An-Najah National University
Faculty of Graduate Studies

Comparative Study of Genetic Diversity in Durum Wheat in Palestine

By

Heba Mu'een Al Fares

Under the supervision of

Dr. Hassan Abu Qau'd

Co-Supervisor

Dr. Sami Yaish

Submitted in Partial Fulfillment of the Requirements for the Degree of Master of Environmental Sciences, Faculty of Graduate Studies at An-Najah National University, Nablus, Palestine

2002

Comparative Study of Genetic diversity in Durum Wheat in Palestine

By
Heba Mu'een al Fares

This Thesis was Successfully defended on 27-11-2002 , and approved by.

Committee members	Signature
1- Dr. Hassan Abu Qau'd	
2- Dr. Sami Yaish	
3- Dr. Feras Sawllah	
4- Dr. Belal Ghareeb	

Dedication

To My parents,

husband , kids

brothers, sisters

and

An-Najah National University

ACKNOWLEDGMENTS

Thanks to Allah for helping me and providing me with strength tolerance and patience to complete this study .

I am grateful to my advisor, Dr.Hassan Abu Qaoud whose assistance, encouragement, guidance, advice supervision and critical review of this study are deeply appreciated...

Thanks, and appreciation should extend to Dr. sami yaish for his encouragement, patience and revision of this study.

And for Dr.Hussein Migdadi deserves special thanks for his guidance and assistance through the Laboratory work and analysis.

For their advise and valuable comments, I express appreciation to member of the examine committee: Dr.

I would like to express my thanks to the NCARTT and all the friends in the Laboratory Fozia..Majdoleen..Ibrahim.. Sobhia..for their help and support.

Special gratitude goes to the Ministry of Agriculture and the UNDP.

List Of Tables

List of Figures

List Of Appendixes

Abstract

Comparative Study of Genetic Diversity in durum wheat in Palestine

By

Heba Mu'een Al Fares

Supervision

Dr. Hassan Abu Qau'd

Co-Supervisor

Dr. Sami Yaish

A total of 11 durum wheat varieties were grown in the green house to study the morphological differences: Six landraces, three commercial cultivars, and two introduced cultivars from ICARDA were further analyzed to detect variations among durum wheat varieties at DNA level using RAPD technique. The following characters were studied: Plant height, Flag leaf length, and Tillering capacity.

Statistical analysis using One Way Anova test showed significant variability among varieties for Tillering number, which ranged between 1 to 3 tiller/plant. However there was non-significant variability among varieties in plant hieght and flag leaf length.

DNA genetic variation was studied using 11 wheat varieties collected from 7 locations in Palestine.

A genetic similarity matrix based on the dice coefficients was constructed using RAPD data to assess the genetic relatedness.

The mean similarity indices presented by the 11 varieties ranged from 0.1 to 0.81 for all varieties with an average of 0.40. The wide range of similarity indices indicated that a wide range DNA polymorphism occur among the 11 varieties.

The results showed also that clustering in the same cluster for the varieties which could have common genomes, but no clustering related to collection site.

Of the 11 varieties used, there were 159 RAPD marker produced by five primers.

This analysis has proven that RAPD-PCR is a useful tool to determine genetic diversity among durum wheat varieties and can provide valuable information for the management and identification of genetic resources.

1. Introduction

The most important cereal crops grown in Palestine is wheat (*Triticum durum, Desf.*); an annual herbaceous plant belonging to Poaceae, The total cereal grain area reaches 469682 dunums table (1). Wheat is the most abundant as it occupies 216672 dunums (statistics of Ministry of Agriculture 1999/2000) table (2). The ability of cereal grain crop to withstand harsh conditions makes them more important in agriculture map in Palestine.

Table (1) basic changes for the field crops sector in the Palestinian territories, 1997/1998-1999/2000.

Crop	2000/1999	1999/1998	1998/1997
Area of field crop	469682	328882	530276

*According to the statistic of Ministry of Agriculture 1999/2000

Table (2): Area of wheat crop, 1999/2000

Crop	Rainfed	Irrigated	Total area (dunums)
	Area (dunums)	Area (dunums)	
wheat	214932	1740	216672

*According to the statistic of Ministry of Agriculture 1999/2000

Investigations carried out in Palestine and neighboring countries indicated the presence of wild relatives of cereals that are grown in pastures, (Isaac and Gasteyer, 2000). There are several local wheat land races that are planted by farmers.

The same applies to isozyme and biochemical markers that provide only poor coverage of the genome with low level of polymorphism that is only detected in narrow based elite breeding materials (Alberte et al., 1994).

The advent of DNA markers obviated the limitation and proved to be a very powerful tool for detailed assessment of genetic diversity in cultivated and wild plant species as plant pathogen populations (Melchinger, 1993).

2.2. Morphological and Agronomical Variation

The variability of *Triticum* species maintained by the Peasants for about 10000 years, is now decreasing due to the development of new "high yielding" varieties which replaced original landraces in many countries (Couderon, 1980).

In the WANA region, durum wheat grown primarily under rainfed conditions, mainly in areas where the annual precipitation is 250 –450 mm. In this region, abiotic stress such as cold, drought and high temperature, prevail during crop growing period, many primitive cultivars and landraces which are still in cultivation are well adapted to harsh environments.

Considerable genetic diversity is known to be present in these populations, and this can be used in durum wheat improvement (Porceddu and Srivastavas; 1990).

Most of the new wheat cultivares have a narrow genetic base, they were produced by crossing an elite line with a line that has similar

agronomic and end use characteristics (Chen et al, 1994 and Demissie et al, 1990).

Some studies on improvement of durum wheat indicated that it depends on the continuous supply of new germplasm material as donors of various genes of agronomic importance (Chapman, 1985). The availability of such germplasm depends on the identification of areas of concentration for landrace traits of agronomic importance (Bekele, 1983; Negassa, 1985).

It is likely that phenotypic variation is mostly genetic and therefore directly useable in breeding program, especially for those characters, which have a rather "high heritability" such as heading, maturity time, plant height, kernel number and weight (Pecetti et al; 1992).

Accessions of *Triticum dicoccum* provide an important genetic reservoir of variability for several useful characters such as earliness, short stem, high number of fertile tillers, long spike, dense spike, high number of seeds per spike, weight of kernels per spike, protein content and tolerance to drought which could be used in breeding wheat for the dry areas. (Hakim et al; 1989).

In a study of morphological variation of 13 quantitative traits of 250-durum wheat landrace genotypes collected from nine districts in Jordan, it was found that 25.23% of total variability was counted for number of spike/plant, fertile tillers/plant and 1000-kernel weight.

16.69% of total variability was found for the following traits; plant height, peduncle length, upper node and spike number which suggested that Jordanian landraces are rich sources of genetic variation and

therefore, it could be used in the reconstruction of a gene pool of germplasm for durum wheat improvement (AL-Ajlouni and Jaradat, 1997).

2.3. Molecular characterization

2.3.1. Application of Polymerase chain Reaction (PCR).

Previously, genetic variation and diversity have been based mainly on the gene products such as morphological traits and proteins. Recently a new technique, Polymerase Chain Reaction (PCR), has attracted interest for identification and evaluation of plant material (Moreno, 1995), because it is simple and the genetic make up of an organism is analyzed directly.

A Further advantage is the possibility of using genomic DNA extracted at any plant growth stage and from any tissue. Consequently, analysis can be shifted to a very early stage of plant growth, saving time and resources (Pagnotta et al., 1995).

PCR, which was introduced in 1985, is a technique that amplifies DNA fragment to billion of copies without needing living tissues (Newton and Graham, 1994). The major advancement that made PCR the key procedure is the introduction of a new enzyme from the bacteria *Thermus aquaticus* (Taq polymerase) (Newton and Graham, 1994). Taq polymerase simplified the procedure, improved the specificity, yield and length of products amplified by PCR (Saiki et al., 1988). PCR rapidly detects the genetic variation in a few hours rather than days or months (Wiliams and Roland, 1994). Its velocity, selectivity, and sensitivity characterize the PCR technique. And it also requires a small amount of

DNA (Weising et al., 1994). The cycles; denaturation, separates the double strands of DNA at temperature around 94°C, annealing, which enables the primer to anneal to single stranded DNA at specified temperature 33°C to 65°C, and extension in which DNA synthesized by a thermostable DNA polymerase at temperature 70-75°C (Weising et al., 1994)

The purity and yield of reaction products depend on several critical parameters such as annealing temperature (Rychlik et al., 1990). Several PCR -based techniques have been established (Labhilili et al., 1997), such as random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), sequence-tagged-site (STS), using primers derived from RFLP clone or gene sequences, and simple sequence repeats (SSR) also known as micro-satellite. Interest in RAPD's is growing rapidly as they are often highly polymorphic, their identification requires no radioactivity, and only very small quantities of plant material are required for this type of analysis (Hancock and Callow, 1994).

