanical classification. RAPD data analysis generated a total of 76 unique markers of which 52 markers were positive and 24 negative with molecular weight ranged from 125 to 2200 bp. Moreover, fourteen RAPD common markers were identified. Among these markers, 13 markers could be used to discriminate wild beet accessions from the other cultivated forms of beet and only 1 marker could be used to discriminate cultivated forms of beet from wild beet accessions. Whereas, AFLP primer combinations used revealed a total of 34 unique markers comprising 21 positive and 13 negative with molecular weight ranged from 60 to 580 bp. In addition, nine AFLP common markers were identified, seven markers could be used to differentiate or discriminate wild type of beet from the other types used in this study (cultivated forms of beet), while two markers could be used to differentiate cultivated forms of beet (sugar, table, fodder and chard) from wild beet accessions.

Key words: Genetic diversity, Wild beet accessions, Cultivated forms of beets, RAPD, AFLP.

INTRODUCTION

The centre of origin of wild beet (*Beta vulgaris* subsp. *maritima*) is widely believed to be the Middle East, near the Tigris and Euphrates Rivers. It is thought that wild beet accessions spread west into the Mediterranean and north along the Atlantic Coast and into the mountains of Turkey [1]. Wild beet is considered very important since, it is salt-tolerant and could be used as a genetic resource for some disease resistance traits [2]. Cultivated beet forms i.e. garden beet, leaf beet (chard & spinach), fodder beet and sugar beet were produced through selective cultivation of wild beet. All of the previous beet forms are members of the family *Chenopodiaceae* [3].

The first collection of wild beet accessions in Egypt was conducted by El-Manhaly in 1990, where twenty-six accessions were collected from three different districts, North Western Coast, Delta of the Nile and Upper Egypt [4]. Although the number of accessions deposited in gene banks is continuously growing, their effective use is limited by breeding programs. A major cause of this discrepancy is due to slow germplasm evaluation and characterization. Opportunities to maximize the benefit of crop genetic resources, in gene banks, have been accessible by molecular marker techniques [5].

Randomly amplified polymorphic DNA (RAPD-PCR) is a classical genetic marker technique resulting from PCR amplification of genomic DNA sequences

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recognized by ten-mer random primers [6]. RAPD technique is attractive because it is technically easy to implement, cheap compared to other marker systems, a non-radioactive assay, no required prior knowledge of the DNA sequence to design the primers, requires a small amount of DNA and fast in terms of obtaining results which makes it accessible to a broad range of biologists all over the world. It can be used in germplasm characterization, assessment of genetic diversity and genetic relationships studies and used to construct genetic maps in a variety of species [7,8,9,10].

RAPD marker data can be used to identify beet materials and avoid the taxonomic uncertainty which can occur due to phenotypic classification. Moreover, the application of RAPD technology has supported this revision of *Beta* section and *Beta* taxonomy, it also provides a rapid and reliable method for the identification of closely related plant material [11]. In addition, RAPD data provides a more accurate picture of relationships at the species level [12]. Moreover, RAPD data could be used for identification and elimination of duplications in a collection, which can save time and money regarding maintenance due to the reduced number of accessions banked. The identification of genetic diversity, using molecular markers in wild relatives of cultivars, provides breeders with genetic resources for crop improvement [13]. In addition, RAPD markers were found to be a useful tool for detecting genetic variation within many genuses like *Beta* [12] and *Chenopodium* [14], they were successfully used to determine genetic distance among beet [8] and saltgrass [15] genotypes. They also, allowed discriminating between all commercial varieties and wild species of plants [16].

Amplified Fragment Length Polymorphism (AFLP) is a powerful DNA fingerprinting technique easy to use, high replicable and very reproducible. It is used for rapid screening of genetic diversity and DNA fingerprinting that combines both hybridization-based and PCR-based technology [17]. Therefore, AFLP markers have emerged as a major new type of genetic markers with broad applications [18]. AFLP is considered a powerful tool for genotyping large number of accessions due to its high polymorphism level. Therefore, it is suitable for genetic diversity assessment in large populations in gene banks like sweet potato [19], and in closely related commercial cultivars like pepper [20]. Moreover, it has been extensively used for discrimination between closely related genotypes within a species and for genotyping methodology for numerous plants [21].

AFLP markers proved to be a valuable tool for cultivar identification, especially in collections having a narrow genetic base i.e. Vanuatu taro germplasm and useful for detecting duplicates and fingerprinting of accessions [22]. AFLP constitutes an attractive approach to generate a nearly unlimited number of markers. The enlargement of the number of primer combinations would supply more useful information to assist the selection and to manage rationally the conservation of the local germplasm [23].