2.3.2 Randomly Amplified Polymorphic DNA (RAPD)

RAPD analysis can be performed on any organism with no DNA sequence information. It is effective with tiny amounts of DNA (Lowe et al., 1996). RAPD amplification was possible with DNA isolated from a single tobacco protoplast (Brown et al., 1993). The technology is relatively simple and cheap, allowing the analysis of a large number of samples in a short time (Williams et al., 1993).

The amplified DNA fragments are detected by the agarose gel electrophoresis. DNA pattern could be visualized directly by UV light

after ethidium bromide staining (Gregor et al., 1994; Sambrook et al., 1998).

The RAPD technique has been used recently in many studies. 1) The taxonomic and genetic relationships between species such as brassica species (Demeke et al., 1992; Ren et al., 1995). 2) Genotype fingerprinting as a powerful and highly accurate tool for distinguishing genotype and cultivars of buffalograss (Wu and Lin, 1994) broccoli and cauliflower (Hu and Quiros, 1991), tomato (Klein-Lankhorst et al., 1991), and 3) the genetic variation existing within and among cultivars and land races of collard (Franham, 1996) eggplant (Karihaloo et al., 1995; Saifan, 1999), and okra (Rawashdeh, 1999).

Also variation produced by this technique has been applied to analyze intra -and inter - specific polymorphism in cereals (Weining and Langridge, 1991; Beyermann et al., 1992).

In wheat, molecular markers were used to indicate genetic diversity in diploid wheat (Vierling and Nguyen, 1992), tetraploid wheat (Joshi and Nguyen, 1993), hexaploid wheat and splet wheat (Liu and Tsuunewaki, 1990; Lubbers et al., 1991;Siedler et al., 1994; Chen et al., 1994; Martin et al., 1995;and Oixin et al., 1998).

Relationships in fifty natural populations of wild species and genetic variability of genus *Aegilops* included the species *Ae.Biuncialis* (UUMM), *Ae.neglecta* (UUMMNN), *Ae.ovata* (UUMM), *Ae.ventricosa* (DDNN) and *Ae.triuncialis* (UUCC) were revealed by RAPD markers (Monte et al., 1999).

According to type of genomes the species were clustered for example, *Ae.ventricosa* appeared to be segregated from the other species, probably owing to the influence of the D genome. *Ae.biuncialis* and *Ae.ovata* were clearly separated, suggesting that the super index system should be used to differentiate the M genomes of both species. The separation of the M and N genomes was also confirmed. The proximity of N to U and C to M was also suggested. Kong et al. (1998) assayed genomic DNA of 29 varieties of two *Ae.tauschii* subspecies from different region using RAPD technique.

The results showed that DNA polymorphism of accessions collected from China was less than the variations found in those collected from Iran and from union of Socialist Sovict Republics (USSR).

Several techniques were compared to evaluate diversity using 147 progenies derived from the crosses between *T.aestivum* line and *T. tauschii [Ae. squarrosa]* after evaluation of yield, grain weight, protein concentration and grain hardness (Fritz et al., 1995). Genotypes were determined by testing polymorphism of one protein, 25 RFLP probes, and eight RAPD markers.

The results have shown that two RAPD markers were associated with consistent effects on grain yield and three markers were associated with grain weight accounting for 16% of the variance among families.

3. Materials and Methods

3.1. Morphological Characterization of Wheat

3.1.1. Plant Material and Cultural Practices

Seed of 6 landraces, 3 commercial, and 2 introduced by ICARDA of wheat were collected from farmer with cooperation with the UNDP and Ministry of Agriculture. These varieties were collected from different regions in Palestine 1999/2000 (table 3).

Seeds were planted in pots containing a soil mixture of 2:1 peatmoss and sand in the green house, growth and development were monitored through the period from April 25th , 2001 to June 14 th , 2001. After two weeks seedlings were transplanted to larger pots in the green house at the An–Najah National University farm of the faculty of Agriculture in Tulkarm.

Each pot contained ten seeds, and irrigation was supplied at the time of planting and when needed. And weeding in the pots was done and continued throughout the period of the study.

3.1.2. Data Collection

Data were obtained from individual plants in each pot. The following characters were studied:

1- Plant height: measured in centimeter from the soil level to the top of upper most spikelet, awn excluded.

2-Flag leaf length: measured from the tip of the flag leaf to its base in centimeter.

3- Tillering capacity: number of reproductive tillers per plant.

Table (3): wheat collected from different regions in Palestine (1999-2000)

Variety name	Variety number	Location
White hiteyah	1	Tobas
F8 hiteyah	2	Tamon
Black hiteyah	3	Tamon
Yellow hiteyah	4	Beit mrean
**Sham 6	5	Beit Qad station
*Acsad 933	6	Beit Qad station
870	7	Jabah
Black dibeyah	8	Hebron/ Dahreieh
*Anbar	9	Arabeh
White dibeyah	10	Hebron
**Sham 5	11	Beit Qad station

*These varieties are commercial cultivars obtained from a private company.

**These varieties are introduced by ICARDA.

The other varieties are Palestinian landraces

3.1.3. Statistical analysis

Analysis of variance was performed for each trait according to Steel and Torrie, (1982) using the computer software system of SPSS (ONE WAY ANOVA TEST). Least significant difference (LSD) at 0.05 probability level was used to assess the difference means. Mean, standard deviations, variance for the varieties were calculated for the characters studied according to the formula described by Steel and Torrie (1982).

3.2. Molecular characterization of wheat varieties

The DNA analysis protocols were done at the laboratory of Jordan National Center for Agricultural Research and Technology Transfer (NCARTT). Solutions and tools used during molecular analysis were autoclaved to avoid possible cross contamination. Protection from chemicals used in DNA extraction and PCR amplification were applied by wearing laboratory coat, mouth mask, latex gloves, and face shield when UV light is used.

3.2.1. Plant material

Six-wheat landrace varieties, two introduced by ICARDA and three-commercial cultivars were used in RAPD analysis (table 1). The seeds were planted in the laboratory in plastic pots. After two weeks DNA was extracted from leaf tissues.

3.2.2.Genomic DNA extraction from plant tissues

Genomic DNA was extracted from the seedlings germinated in the growth chamber under totally dark condition maintained at 21° C. Promega Wizard genomic DNA purification kit was used according to instructions provided by the manufacturer.

40mg of leaf tissue processed by freezing with liquid nitrogen and ground into a fine powder-using screwdriver in 1.5ml microcentrifuge tube. 600 µl of nucleilysis solution was added to the fine powder and mixed by vortexing for 3 seconds to wet the tissue .The mixture was incubated at 65°C in the water bath for 15 minutes. 3 µl of RNase were added to the cell lysate and mixed by inverting the tubes 25 times.

The mixture then was incubated at 37° C for 15 minutes. The samples were allowed to cool at room temperatures for 5 minutes before proceeding. A 200 µl of protein precipitation solution was added and the mixture and vigorously vortexed at high speed for 20 seconds.

The tubes were put in a microcentrifuge and the samples were spun for 3 minutes at 13000*Xg*. The precipitated proteins formed a tight pellet and the supernatant containing the DNA was carefully removed from the tubes (leaving protein pellet behind) and transferred to a cleaned 1.5 ml microcentrifuge tubes contained 600 µl of room temperature isopropanol.

The solution was gently mixed by inversion until thread-like strands of DNA were visible. The solution then was centrifuged at 13000*Xg* for one minute at room temperature. The supernatant was carefully decanted leaving the DNA pellet adhered at the bottom of the tube. A 600 µl of room temperature 70% ethanol was added to the tubes containing DNA pellet and gently inverted several times to wash the DNA. The samples were then centrifuged at 13000 *Xg* for one minute at room temperature. The ethanol was carefully aspirated leaving loosed DNA pellets in the tubes.

The tubes were inverted on the cleaned absorbent paper and pellet was left to air dry for 15 minutes. 100 µl of DNA rehydration (TE buffer) solution was added to each tube and then incubated at 65° C in water bath with gentle shaking for one hour. Then DNA was stored at -20° C in the freezer, for use later.

3.2.3 Characterization of Genomic DNA

Genomic DNA quality was examined by agarose gel electrophoresis. Five µl of DNA solution from different varieties and 0.2 volume (1µl) of loading buffer were loaded in each slot in the 0.7% agarose gel containing ethidium bromide. The gel was run for 15-30 minute at 100 volt. DNA quality and relative quantity was detected under UV-light and photographed by Polaroid camera using black and white 667 (3.25x4.25 inch) film.

The DNA concentration was determined spectrophotometrically using GeneQuant II, RNA/DNA calculator (Pharmacia Biotech) table (4). 5µl of DNA sample in 100 µl of deionized water was injected and the double stranded DNA concentration was measured and automatically given in ng/µl.

3.2.4. Polymerase Chain Reaction (PCR)

The standard RAPD protocol recommended by Williams *et al.* (1990) was performed. A total reaction volume of 25 µl containing 2.5 µl of 10x already prepared PCR buffer; [100 mM Tris-Cl, pH 8.3,500 mM KCl, 15 mM $MgCl_2$, 0.01% gelatin (from Promega, catalog # M2661, Madison)]. 2.5 µl of dNTPs stock; containing [2mM of 2′ - deoxyadenosine 5′- triphosphate (dATP), 2mM of 2′ - deoxycytidine 5′- triphosphate (dCTP), 2mM of 2′ - deoxyguanosine 5′- triphosphate (dGTP) and 2mM of 2′ - deoxythymidine 5′- triphosphate (dTTP) from Promega, catalog # U1330]. 5 picomoles of a single 10-base primer (Operon Technolgies, Inc. Alameda, USA) (primers are supplied as 0.5 OD that are resuspended in 1ml water and used 1µl per reaction), 0.2 µl of (5u/µl) *Taq* DNA polymerase (Promega, catalog # M2661) 1 µl (75-100

ng) of genomic DNA template, double distilled and sterile water was added to make up the final volume of 25 µl.

A reaction mixture for each primer (master mix) was used. A negative control to which water was added instead of DNA was used for each primer. The master mix was divided to labeled PCR reaction tubes (0.2 ml) and 1µl of template DNA solution or water (negative control) was added.

The contents were mixed and spun briefly. DNA amplification was carried out with the MJ Research model PTC-100 DNA thermocycler. The PCR program was set as:

1. two minutes at 95°C (initial denaturing step)
2. forty cycles consisting of:
   - 1 minute 94°C (denaturing)
   - 1 minute 36°C (annealing)
   - 2 minutes 72°C (extension, ramp time 2 minutes, 0.3°C/second)
3. Two minutes and thirty second at 72°C (final extension step).

After amplification the tubes were stored at 4°C overnight for further use in gel electrophoresis.

3.2.5. Gel Electrophoresis and documentation

1.4% agarose was used for gel electrophoresis in 0.5xTBE buffer in a glass bottle and the suspension was boiled in a microwave oven for 2 minutes and the bottle swirled many times to completely dissolve the agarose.

The melted agarose cooled to about 60^0C and 8 µl of ethidium bromide [stock solution (10mg/ml in water)] was added and mixed thoroughly.

The warmed agarose was carefully poured into a previously prepared horizontal casting tray (the slot-forming comb was inserted about 1 mm above the plate to form a complete well).

After the gel was completely set (20-30 minutes at room temperature) the comb was carefully removed and a running buffer of 0.5x TBE was added to a level of around 0.5 cm above the gel.

A 0.2 volume (1:5 dye to DNA solution) of loading buffer; [Blue/orange 6x loading dye composed of 15% Ficoll 400, 0.03% bromophenol blue, 0.03% xylene cyanol FF, 0.4% orange G, 10mM Tris-HCl (pH 7.5) and 50 mM EDTA (from Promega)] added to the DNA samples, mixed and centrifuged for few seconds in a microcentrifuge in order to collect the sample at the bottom of the tubes. 10 µl DNA samples slowly loaded into the submerged wells.

Molecular size of the amplification products were estimated by utilizing a 1-kb DNA ladder (from Promega).

The gel tank was closed by its lid and attached with power supply in away that DNA will migrate toward the anode (red lead) and applied a constant voltage of 100 volts for 1.5 hour was applied. After that the gel was placed on a UV transilluminator (254nm) it was photographed using a black and white Polaroid film type 667.

3.2.6. Data Analysis

Data were scored for analysis on the bases of the presence or the absence of the amplified products. If the product was present in a genotype it was scored as (1), if it was absent it was designed as (0). Pair-wise comparisons of varieties based on presence /absence of unique and shared polymorphic products, were used to generate Dice similarity coefficients employing the following equation (Dice, 1945): $(2N_{ab})/(N_a+N_b)$, where; N_{ab}, N_a and N_b are the number of shared bands between sample A and B, total number of bands detected in sample A and sample B respectively.

The similarity coefficients were then used to construct a dendrogram using the SPSS-10.0, PC software for Window computer program.

4. Result and discussion

4.1 Morphological Variation among Wheat in Palestine

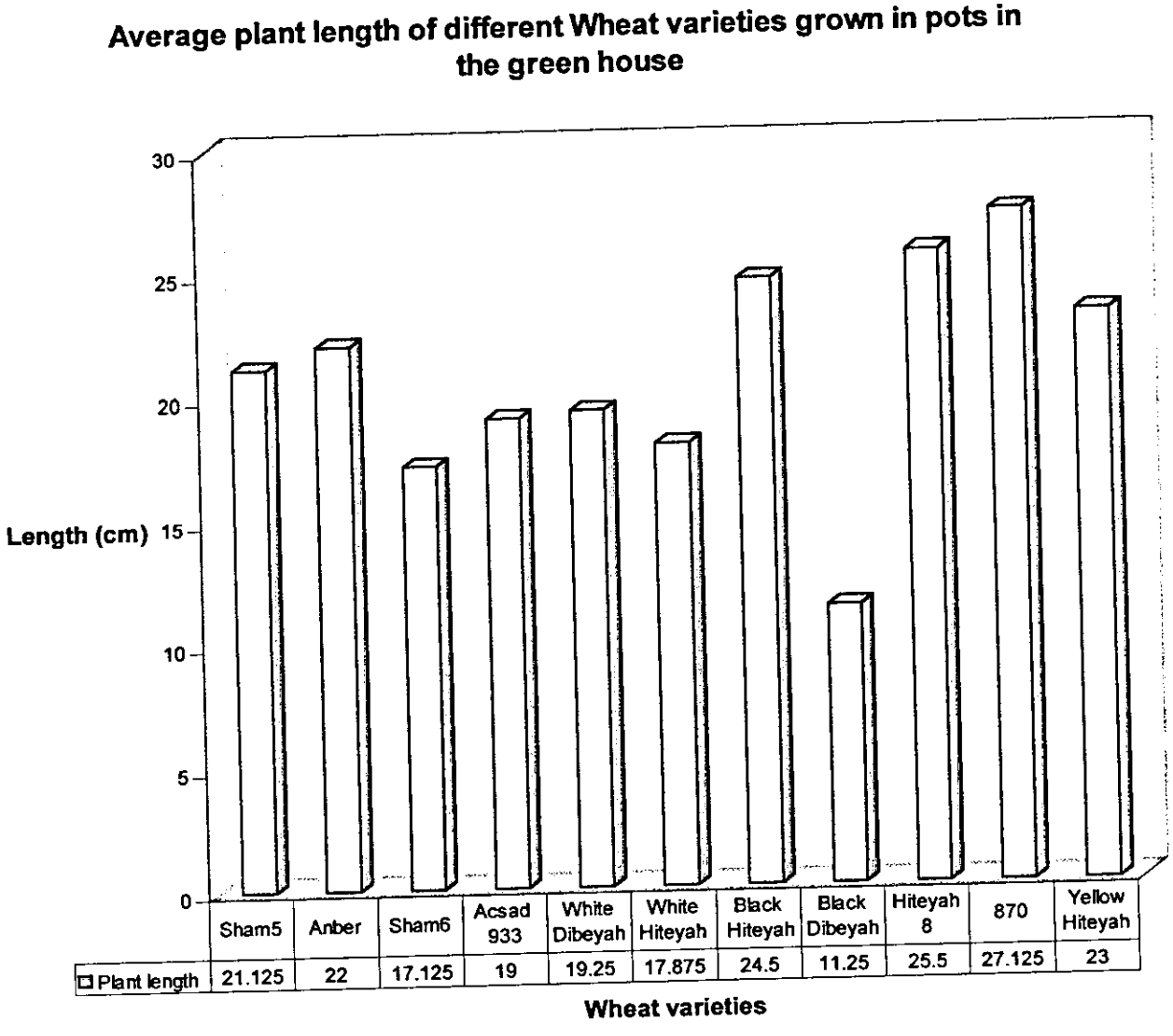
Analysis of variance for the morphological characters studied of the 11 wheat varieties in green house showed that there was no significant difference for plant height, flag leaf length, but there was a significant deference for tillering capacity.

4.1.1. Plant Height

Average plant height for 11 wheat varieties are presented in **Fig (1)**. Among wheat varieties plant height ranged from 11cm for Black Dibeyah to 27cm for 870. There was no significant difference in plant height among the different varieties.

These results are in agreement with the finding of Duwayri, (1984); Ismail, (1996) and Migdadi, (1990). (Hassan, 1982) Who found that plant height of different wheat genotypes reduced significantly when grown under drought condition or artificially stressed under green house by 50% and by 43%.