RAPDs and AFLPs appear to be valuable tools for assessing genetic diversity levels in Azuki [24], and in *Phaseolus* accessions [25]. Among other commodities AFLPs proved to be more efficient than RAPDs for generating polymorphisms and, therefore, were more efficient for estimation of genetic diversity among accessions [24].

This study was conducted to estimate the level of polymorphism, genetic similarity within and among five types of beet (wild, table, sugar, fodder and chard) and to identify unique DNA markers to generate a fingerprint for each accession.

MATERIALS AND METHODS

Plant Material: Six Egyptian wild beet accessions (WB-1003, WB-1006, WB-1007, WB-1013, WB-1021 and WB-1026 kindly provided by Dr. Alan L. Hodgdon, USDA, ARS, Regional Plant Introduction Station (WRPIS), Washington State University, USA), in addition to other cultivated forms of beet (sugar beet, Top and Oscar poly cultivars; table beet, Detroit and CrosbyEgy cultivars; fodder beet, Frochenigar cultivar and chard, Balady cultivar obtained from local market) were used in this study to investigate the phylogenetic relationships within and among them through RAPD and AFLP makers.

Twenty-five seeds from each wild beet accession and each cultivated form of beet were planted in a 25 cm diameter plastic pot filled with mixture of peat moss: vermiculite, 1:1 (v/v) media. Afterwards, pots were kept in the greenhouse and regularly irrigation till seeds completely germinated and produced young beet seedlings which were used as plant material for DNA extraction. After two weeks of planting date young beet seedlings from each genotype were collected, bulked and used directly for DNA extraction.

Extraction of Genomic DNA: Genomic DNA was extracted from young beet seedlings using DNeasy® Plant mini Kit for DNA isolation from plant tissue (Cat # 69104, QIAGEN Inc., California, USA). Extraction was carried out according to the manufacturer's protocol.

Random Amplified Polymorphic DNA (RAPD): The DNA amplification protocol was performed as described by Welsh and McClelland [26] and Driessen *et al.* [27].

Primers Used in Rapd Analysis: A total of thirty-two random 10-mer Operon primers Table (1) were used in the detection of polymorphism among the six wild beet accessions and other relative beet forms.

Preparation of PCR Reactions: Reactions were carried out in a total volume of 25 µl containing 3 µl of 10 ng of genomic DNA as a template, 3 µl of 10 pmoles of random primer, 2.5 µl of 2mM of dNTP's mix (ABgene, Surrey, UK), 2.5 µl of 10X PCR buffer, 1.5 µl of 25 mM $MgCl_2$, and 2 units *Taq* DNA polymerase (MBI Fermentas Inc., Wisconsin, USA). An aliquot of 22 µl master mix solution was dispensed in each PCR tube (0.2 ml eppendorf tube), containing 3 µl of the appropriate template DNA.

PCR Program and Temperature Profile: PCR amplification was performed in a GeneAmp® PCR System 9700 (Applied Biosystems, California, USA), programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94ºC. Complete denaturation of DNA results in the efficient utilization of template in the first amplification cycle and in a good yield of PCR product. Each cycle consisted of 3 steps, a denaturation step at 94ºC for 45 sec, an annealing step at 36ºC for 1 min, and an elongation or extension step at 72ºC for 2 min. After the last cycle the primer extension segment was extended to 7 min at 72ºC in the final extending cycle then followed by soaking at 4ºC until reaction removed from PCR machine.

ElectropHoresis of PCR Products: The amplification products were resolved by electrophoresis in a 1.4% agarose gel containing ethidium bromide (0.5 µg/ml) in 1X TBE buffer. 15 µl of each PCR product were mixed with 3 µl of loading buffer (tracking dye), and loaded onto the wells of the gel. The gel was run at 85 volts for about 3 hrs or until tracking dye reached to the end of the gel.

Visualization and Photograph of Pcr Products Pattern: After electrophoresis, the amplified RAPD-PCR product patterns were visualized under UV transilluminator. The gels were photographed using a Polaroid camera (MP4 Land Camera) and Polaroid films type 57 (ASA3000). In addition, Gel Documentation System (Gel-Doc 2000, Bio-Rad Laboratories, California, USA) was used for gel documentation and gel analysis.

Amplified Fragment Length Polymorphism (AFLP): Five AFLP primer combinations, E-AAC X M-CAA, E-ACA X M-CAG, E-ACC X M-CAT, E-AGC X M-CTG and E-ACA X M-CTC were used in AFLP analysis utilizing the Invitrogen AFLP® Analysis system I, and AFLP Starter primer kit (Cat # 10544-013 and 10483-014, respectively, Invitrogen Corporation, California, USA).

Table 1: Sequence of thirty-two decamer arbitrary (10-mer) primers assayed in RAPD- PCR marker.