Fig (1):



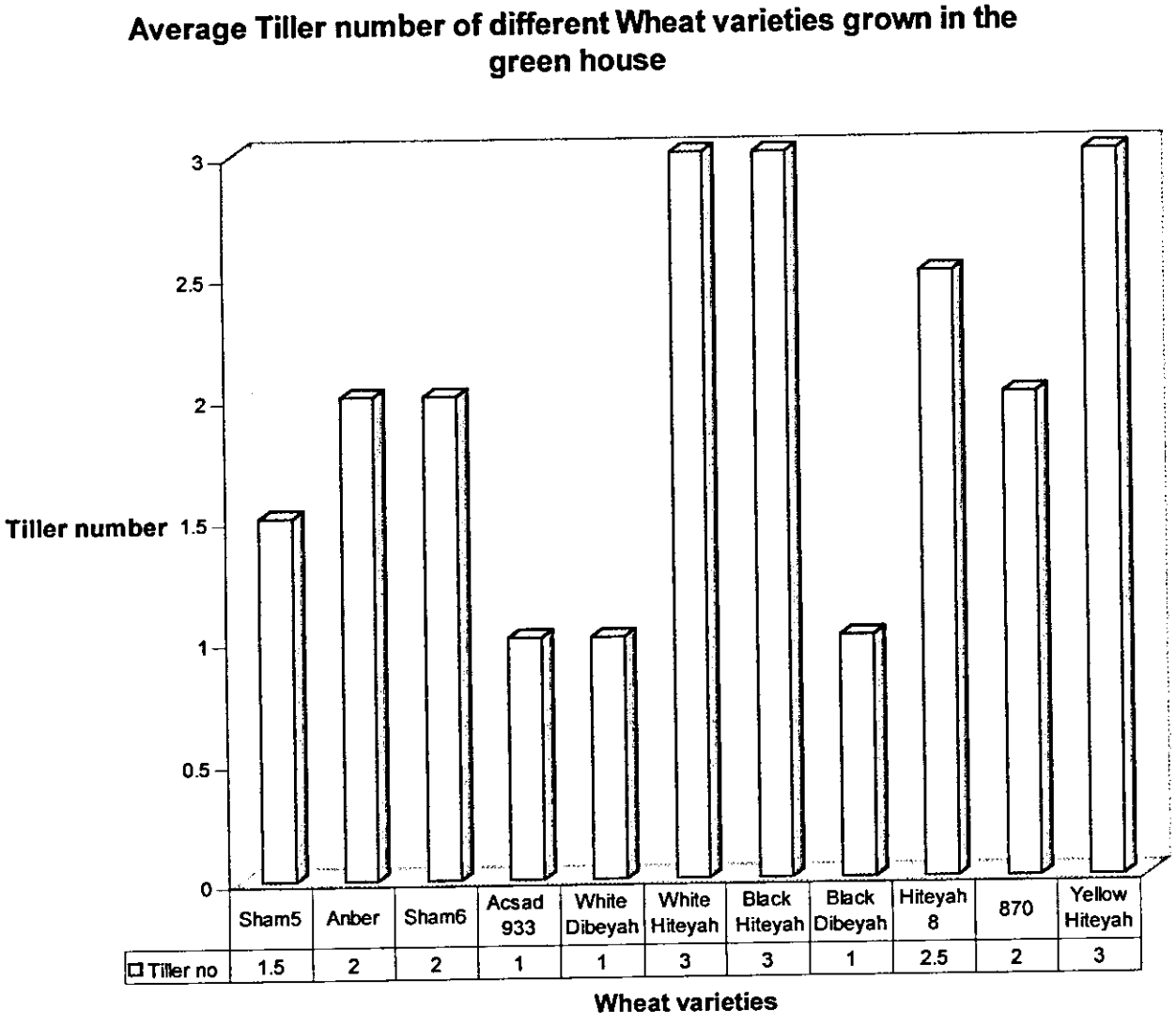
4.1.2. Tillering capacity

The importance of tiller survival and spike number in sustaining yield is considered as critical for yield. Average number of tillers per plant - for the growing period is shown in Fig (2).

Analysis of variance showed that significant differences among varieties are present. The mean values ranged from 1 for Acsad933, White Dibeyah, and Black Dibeyah to 3 for Black Hiteyah, Yellow Hiteyah and White Hiteyah. This wide range of variability indicated the high variation between varieties.

Some varieties did not show any tillering and that could be due to short period of the experiment or due to environmental factors in the green house and/or genetic variation.

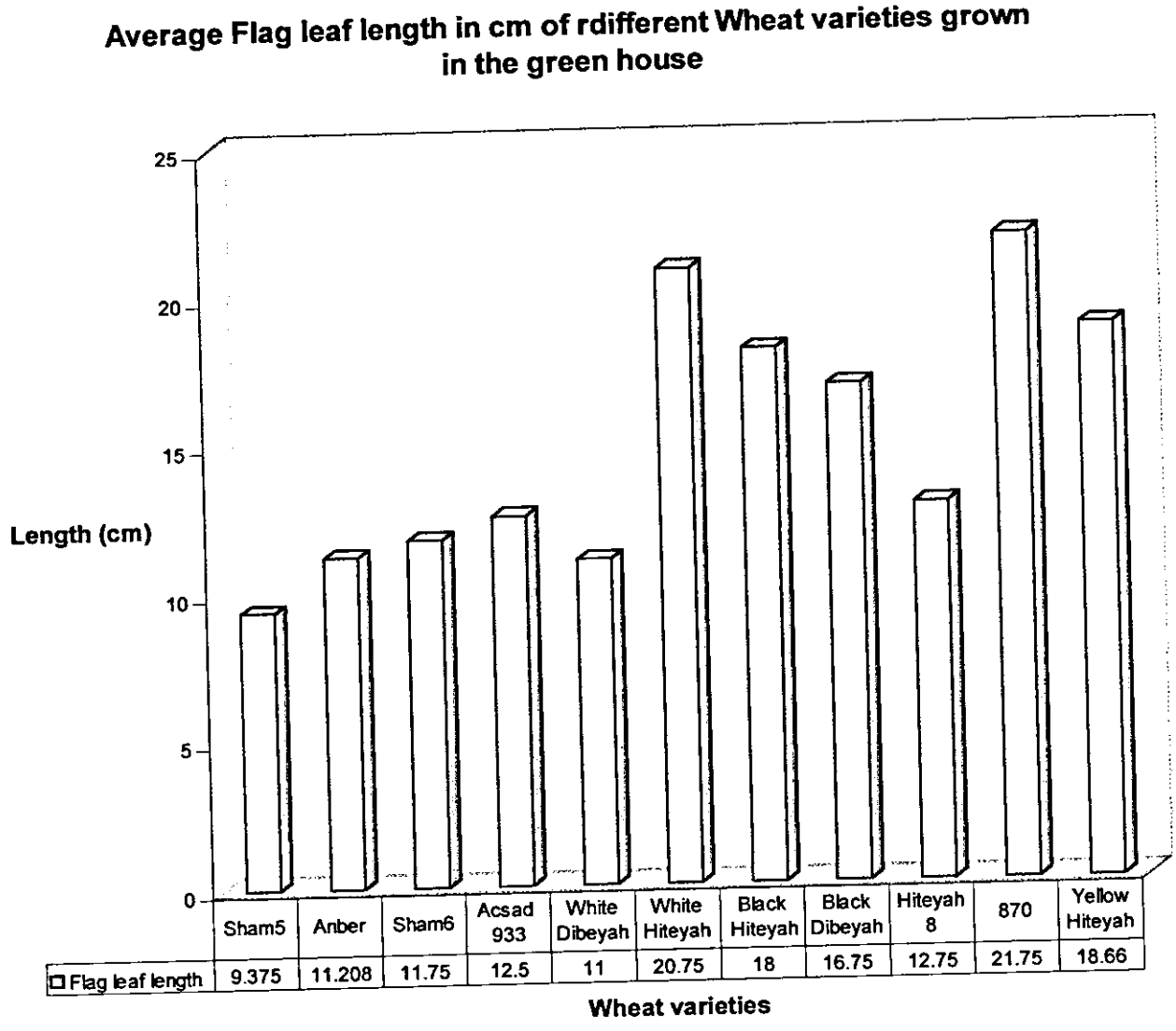
Fig (2):



4.1.3. Flag Leaf Length

Average values of flag leaf length are presented in Fig (3).The mean range from 9.4cm for sham5 to 23.75 cm for variety 870. The analysis of variance showed that there was no significant difference among varieties in this trait.

Fig (3):



4.2.1.2. PCR Reaction

4.2.1.2.1. Annealing Temperature

Three annealing temperature were used to establish the best annealing temperature which were 25, 36 and 55°C. The results showed that 36°C for one minute was the best. Abo-elwafa, Murai, and Shimada (1994) found that the optimum condition of template DNA concentration 10ng/20µl reaction mixtures and annealing temperature 36°C. Williams et al. Suggested that temperature for annealing 36°C.

4.2.1.2.2. Taq Polymerase and Primer Concentration

Three concentrations for Taq polymerase were used; 0.2, 0.3 and 0.5µl for each 25µl PCR reaction. The result indicated that 0.2µl per reaction produced the best amplification.

The 1, 1.5 and 2µl of OPERON 10 base primer were used to standardize the primer concentration. The result showed that 1µl was the best concentration and gave reproducible patterns.

4.2.3. Primer screening

A total of 70 random primers were evaluated for their ability to prime PCR amplification of 11 Palestinian wheat that was randomly selected. The 5' to 3' sequences of these primers are presented in table (8).

Out of 70 primers, 27(37%) yield RAPD gels that showed amplified fragments with mean of 3 fragments per each primer and 43 (62%) didn't amplify detectable products. For the choices of RAPD primers, unambiguous and qualitative (present or absent) fragments that gave

repeatable patterns when tested four to five times with the same cultivars were considered. Five primers OPA-13, OPN-14, OPN-16, OPN-18, and OPT-1 which showed consistent reproducible polymorphisms were selected and used to analyze all of the 11 durum wheat varieties collected in this study from different locations in Palestine.

The fragment sizes of amplified products were estimated using standard curves figure (5) for bands in the molecular weight marker used in each gel. Naming polymorphic bands was according to primer-manufacturer recommendation using the name of the primer and estimated size of the band as a subscript table (5).

Table (5) 10-Mer operon DNA technologies (Alameda, U.S.A) primers used in the detection of the DNA polymorphism in the wheat varieties

Primer code	5′ to 3′ sequence	Primer code	5′ to 3′ sequence
OPA-4	AATCGGGCTG	OPD-16	AGGGCGTAG
OPA-8	GTGACGTAGG	OPD-17	TTTCCCACGG
OPA-11	CAATCGCCGT	OPD-18	GAGAGCCAAC
OPA-13	CAGCACCCAC	OPD-20	ACCCGGTCAC
OPA-14	TCTGTGCTGG	OPF-1	ACGGATCCTG
OPA-19	CAAACGTCGG	OPF-9	CCAAGCTTCC
OPB-10	CTGCTGGGAC	OPM-5	GGGAACGTGT
OPB-19	ACCCCCGAAG	OPM-7	CCGTGACTCA
OPB-18	CCACAGCAGT	OPN-1	CTCACGTTGG
OPC-1	TTCGAGCCAG	OPN-2	ACCAGGGGCA
OPC-2	GTGAGGCGTC	OPN-3	GGTACTCCCC
OPC-3	GGGGGTCTTT	OPN-4	GACCGACCCA
OPC-4	CCGCATCTAC	OPN-5	ACTGAACGCC
OPC-5	GATGACCGCC	OPN-7	CAGCCCAGAG
OPC-7	GTCCCGACGA	OPN-8	ACCTCAGCTC
OPC-9	CTCACCGTCC	OPN-9	TGCCGGCTTG
OPC-10	TGTCTGGGTG	OPN-10	ACAACTGGGG
OPC-11	AAAGCTGCGG	OPN-11	TCGCCGCAAA
OPC-12	TGTCATCCCC	OPN-12	CACAGACACC
OPC-13	AAGCCTCGTC	OPN-13	AGCGTCACTC
OPC-14	TGCGTGCTTG	OPN-14	TCGTGCGGGT
OPC-15	GACGGATCAG	OPN-15	CAGCGACTGT
OPC-17	TTCCCCCCAG	OPN-16	AAGCGACCTG
OPC-18	TGAGTGGGTG	OPN-17	CATTGGGGAG
OPC-19	GTTGCCAGCC	OPN-18	GGTGAGGTCA
OPD-3	GTCGCCGTCA	OPN-19	GTCCGTACTG
OPD-4	TCTGGTGAGG	OPN-20	GGTGCTCCGT
OPD-5	TGAGCGGACA	OPO-2	ACGTAGCGTC
OPD-7	TTGGCACGGG	OPO-19	GGTGCACGTT
OPD-8	GTGTGCCCCA	OPP-6	GTGGGCTGAC
OPD-9	CTCTGGAGAC	OPP-13	GGACTGCCTC
OPD-11	AGCGCCATTG	OPT-1	GGGCCACTCA
OPD-12	CACCGTATCC	OPT-7	GGCAGGCTGT
OPD-13	GGGGTGACGA	OPW-3	GTCCGGAGTG
OPD-15	CATCCGTGCT	OPW-6	AGGCCCGATG
		OPZ-10	CCGACAAACC

4.2.3 Genetic Variation Among 11 durum wheat varieties Collected from Different Locations

The five primers, which were selected, produced total (159) of markers; all of them polymorphic using the 11-durum wheat varieties, table (6). Each primer produced 19 to 39 polymorphic bands with an average of 29 markers per primer. Representative amplified DNA patterns using the 11 wheat varieties are shown in figures (6,7,8,9,10).

Polymorphic band ranged in size 348 to 1908 bp. The size out of this range was not considered in the analysis. For example, variety number 4-showed DNA band out of the adapted range of DNA sizes using the OPN14. Both faint as well as strong markers are considered in scoring. The strong RAPD fragments, which was shown in some primers, i.e. primers OPN18 and OPA13 could have arisen from amplifying two or more products of similar sizes. Variation in the brightness of the bands was also observed in few samples.

The possible causes include difference in template sequence copy number and varying degrees of mismatch between the primer and the binding site (Ratnaparkhe et al., 1995). Previous studies considered RAPD markers ranging from 200 to 1500 bp as suitable for use in further experiments as probes in southern blots or in situ hybridization (Wei and Wang, 1995).

The total markers for each primer ranged from 19 for primer OPN-16 to 39 markers for OPT-1 using 11-durum wheat. There are many reactions that failed to produce PCR products. It was found that the six wheat landraces showed the highest number of polymorphic bands, which was (90), while the cultivars showed the lowest (69).

Table (6) the total number of polymorphic bands produced using the five selected primers in RAPD –PCR for the 11-durum wheat variety.

Name of primer	Polymorphic bands
OPA-13	37
OPN-14	38
OPN-16	19
OPN-18	26
OPT-1	39
Total	159

The variety 8, 10 and 11 did not show any amplification using primer OPA-13. Also variety 11 using primer OPN-16 varieties 2, 6 and 9 using primer OPN-18 and varieties 1 and 7 using OPT-1 did not show any amplification. This is expected and shows typical examples when some reactions failed for no apparent reason.

These genotype showed amplification by using the same DNA and other primers. Such variability could be due to experimental errors that are often reported using RAPD-PCR. But also genetic variability between varieties is possible at threshold PCR- cycle settings.

Wheat collected in this study of 11 varieties can be divided as: 6 wheat landraces white hiteyah, f8, black hiteyah, yellow hiteyah, black dibeyah and white dibeyah, 3 commercial wheat Acsad933, 870, Anbar and two introduced by ICARDA sham6, sham5.

Based on the dice coefficients (Okuno et al., 1998), a genetic similarity matrix was constructed using the RAPD data to assess the genetic relatedness among the 11 wheat varieties. The mean similarity indices ranged from 0.1 between varieties 11and 2 (sham5 and F8) to 0.81 between varieties 3 and 4 (Black hiteyah and Yellow hiteyah). All varieties showed an average of 0.40, which could mean that the varieties

share on average 40% of their RAPD fragments. The wide range of similarity indices indicated that a high polymorphism at the DNA level among varieties and hence a large amount of genetic variation exists among the varieties.

High polymorphisms were recorded in *Apium graveolens* cultivars and *Lycopersicon esculentum* (Karihaloo et al., 1995), Tibetan wheat (Sun et al., 1998), among wild pigonpea accessions (Ratnaparkhe et. al., 1995), *Aegilops* species (Migdadi, 2001) and *hordeum* species (Gonzalez and Ferrer, 1993).

Based on RAPD product data, the results showed that most landraces grouped in one cluster and three-sub cluster. The commercial varieties grouped in one cluster; whereas varieties having common genomes tend to cluster together in the same sub cluster (fig11). So corresponding to the genome, the 11 varieties grouped in four main clusters, two out of them consist of one variety in one cluster sham5 introduced from ICARDA (1999) and White hiteyah in the second cluster.

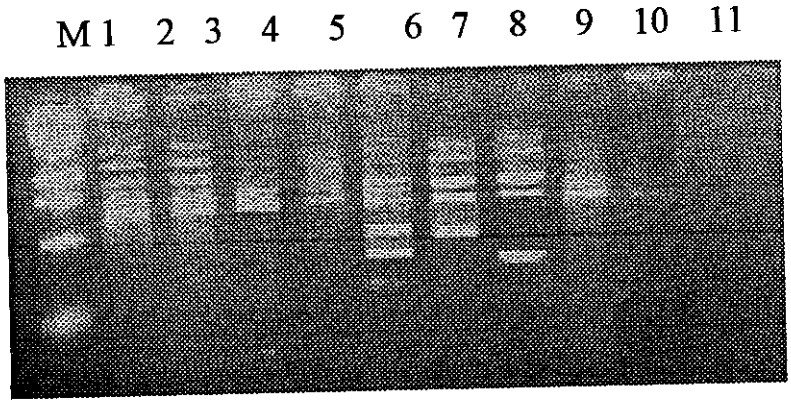


Fig (7) RAPD pattern obtained among 11 durum wheat varieties collected from different location in Palestine using primer OPN14. M = molecular weight marker (1kb DNA ladder). Lane1 White hiteyah, lane2 f8, lane3 Black hiteyah, lane4 Yellow hiteyah, lane5 Sham6, lane6 Acsad933, lane7 870, lane8 Black dibeyah, lane9 Anbar, lane10 White debeyah, lane11 Sham5

The coefficient of similarity between these clusters was (13%). The third cluster consisted from three sub clusters included Black Hiteyah, Yellow Hiteyah and F8 (hiteyah8) in one sub cluster with coefficient of similarity ranging from 66-81%. Black Dibeyah in the second sub cluster and white Dibeyah in the third sub cluster.