Primer	Sequence (5′ - 3′)	Primer	Sequence (5′ - 3′)
OPA-02	TGCCGAGCTG	OPB-18	CCACAGCAGT
OPA-04	AATCGGGCTG	OPC-07	GTCCCGACGA
OPA-07	GAAACGGGTG	OPC-13	AAGCCTCGTC
OPA-08	GTGACGTAGG	OPC-15	GACGGATCAG
OPA-10	GTGATCGCAG	OPC-20	ACTTCGCCAC
OPA-12	TCGGCGATAG	OPE-05	TCAGGGAGGT
OPA-13	CAGCACCCAC	OPE-10	CACCAGGTGA
OPA-14	TCTGTGCTGG	OPO-02	ACGTAGCGTC
OPA-16	AGCCAGCGAA	OPO-03	CTGTTGCTAC
OPA-18	AGGTGACCGT	OPO-04	AAGTCCGCTC
OPA-20	GTTGCGATCC	OPO-07	CAGCACTGAC
OPB-06	TGCTCTGCCC	OPO-15	TGGCGTCCTT
OPB-07	GTCCACACGG	OPO-16	TCGGCGGTTC
OPB-11	GTAGACCCGT	OPO-19	GGTGCACGTT
OPB-12	CCTTGACGCA	OPO-20	ACACACGCTG
OPB-13	TTCCCCCGCT	OPZ-04	AGGCTGTGCT

The success of the AFLP technique depends upon complete restriction digestion of DNA, therefore, much care should be taken in consideration to isolate high quality (purity) genomic DNA, intact without contaminating nucleases or inhibitors. AFLP technique was carried out according to Vos *et al.* [17].

Restriction Digestion of Genomic DNA: Restriction digestion of genomic DNA step generates the required substrate for ligation and subsequent amplification. Genomic DNA, 20 µl (300 ng/20 µl) was digested with 2 µl of two restriction enzymes simultaneously *EcoR1*/*Mse1* (1.25 units/µl each), 5 µl of 5x reaction buffer and 3 µl distilled water. These enzymes generate small DNA fragments that will amplify well and are in the optimal size range (<1 kb). A total of 30 µl was mixed well and incubated overnight at 37ºC, followed by a 15 min at 70ºC to inactivate restriction enzymes, then immediately put on ice.

Ligation of Adapters: *EcoRI* and *MseI* adapters were ligated to the digested DNA fragments to generate template DNA for amplification. This reaction was performed in 55 µl total volume as follows: 24 µl adapter/ligation solution (*EcoR1/Mse1* adapters) and 1 µl of T4 DNA ligase (1unit/µl) were added to 30 µl of digested DNA samples, then incubated at 20 ± 2ºC for at least 2 hours. Ligated reaction mixture was diluted 10-folds with TE, after loaded onto 2% agarose gel to make sure that DNA samples were completely digested and 10-folds dilution were performed according to the samples concentration obtained from the gel.

Amplification of the Restriction Fragments: Two consecutive PCR reactions were performed as follow:

Pre-amplification of DNA: The first PCR reaction was performed in a total volume of 51 µl which consisted of 5 µl of the 10-folds diluted ligation mix, 40 µl pre-amp primer mix, 5 µl of 10X PCR buffer plus $MgCl_2$ and 1 µl *Taq* DNA polymerase (MBI Fermentas Inc., Wisconsin, USA). The mixture was mixed gently and centrifuged briefly to collect reaction contents. PCR conditions were 20 cycles at: 94ºC for 30 sec, 56ºC for 1 min, and 72ºC for 1 min, and soaked at 4ºC. The pre-amplified products were diluted in a ratio of 1:50 according to samples concentration obtained from the gel after running samples in a 2% agarose gel.

Selective Amplification of DNA: One to fifty dilutions of pre-amplified products was used as templates for selective amplification using two AFLP primers, each containing 3 selective nucleotides. Two mixes were prepared; Mix 1 (primers/dNTPs), with a total volume of 65 µl (6.5 µl *EcoR1* primer and 58.5 µl *Mse1* primer contains dNTPs), Mix 2 (*Taq* DNA polymerase/buffer), with a total volume of 130 µl (102.7 µl ddH_2O, 26 µl 10X PCR buffer plus $MgCl_2$ and 1.3 µl *Taq* DNA polymerase). The reaction was performed in a 20 µl total volume of 5 µl diluted pre-amp product (1:50 dilution), 5 µl Mix 1 and 10 µl Mix 2, gently mixed and fast spin to collect reaction components. The reactions were carried out using the following cycling parameters: one cycle at 94ºC for 30 sec, 65ºC for 30 sec, 72ºC for 1 min followed by 12 cycles in which the annealing temperature decreases 0.7ºC, per cycle as touch down PCR, followed by 23 cycles at 94ºC for 1 min, 56ºC for 30sec, and 72ºC for 1 min. After completion of the PCR program, the reaction was stored at –20ºC until loaded onto a 6% denaturing polyacrylamide gel.