The coefficient of similarity among these varieties ranged from 43% between White Dibeyah and F8 (Hiteyah8) to 55% between Black Hiteyah and White Dibeyah.

In the fourth cluster, two sub clusters were formed and grouped in Sham6 and 870 in sub cluster and Acsad933 and Anber in the second sub cluster. The coefficient of similarity among sub clusters ranged from 53% between sham6 and Anber to 75% between Sham6 and 870.

However the similarity within sub clusters was 75%. This means that genetic variation among sub cluster was low. It is high within sub clusters. This indicate that, the relatedness among variety within sub cluster is high which can be due to less genetic variation among the varieties and may originate from the same origin, or may be due to the adaptability of these varieties to the same environment.

The presence of high genetic diversity among varieties could be used in wheat improvement for abiotic stress for example (drought and or salt tolerance) in the one hand and to biotic stress (disease, insect ...etc) on the other hand.

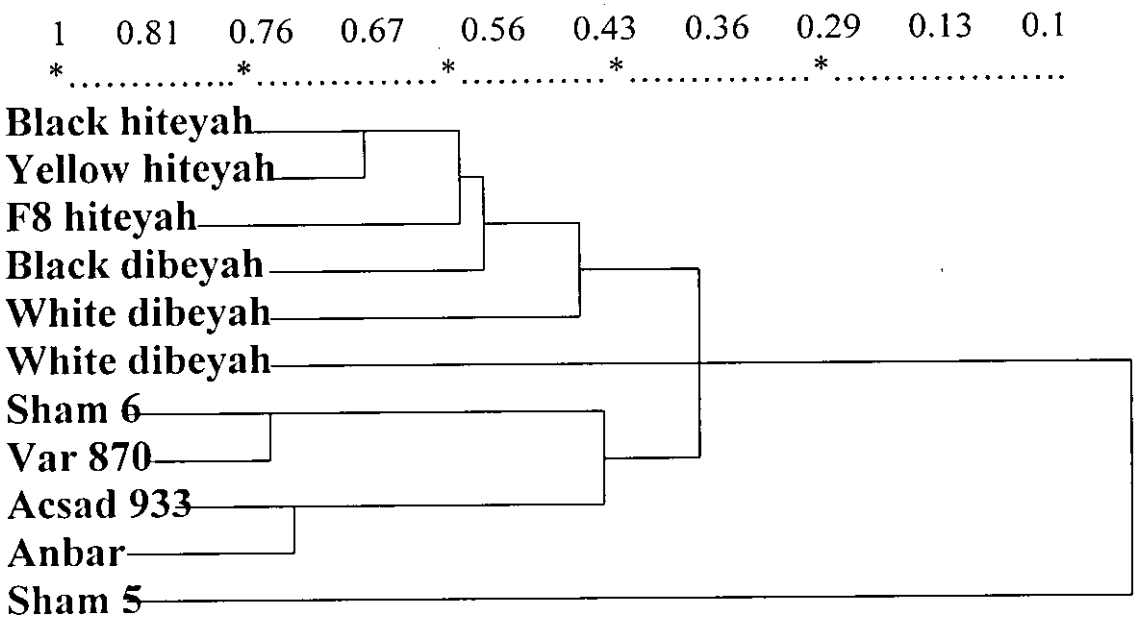


Fig (11) Dendrogram durum of 11 wheat varieties based on similarity for 159 RAPD Marker produced by five single primers

Table (7). .Similarity index for 11 wheat varieties according to DICE (Czekanowski or Sorenson)

Measured used.

Variety	HEW	HE8	HEB	HEW	SH6	AC933	V870	DEB	ANB	DEW	SH5
HEW	1										
HE8	0.58	1									
HEB	0.56	0.76	1								
HEW	0.52	0.67	0.81	1							
SH6	0.43	0.52	0.61	0.52	1						
AC933	0.4	0.57	0.56	0.51	0.68	1					
V870	0.41	0.29	0.46	0.47	0.75	0.55	1				
DEB	0.32	0.53	0.71	0.67	0.61	0.48	0.36	1			
ANB	0.44	0.5	0.55	0.36	0.53	0.74	0.54	0.36	1		
DEW	0.26	0.43	0.55	0.5	0.35	0.3	0.23	0.55	0.3	1	
SH5	0.13	0.1	0.19	0.2	0.15	0.11	0.11	0.29	0.17	0.33	1

HEW= White Hiteyah, HE8= Hiteyah 8, HEB= Black Hiteyah, HEY= Yellow Hiteyah SH6= Sham 6, AC933= Acsad933, V870 =Variety 870, DEB= Black Dibeyah ANB= Anber, DEW= White Dibeyah, SH5= Sham 5.

Conclusions and recommendations

1- Based on morphology the varieties are considered as a source of desirable traits that can be used in breeding programs.

2- The high effect of environmental condition should be taken in consideration through traditional classic selection so using DNA fingerprinting technique avoid this effect.

3- The results showed that the relatedness between varieties in the same cluster might be due to their common genome.

4- According to morphological traits there is a significant variation between the varieties in tillering capacity and this indicates that there is high biodiversity between them.

5- Primers A13, N16, N18, N14, T1 were efficient to amplify DNA fragments which could be useful to show polymorphism among *Triticum durum* varieties.

6- RAPD technique could be a useful tool for studying variation among wheat varieties.

7- The wide range of similarity indices indicated that a wide range of DNA polymorphism occurs among the varieties collected in this study indicates high genetic variation among variety.

8- RAPD-PCR has proven to be a valuable and useful tool to determine the extent of genetic diversity and variation among wheat *(Triticum durum)* varieties.

9- It could be useful to study durum wheat varieties under different controlled environmental conditions such as water stress comparing to other molecular technology.

REFFRENCES

REFFRENCES:

1. AL_Ajlouni, M.M and Jaradat, A.A.1997: **Diversity in durum wheat landraces collected from Jordan.**I.Quantitative traits. Cereal Res.comm.**25**:17-23.

2. Al berte, R.S., G.S. suba, G. Procaccini, Zimmerman and R.S. Fain 1994. **Assessment of genetic diversity of seagrass populations using DNA fingerprinting implications for population stability and management.** Proc. Natl. Acad. Sci. U. S.A., **91**:1049-1053.

3. Abo-Elwafa, A. Muria, k. and Shimada, T. 1995. **Intra- and inter -specific variation in lens revealed by RAPD markers.** Thero. Appl. Genet., **90**:335-340.

4. Asins, M.J. and Carbinell, E.A. 1989. **Distribution of genetic variability in a durum wheat world collection.** Theor. Appl. Genet., 77:287-294.

5. Bekele, E.1983. **Some measures of gene diversity analysis on Landrace populations of Ethiopian barley.** Heredities, **21**:547:563.

6. Beyermannet, B. Nurnberg, A. weihe, M. Meixner, J.T. Epplen and T. Borner, 1992. **Fingerprinting plant genomes with oligonucleotide probes for simple repetitive DNA sequences.** Theor. Appl. Genet., **83**: 691-694.

7. Brown, P.T. H., F.D. Lange, E. Kranzand H. lorz.1993.**Analysis of single protoplasts and regenerated plants by PCR and R APD technology** .Mol. Gen. Genet . **237**:311-317.

8. Cauderon, A. 1980. **Surla protection des resources genetiques. Enrelation avec leur surveillance leur modeloge et leur utilisation.** C.R.A cad. J'Agric.de France **66(12):** 1051-1068.

9. Chapman, C.D.G. 1985. **The genetic resources of wheat. A survey and strategies for collecting**. International Board of plant Genetic Resources, Rome, Italy.**39.**

10. Chen, H.B. Martin, J.M. Lavin, M. and Talbert, L.E. 1994. **Genetic diversity in hard red spring wheat based on sequence-targeted-site PCR markers**. Crop Sci., **34:**1628-1632.

11. Choumane, W. Achtar, S. Valkoun, J. and weigand, F. 1998. **Genetic variation in core and base barley collections from WANA as revealed by RAPDs**. In Jaradat, A.A. Editor, Triticea III. Proceedings of The Third International Triticeae Symposium, Aleppo, Syria, 4-8 May 1997. 53-56.

12. Demeke, T., R.P. Adams, and R. Chibbar. 1992. **Potential taxonomic use of random amplified polymorphic DNA. (RAPD)**: A case study in Beassica. Theor. Appl. Genet., **84:**991-994.