Gel Electrophoresis and Gel Analysis: Products from the selective amplification were separated using Sequi-Gen® Sequencing Cell (Bio-Rad Laboratories, California, USA) on a 6% denaturing polyacrylamide gel.

After completion of loading the samples, the gel was run at 50 Watt for 2 hours or until xylene cyanol (slower dye) was two-thirds down the length of the gel. A 50ºC temperature was maintained throughout the run. Then, the gel was silver stained.

Silver Staining: The SILVER SEQUENCE™ DNA Staining Reagents Cat # Q4132 (Promega Corporation, Wisconsin, USA) was used to detect bands in a polyacrylamide gel. Staining steps were carried out according to the manufacturer's protocol.

Data Analysis: Amplified products for RAPD and AFLP markers were scored on the basis of the presence or absence of bands as '1' or '0' respectively. In order to determine the genetic relationships of the six selected wild beet accessions and other relative forms of beet. Both weak bands with negligible intensity and smeared bands were excluded or ignored from final data analysis. Moreover, Gel Documentation System (Gel-Doc 2000, with Diversity Database Fingerprinting Software, version 2.1, Bio-Rad Laboratories, California, USA) was used for gel analysis, scoring, data handling, cluster analysis and construction of dendrograms.

Pair-wise comparisons of wild accessions and cultivated forms of beet were used to determine similarity coefficients according to Dice's similarity coefficient (DSC's). The similarity coefficient was then used to construct dendrograms using the Un-weighted Pair Group Method with Arithmetic Averages (UPGMA) [28].

Unique positive and(or) negative markers as well as their sizes were identified from 0, 1 scoring data generated from each primer or each primer combination for RAPD and AFLP, respectively. In addition, common RAPD and AFLP markers that could be used to discriminate between wild beet accessions and other cultivated forms of beet were identified.

RESULTS AND DISCUSSIONS

Level of Polymorphism Within and among the Five Beet Types Revealed by RAPD: The set of 32 RAPD primers successfully amplified bands that were highly informative. They produced multiple banding profiles with a number of amplified DNA fragments ranging from 7 to 22, with an average of 13.13 bands per primer. The number of polymorphic fragments ranged from 6 to 21 with an average of 11.97 polymorphic bands per primer (Figure 1a). A maximum number of 22 fragments were amplified with primer OPA-13 and a minimum number of 7 fragments were amplified with primer OPC-15. The total number of reproducible fragments amplified by the thirty-two primers reached 420 bands, of which 383 were polymorphic fragments. This represented a level of polymorphism of 89.64% and an average of 11.97 markers per primer, which indicates a very high level of polymorphism among the genotypes studied. The size of the amplified fragments also varied with the different primers and ranged from 125 to 2250 bp.

RAPD analysis revealed a high level of polymorphism among the various genotypes studied, which enabled accurate analysis of the genetic distance. The obtained results are in good accordance with those of [8,9,10,11,12,27,29,30]. All these authors demonstrated that

RAPD primers produced reliable and reproducible banding patterns and the number, size of amplified DNA fragments and percentage of generated polymorphic bands differed with the different primers tested.

Levels of Polymorphism Within and among the Five Beet Types Revealed by AFLP: The AFLP primer combinations used generated 341 selectively amplified DNA fragments and identified 275 (80.65%) polymorphic markers. An average of 68.2 distinguishable bands was observed after selective amplification with each primer combination, and an average of 80.61% of these AFLP bands were found to be polymorphic among beet genotypes, while, the percentage of polymorphism ranged from 70.42 to 88.09%. The highest percentage of polymorphism (88.09%) was obtained with primer combination, E-ACA X M-CAG, while, the lowest percentage (70.42%) was obtained with the primer combination E-AGC X M-CTG. However, the maximum number of bands per primer combination was found to be 84 which was generated with primer combination E-ACA X M-CAG, whereas, the minimum numbers of bands per primer combination (49) were obtained with primer combination E-ACA X M-CTC. The obtained results confirm the high multiplex ratio produced by AFLP markers. The size of selectively amplified DNA fragments ranged in size from 50 to 600 bp as shown in Figure (1b).

These results are in good accordance with the results obtained by Zhang *et al.* [19] on sweet potato core collection, [31] on sugar beet and wild *Beta* species, [32] on wild carrot and cultivated varieties, [33] on wild and cultivated cardoon and globe artichoke and [34] on common bean accessions.

Genetic Similarity Within and among the Five Beet Types Revealed by RAPD: The genetic similarity within and among five types of beet was estimated in terms of using Dice's similarity coefficients (DSC's) to compute the similarity matrix based on the scored data matrix. This similarity matrix was used to generate a dendrogram using the UPGMA method. RAPD data analysis showed that the genetic similarity among the five types of beet ranged from 49.3 to 86.9 as shown in Table (2), with an average value of 68.1. Also, the highest similarity level (86.9%) was detected between wild beet accession WB-1003 and WB-1006 which are closely related accessions. While, the lowest genetic similarity (49.3%) was detected between wild beet accession WB-1003 and table beet Detroit cultivar.

The obtained results are in agreement with that obtained by Sitthiwong *et al.* [35] on pepper accessions, [9] on cherry species and [10] on Tunisian olive accessions.

Genetic Similarity Within and among the Five Beet Types Revealed by AFLP: AFLPs generated data showed that the highest genetic similarity value was 89.8 and the lowest value was 58.7 with an average of 74.25 as shown in Table (3). Based on AFLP analysis, the highest similarity percentage (89.8%) detected by the AFLP assay was between

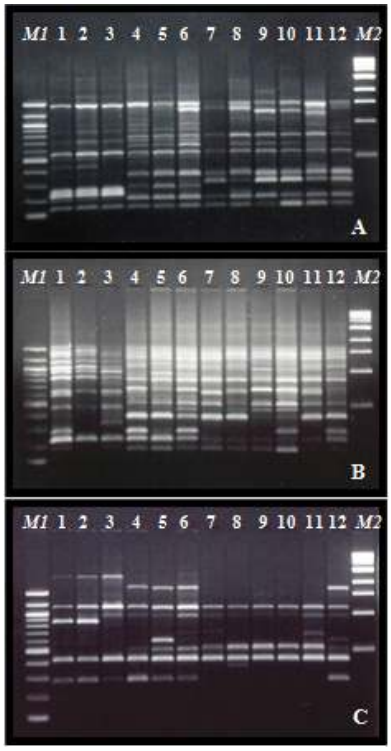

Fig. (1a): RAPD profiles of 12 beet genotypes (1-12) as detected with primers (A) OPA-16, (B) OPA-18 and (C) OPB-13. Lanes 1 to 6 represent: wild beet accessions (WB-1003, WB-1006, WB-1007, WB-1013, WB-1021 and WB-1026) and lanes 7 to 12 represent: Wild beet relatives (sugar beet, Top and Oscar poly cultivars, table beet, Detroit and CrosbyEgy cultivars, fodder beet, Frochenigar cultivar and chard, Balady cultivar. DNA molecular weight standards (M1) 100 bp DNA ladder and (M2) 1 Kb DNA ladder.

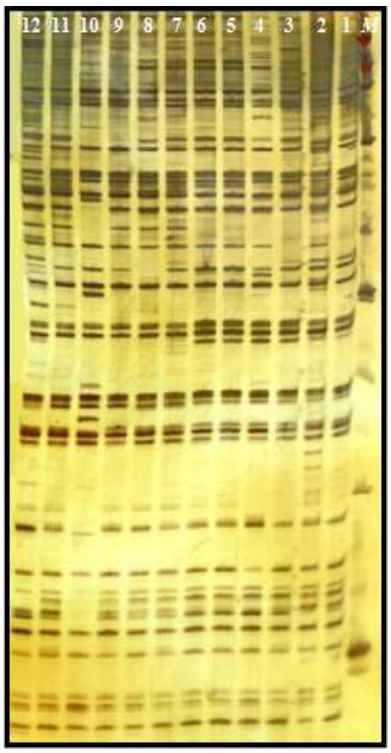

Fig. (1b): AFLP profiles of 12 beet genotypes (1 - 12) as revealed by AFLP primer combination E-AACXM-CAA. Lanes 1 to 6 represent: wild beet accessions (WB-1003, WB-1006, WB-1007, WB-1013, WB-1021 and WB-1026) and lanes 7 to 12 represent: wild beet relatives (sugar beet, Top and Oscar poly cultivars, table beet, Detroit and CrosbyEgy cultivars, fodder beet, Frochenigar cultivar and chard, Balady cultivar. M, DNA molecular weight standard, 100 bp DNA ladder.

wild beet accession WB-1003 and WB-1006, both share the same genetic background and geographical region since they were collected from The North-Eastern Coast of Egypt. While the lowest percentage of similarity (58.7%) was detected between wild beet accession WB-1006 and table beet CrosbyEgy cultivar. These results are in agreement with those of Roa *et al.* [36] on genus *Manihot*, [25] on commercial varieties and landraces of bean and [34] on common bean accessions.