13. Demissie, A. G.H. Mariam, Fayssa. 1990. **Ethiopian wheat germplasm collection and utilization. In Regional wheat workshop for eastern, central and southern Africa**. Mexico, DF (Mexico) CIMMYT.P.327-334 Tanne, DGM.Ginkal, Mvan and W.H wangi(ed).

14. Dwayri, M. 1984. **Effect of flag leaf and awn removal on grain yield and yield components of wheat grown under dry land conditions.** Field crop Res., **8**:307-313.

15. Dweikat, I. S. Mackenzie, M. Levy, and H. Ohm. 1993. **Pedigree assessment using RAPD - DGGE in cereal crop species.** Theor. Appl. Genet., **83**:497-505.

16. Farnham, M.W. 1996 .**Genetic Variation among and within United States collard cultivars and landraces as determined by Randomly Amplified Polymorphic DNA markers.** J. Amer. Soc. Hort. Sci., **121(3)**:374-379.

17. Frankel, O.H. 1977. **Natural variation and its conservation in Muhammed**. skel and R.Von Borstel (Eds). Genetic Diversity in plants .P. 21-44. Plenum, New York.

18. Fritz, A.K., Cox, T.S., Gill B.S., and sears, R.G. 1995. **Marker based analysis If quantitative traits in winter wheat x Triticum *tauchii population*** crop sci. **35**: 1695-1699.

19. Gill, B.S., Wilson, D.L., Sharma, H.C., Browder, L.E., Hatchett, J.H., Harrey, T.L., Moseman, J.G., and Waines, J.G. 1986 . **Resistance in *Aegilops* squarrosa to wheat leaf rust, wheat powdery mildew, greenbag and Hessian fly**. Plant Disease, **70**: 553-556.

20. Gorizalez, J.M. and Ferrer, E. 1993. **Random amplified polymorphic DNA analysis in *Hordeum* species**. Genome, **36**: 1029-1031.

21. Gregor, D., Hartmann, W. and Stosser, R. 1994. **Cultivar Identification in prunus Domestica using random amplified polymorphic DNA**. Markers Acta horticulture, 33-91.

22. Hakim, S., A.B. Damania and M. Y. Moualla. 1991/92. **Genetic variability in *Triticum dicoccum schubl* for use in breeding wheat for the dry areas plant genet.** Resources Newsletter, **88/89:**11-15.

23. Hancock, J.F. and P.A. Callow.1994. **Randomly amplified polymorphic DNAs in the cultivated strawberry, *fragaria* X *ananassa*.** J. Amer. soc. Hort. Sci., **119(4)**: 862-864.

24. Havey, M.J., and Muehlbaner, F.J. 1989. **Variability for restriction Fragment Length and phylogenesis in lenil.** Theor Appl. Gent., 77:839-843.

25. Hoelzel, A.R. 1992. **Molecular genetic analysis of populations: A practical approach.**1st Edition. Oxford University Press. New York.

26. Hu, J. and C.F. Quiros. 1991. **Identification of broccoli and cauliflower cultivars with RAPD markers.** Plant Cell Rpts., **10**:505-511.

27. Jaradat, A.A. 1989. **Diversity within and between populations of two sympatricaily distributed Hordeuin species in Jordan.** Theor. Appl. Genet., **78**:653-656.

28. Joshi, C.P., and Nguyen, H.T. 1993. **Application of the random amplified polymorphic DNA technique for the detection of polymorphism among wild and cultivated tetraploid wheats.** Genome, **36**: 602-609.

29. Karihaloo, J.L., and L.D. Golttieb. 1995. **Allozyme variation in the egg plant, *Solanum melongena L.* (Solanaceae).** Thear. Appl. Genet., **90**:578-583.

30. Keim, P., Shoemaker, R.C., and Palmer, R.G., 1989. **RFLP diversity in soybean.** Theor .Appl. Gen t., 77:786-792.

31. Kong, L., Dong, Y., Jia, J., Kong, L.R., Dong, Y.C. and Jia, JZ. 1998. **Random amplified polymorphism of DNA analysis in *Aegilops taushii.*** Acta Botanica Sinica, **40**: 223 - 227.

32. Klein-lankhorset, R.M., A. Vermunt, R. Weide, T. Liharska and P. Zabel. 1991. **Isolation of molecular markers for tomato (L. esculentum) using random amplified polymorphic DNA (RAPD).** Theor. Appl. Genet., **83**:108-114.

33. Kuckuck, H., Kobabe, G., and Wenzel, G., 1991. **Fundamental of plant Breeding.** First Edition Spingerverlag Berlin Heidilberg. pp: 3-5.

34. Labhilili, M., E. Lmad, M. Baum, and M.M. Nachit. 1997. **Techniques in Biomolecular Analysis.** Wana Newsletter, **14**:4-5.

35. Lowe, A.J., O. Hanotte and L. Guario. 1996. **Standarization of molecular genetic techniques for characterization of germplasm collection: The case of random amplified polymorphic DNA (RAPD).** Plant. Genet. Resources Newsletter. **107**:50-54.

36. Lagudah, E.S., and Halloran, G.M., 1988. **I'Isylo genetic relationships of *Triticum tauschi*, the D genome donor to hexaploid wheat. 1. Variation in HMW subunits of glutenin and gliadirts.** Theor. Appl. Genet., **75**:592-598.

37. Liu, Y.G., and Tsunewaki, K. 1990. **Restriction fragment length polymorphism (RFLP) analysis in wheat. I. Genomic DNA library construction and RFLP analysis in common wheat.** Jap J. Genet., **35**:104 - 108.

38. Lubbers, E.L., Gill, K.S., Cox, T.S., and Gill, B.S. 1991. **Variation of molecular markers among geographically diverse accessions of *Triticum tauschii*.** Genome, **34**:354-361.

39. Martin, J.M., Talbert, L.E. Lanning, S.P., and Blake, N.K. 1995. **Hybrid performance in wheat as related to parental diversity.** Crop Scin., **35**:104 - 108.

40. Mcintyre, C.L. 1988. **Variation at isozyme Loci in *Triticeae*.** Plant syst. Evol., **160**:123-142.

41. Migdadi, H.M. 2001. **Genetic variation in some *Aegilops* Species as revealed by Morphological and molecular Techniques.** PHD. Sc. Thesis, University of Jordan, Amman, Jordan.

42. Monte, J.V., Gasanova, C. and Soler, C. 1999. **Genetic variation in Spanish populations of the genus Aegilops revealed by RAPDs.** Agronomie, **19**:419, 427.

43. Moreno, S., Gogorcena, Y., and Ortiz, J.M. 1995. **The use of RAPD Markers for Identification of cultivated Grapevine *(Vitis vinifera* L.).** Seientia Horticulturae, **62**: 237-243.

44. Nevo, E., and Beiles, A. 1998. **Genetic diversity of wild emmer wheat in Israel and Turkey**. Theor. Appl. Genetic., 77: 421-455.

45. Newton, C.R. and Graham, A. 1994. **PCR**: Bios Scientific Publisher Ltd, pp: 2-7.

46. Okuno, K., Ebana, K., Noov, B. and Yoshida, H. 1998. **Genetic diversity of Central Asian and North Caucasian *Aegilops* species as revealed by RAPD markers.** Genetic Resource and crop Evaluation, **45**:389-394.

47. Oixin, S., Zhongfu, Ni., Zhiyong, L., Jianwei, G., and Tiecheng, H. 1998. **Genetic relationships and diversity among Tibetan wheat, common wheat and European spelt wheat, revealed by RAPD Markers.** Euphytica, **99**:205-211.

48. Pagnotta, M.A., Nevo, E. Beiles, A. and Porceddu, E. 1995. **Wheat storage proteins: glutenin diversity in wild emmer, *Triticum diccoides*, in Israel and Turkey DNA diversity detected by PCR.** Theor. Appl. Gent., **91**:409-414.

49. Pecetti, L., A.B. Damania and G.K. Lashour. 1992 **Geographical variation for spike and grain characteristics in durum wheat germplasm adapted to dryland conditions.** Genete. Resources and crop Evaluation **39(2):** 97-105.

50. Ratnaparkhe, M.B., Gupta, S. and Venmathg, M.R. 1995. **Genetic fingerprinting of pigeonpea (*Cajanus cajan* L. Millsp.) and its wild relatives using RAPD markers.** Theor. Appl. Gente., **91**:893-898.

51. Rawashdeh, I.M. 1999. **Genetic variation among and within Okra *(Abelmoschus esculentus L.)* Landraces in Jordan.** MSc. Thesis, University of Jordan, Amman, Jordan.

52. Ren, J., J.R. Mcferson, R. Li, S. Kresovich and W.F. Lamboy. 1995. **Identities and relationships among Chinese vegetable Brassicas as determined by random amplified polymorphic DNA markers.** J. Amer. Soc. Hort. Sci., **120**: 548 - 555.