Genetic Relationships Within and among the Five Beet Types as Revealed by RAPD: Genetic relationships based on the similarity matrix derived from each type of markers was carried out using the un-weighted pair group method arithmetic averages (UPGMA) method to construct dendrograms revealing the genetic relationships among beet genotypes. The constructed dendrogram clearly discriminated between wild beet accessions and the other cultivated forms of beet.

The dendrogram separated the five types of beet into three major clusters, according to the geographic distributions of wild accessions and to traditional classification of cultivated forms of beet [11]. The first cluster contained wild beet accessions collected from The North-Western Coast (WB-1003, WB-1006 and WB-1007), while the second cluster contained wild beet accessions collected from The North-Eastern Coast and South of Egypt regions (WB-1013, WB-1021 and WB-1026) in addition to chard cultivar (Balady). On the other hand, the third cluster, which contained all cultivated forms of beet except chard, could be divided into 3 subclusters. Table beet cultivars (Detroit and CrosbyEgy) are grouped together in the first subcluster, while sugar beet cultivars (Top and Oscar poly) grouped together in the second subcluster. The third subcluster contain only fodder beet cultivar (Frochenigar) as shown in Figure (2).

The obtained results are in good agreement with those of Ram *et al.* [15] on saltgrass genotypes**,** [16] on alstroemeria, [9] on cherry speies and [10] on Tunisian olive accessions. Also, these results are in congruence with those obtained by [11, 12, 27] on wild beet accessions. RAPD markers were shown to reveal a high degree of genetic diversity and are capable of distinguishing between different accessions in different locations [10, 37]. Such genetic diversity studies will be useful for future genetic analysis and provide breeders with a genetic basis for selection of parents for beet improvement.

Genetic Relationships Within and among the Five Beet Types as Revealed by AFLP: The dendrogram constructed from the AFLPs data clearly discriminated between wild beet accessions and other cultivated forms of beet. The topology of the AFLP data based dendrogram was highly similar with that dendrogram constructed based on RAPD data with a few discrepancies in the values of similarity coefficients. The constructed dendrogram also separated five types of beet into three major clusters, according to the geographic region of wild accessions and to classical botanical classification of cultivated forms of beet. The first cluster contained wild beet accessions collected from The North-Western Coast of Egypt (WB-1003, WB-1006 and WB-1007), while, the second cluster contained wild beet accessions collected from The North-Eastern Coast and South of Egypt regions (WB-1013, WB-1021 and WB-1026) in addition to, chard cultivar (Balady). On the other hand, the third cluster, which contained all cultivated forms of beet except chard, could be divided into 3 subclusters. Table beet

Table 2: Genetic similarity matrix within and among the five types of beet represented by 12 beet accessions as computed according to Dice's similarity coefficient from RAPD data.

WB-1003	100.0											
WB-1006	86.9	100.0										
WB-1007	79.4	83.0	100.0									
WB-1021	58.5	57.0	56.1	100.0								
Chard	57.4	56.3	58.4	70.9	100.0							
WB-1013	56.6	56.5	56.6	78.5	68.6	100.0						
Fodder	55.9	54.2	54.8	64.4	72.3	64.8	100.0					
WB-1026	55.6	56.5	56.5	81.9	68.8	72.9	64.8	100.0				
Top	55.0	54.5	56.0	63.0	68.2	65.8	73.1	69.0	100.0			
Oscar poly	53.6	53.0	53.6	60.8	66.1	63.2	73.9	64.7	81.8	100.0		
CrosbyEgy	51.0	50.4	50.5	61.5	66.3	62.8	74.7	62.3	69.3	70.4	100.0	
Detroit	49.3	49.7	51.4	61.5	67.0	62.9	67.6	62.9	66.8	69.0	77.0	100.0

Table 3: Genetic similarity matrix within and among the five types of beet represented by 12 beet accessions as computed according to Dice's similarity coefficient from AFLP data.

WB-1003	100.0											
WB-1006	89.8	100.0										
WB-1007	84.5	87.1	100.0									
WB-1021	65.6	63.2	63.0	100.0								
Chard	63.8	63.1	65.1	75.3	100.0							
Top	63.2	63.0	65.5	69.0	74.2	100.0						
Fodder	62.9	62.7	63.6	69.8	77.8	80.8	100.0					
WB-1013	62.8	62.0	62.3	81.5	73.2	69.4	70.2	100.0				
WB-1026	62.4	62.2	63.0	85.3	73.6	73.2	70.3	76.2	100.0			
CrosbyEgy	61.8	58.7	60.1	67.4	71.0	77.1	81.8	67.7	69.1	100.0		
Detroit	60.9	61.3	62.7	70.5	73.6	74.4	76.3	69.1	69.3	83.4	100.0	
Oscar poly	60.6	59.9	61.8	67.2	72.5	85.6	80.5	68.1	70.5	78.4	76.8	100.0

cultivars (Detroit and CrosbyEgy) are grouped together in the first subcluster, while, sugar beet cultivars (Top and Oscar poly) grouped together in the second subcluster. The third subcluster contain fodder beet cultivar (Frochenigar) only as shown in Figure (3).

The obtained results are in good accordance with those obtained by Paran *et al.* [20] on different types of pepper, [38] on melons, [33] on wild and cultivated cardoon and globe artichoke and [23] on pomegranate cultivars. The obtained results are also confirmed by Yee *et al.* [24] on Azuki accessions and [32] on wild carrot and cultivated varieties.

The constructed dendrogram indicates that cultivars from the same geographic origins tend to group together. Due to the high polymorphism of AFLP it is considered a powerful tool for genotyping large number of accessions. Therefore, it is suitable for genetic diversity assessment [19, 23].

Both RAPD and AFLP dendrograms were in accordance to each other, which confirms the results obtained throughout this work and verifies the positions of cultivated and wild beet accessions in relevance to each other.

Unique Markers and Fingerprints as Revealed by RAPDS: In RAPD analysis, among the thirty-two RAPD primers used only four RAPD primers (OPA-12, OPC-13, OPC-15, and OPO-15) failed to generate unique markers while the rest, 28 primers were able to generate unique markers. Two RAPD primers produced unique negative markers (OPB-07and OPO-07). While, nine primers produced, unique positive markers (OPA-07, OPA-08, OPA-14, OPB-13, OPB-18, OPE-05, OPE-10, OPO-03 and OPO-04) and seventeen RAPD primers produce both types of unique markers (positive and negative). A total of 76 unique markers were generated from twenty-eight primers of which fifty-two markers were unique positive ones and twenty-four markers were unique negative ones with molecular weight ranging from 125 to 2200 bp. These unique markers were able to identify only ten genotypes out of the twelve beet genotypes used. Wild beet accession WB-1006 and fodder beet cultivar (Frochenigar) could not be identified well since, the lowest numbers of unique markers were detected with them. In the same regard, the highest numbers of unique markers were detected with WB-1007 and chard. Nevertheless, the banding patterns obtained by the different RAPD primers yielded unique fingerprints, which characterize each genotype.

In addition, fourteen unique common RAPD markers (unique common bands presented in all wild beet accessions but not in the other cultivated forms of beet or contrast) that could be used to discriminate between wild beet accessions and cultivated forms of beet were identified. Among these unique markers, 13 could be used to discriminate wild beet accessions from the other cultivated forms of beet and only 1 marker could be used to discriminate the cultivated forms of beet from the wild beet accessions (Table 4).

The obtained results are in agreement with that obtained by Rodriguez *et al.* [29] on *Capsicum* species and [16] on vegetative propagated crops such as *alstroemeria*.

In the same regard, using these specific markers, species and varieties could be identified as shown in a study by Cai *et al.* [9] on cherry and [13] on wild taxa of the *Brassica*.

Unique Markers and Fingerprints as Revealed by AFLPS: All the AFLP primer combinations used detected unique markers, whether positive or negative. They revealed a total of 34 unique markers comprising 13 negative and 21 positive ones. The highest number of unique markers (8 markers) was obtained with primer combinations E-AAC X M-CAA and E-ACC X M-CAT whereas, the lowest number (5 markers) was obtained with primer combination E-ACA X M-CTC with molecular weight ranging from 60 to 580 bp. These unique markers can be used to identify eight genotypes out of the twelve studied beet genotypes. This means that RAPD markers had the potentiality to characterize a higher number of genotypes as compared to the capacity of the AFLP markers under the conditions of this study. This may be due to the lower number of AFLP primer pairs (5) used than number of RAPD primers (32) and/or to the higher number of polymorphic fragments obtained with RAPD marker than that obtained with AFLP, 383 and 275 with a percentage of 89.64 and 80.61 for RAPD and AFLP markers, respectively.

The highest numbers of unique markers were detected with WB-1007 and WB-1026, while, the lowest numbers of unique markers were detected with WB-1003 and fodder beet (Frochenigar). Also, there were no unique markers identified with Oscar poly and chard. The banding patterns obtained by the different AFLP primer combinations yielded unique fingerprints that characterized each genotype.

Moreover, nine unique common AFLP markers were identified. These markers could be used to differentiate between wild beet accessions and cultivated forms of beet. Seven out of nine markers could be used for differentiation or discrimination of wild beet from the other types used in this study, while two markers could be used for the differentiation between cultivated and wild beet accessions (Table 5).

The obtained findings are in good accordance with Lanteri *et al.* [39] on accessions of cardoon and artichoke and [25] on commercial varieties and landraces of bean. While, Zhang *et al.* [19] declared that there were no region specific markers identified when assessed genetic diversity in 80 cultivars of sweet potato from different geographical regions.

We could reveal from the obtained results that RAPDs were more efficient than AFLPs for generating polymorphisms in the studied beet types, while AFLPs were more efficient for distinguishing among accessions used. Estimation of genetic diversity are highly influenced by the accessions selected for evaluation and by the number of markers assayed.

The numbers of RAPD and AFLP markers evaluated in this study were insufficient to permit a through comparison of the two marker techniques. However, the ultimate goal of genetic similarity studies is not in the comparison of marker techniques themselves, but rather in the ability to use these techniques for identification of genetic variation and to find relationships between marker systems and traits of interest.

The obtained results concluded that RAPDs and AFLPs appear to be valuable tools for assessing genetic diversity and estimating of genetic relationships within and among beet genotypes. This is essential for the maximization benefits and utilization of plant genetic resource collections and could be of great benefit in genotype identification and protection, germplasm conservation and breeding programs.

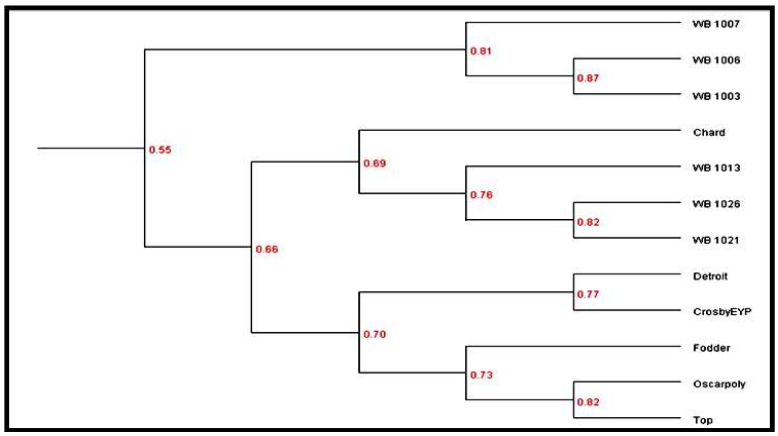

Fig. 2: Dendrogram for the 12 beet genotype accessions constructed from the RAPDs generated data using UPGMA method and similarity matrices computed according to DSC's.

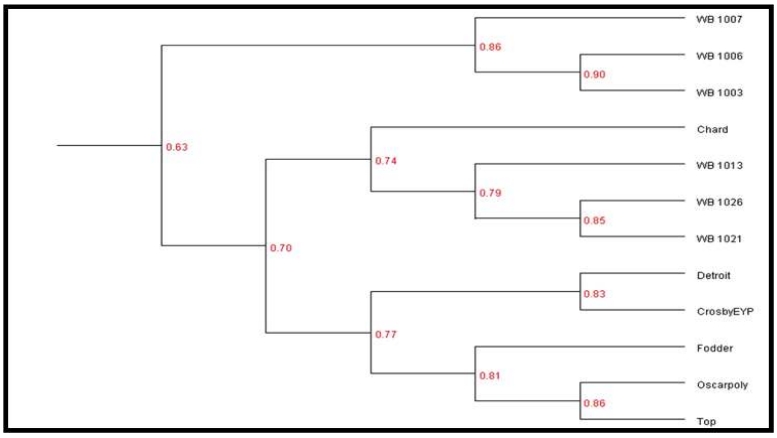

Fig. 3: Dendrogram for the 12 beet genotype accessions constructed from the AFLPs generated data using UPGMA method and similarity matrices computed according to DSC's.

Table 4: Unique RAPD markers and marker size used for discrimination and identification between the wild and cultivated beet accessions.

Primers	Wild beet accessions	Marker size (bp)	Cultivated forms of beet	Marker size (bp)
OPA-08	+	230	–	–
OPA-16	+	900	–	–
+	700	–	–	
OPB-06	+	400	–	–
OPB-13	+	300	–	–
OPB-18	+	250	–	–
OPC-15	+	1600	–	–
+	1200	–	–	
OPE-05	+	700	+	400
+	250	–	–	
OPO-03	+	700	–	–
OPO-04	+	500	–	–
OPO-20	+	900	–	–

Table 5: Unique AFLP markers and marker size used for discrimination and identification between the wild and cultivated beet accessions.

Primer combinations	Wild beet accessions	Marker size (bp)	Cultivated forms of beet	Marker size (bp)
E_{AAC} X M_{CAA}	+	285	+	350
E_{ACA} X M_{CAG}	+	175	+	110
E_{ACC} X M_{CAT}	+	550	–	–
E_{AGC} X M_{CTG}	+	420	–	–
	+	210	–	–
E_{ACA} X M_{CTC}	+	265	–	–
	+	250	–	–

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