53. Rychlik, W., Spencer, W.J. and Rhoads, R.E. 1990. **Optimization of the annealing temperature for DNA. Amplification in vitro.** Nucleic Acids Research, **18(21)**: 6909 - 6412.

54. Saifan, S.M. 1999. **Genetic variation among and within Eggplant *(Solanum melongena L.)* Landraces in Jordan.** MSc. Thesis, University of Jordan, Amman, Jordan.

55. Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullus, K. B. and Erlich, H.A.1988. **Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA polymerase.** Science. **239**:487-490.

56. Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. **Molecular cloning: a Laboratorry Manual.** Second Edition Cold Spring Harbor Laboratory Press.U.S.A.

57. Siedler, H., Messmer, M.M., Schachermayr, G.M., Winzeler, H. and Kellerl, B. 1994. **Genetic diversity in European wheat and spelt breeding materials based on RFLP data.** *Theor. Appl.* Genet, **88**:994-1003.

58. Song, K, Osborn, T.C. Williams P.H. 1988. **Brassica taxonomy based on nuclear Restriction Fragment Length Polymorphisms (RFLP). I. Genome evolution of dipolid and amphidiploids species.** Theor. Appli. Genet., **75**: 784-794.

59. Steel, R.G.D., and Torrie, J.H. 1980. **Principles and procedures Statistics a biometrical Approach**, Second Edition. Mc Graw-Hill Co. INC.633.pp.

60. Sun, Q., Huang, T.C., Ni, Z. E. and Procunier. J.D. 1996. **Study on wheat heterotic group. I. genetic diversity revealed by random amplified polymorphic DNA (RAPD) in elite wheat cultivars.** J. Agric. Biotech., **4**: 103-110.

61. Vazquez, F.J., Jimenez, J. M.C. and Vico, F.R. 1996. **RAPD Fingerprinting of pepper (*Capsicum annuum* L.) Breeding Lines. Capsicum and Egg plant.** Newsletter, **15**: 37- 40.

62. Vierling, R.A. and H.T. Nyuyen.1992. **Use of RAPD markers to determine the genetic diversity of diploid wheat genotypes.** Theor. Appl. Genet., **84**: 835-838.

63. Weining, S. and P. Langridge. 1991. **Identification of mapping of polymorphisms in cereals based on polymerase chain reaction.** Theor. Appl. Genet., **80**: 209-216.

64. Weising, K., H. Nybom, K. Wolff and W. Meyer .1994. **DNA Fingerprinting in plants and Fungi.** BocaRaton Ann Arbor London Tokyo. PP: 23-25.

65. Wu, L. and H. Lin. 1994. **Identifying buffalograss [*Buchloe dactyliodes* (Nutt.) Engelm.] cultivar breeding lines using random amplified polymorphic DNA (RAPD) markers.** J. Amer. Soc. Hort. Sc: **9119(1)**: 126-130.

Appendixes

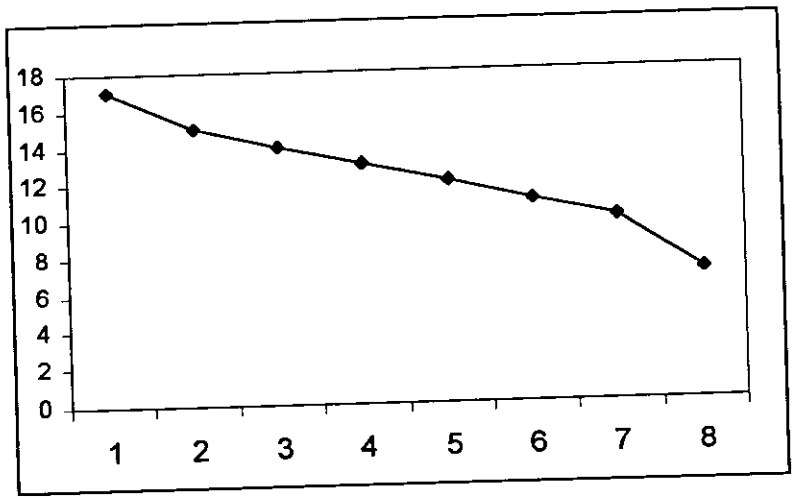
Appendix (A) Classification of *Triticum* according to the various proposed systems.

***Triticum* L.**

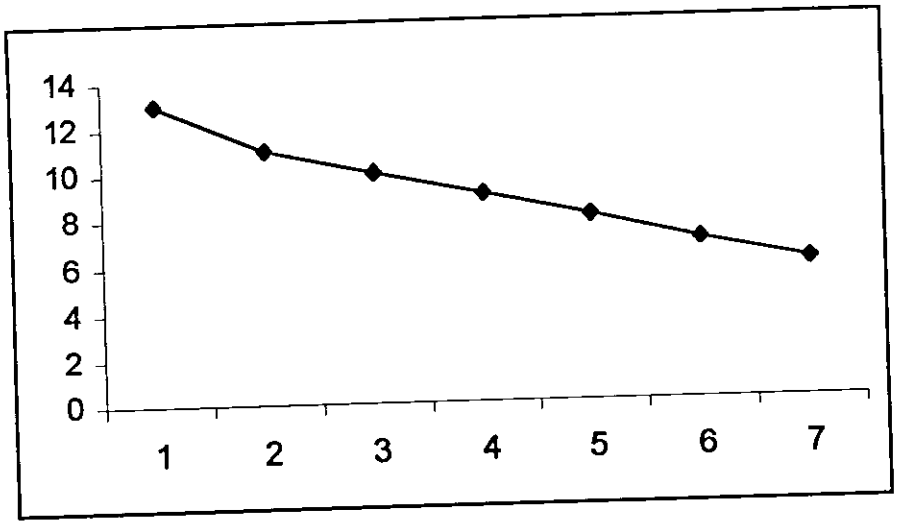
Taxonomic treatment according to			
Van Slageren 1994	Kimber and Sears 1984	Mac Key 1975	Derofeev and migushova1979
Section *Monococcon* Dumort.		Section *Monococca* Flaksb.	Section *Monococcon* Dum.
Triticum monococcum L.		*Triticum monococcum* L.	
ssp. *monococcum*	*Triticum monococcum* L.	ssp. *monococcum*	*Triticum monococcum* L.
ssp. *Aegilopoides* (Link) Thell.	*Triticum monococcum* L.	ssp. *boeoticum* (Boiss.) Á.Löve et D.Löve	*Triticum boeoticum* Boiss.
		var. *aegilopoides* (Link) MacKey	
		var. *thaoudar* (Reut.) Percival	
			Triticum sinskajae A. Filat. & Kurk.
			Section *Urartu* Dorof. et A. Filat.
Triticum urartu Tumanian ex Gandilyan	*Triticum monococcum* L.	*Triticum urartu* Tum.	*Triticum urartu* Tum. ex. Gandil.
Section *Dicoccoidea* Flaksb.		Section *Dicoccoidea* Flaksb.	Section *Dicoccoides* Flaksb.
Triticum turgidum L.		*Triticum turgidum* (L.) Thell.	*Triticum turgidum* L.
ssp. *turgidum*	*Triticum turgidum* L.	ssp. *turgidum*	
		conv. *turgidum*	
ssp. *durum* (Desf.) Husn.	*Triticum turgidum* L.	conv. *durum* (Desf.) MacKey	*Triticum durum* Desf.
		conv. *turancium* (Jakubz.) MacKey	
ssp. *polonicum* (L.) Thell.	*Triticum turgidum* L.	conv. *polonicum* (L.) MacKey	*Triticum polonicum* L.
ssp. *carthlicum* (Nevski) Á.Löve & D.Löve	*Triticum turgidum* L.	ssp. *carthlicum* (Nevski) Á.Löve et D.Löve	*Triticum carthlicum* Nevski
ssp. *dicoccum* Schrank ex Schübler	*Triticum turgidum* L.	ssp. *dicoccum* (Shrank ex Schübler) Thell.	*Triticum dicoccum* (Schrank) Schübler
		ssp. *georgicum* (Dekapr. & Menabde) MacKey	
ssp. *paleocolchicum* (Menabde) Á.Löve & D.Löve			
ssp. *turanicum* (Jakubz.) A.Löve & D.Löve			

ssp. *dicoccoides* (Körn. ex Aschers. & Graebn.) Thell.	*Triticum turgidum* L.	ssp. *dicoccoides* (Körn.) Thell.	*Triticum dicoccoides* (Körn. ex Aschers. & Graebn.) Schweinf.
			Triticum karamyschevii Nevski
			Triticum ispahanicum Heslot
			Triticum jakubzineri Udacz. et Schachm.
			Triticum turanicum Jakubz.
			Triticum aethiopicum Jakubz.
			Section *Timopheevii* A. Filat. et Dorof.
Triticum timopheevii (Zhuk.) Zhuk.		*Triticum timopheevii* Zhuk.	
ssp. *timopheevii*	*Triticum timopheevii* (Zhuk.) Zhuk.	ssp. *timopheevii*	*Triticum ttimopheevii* Zhuk.
ssp. *armeniacum* (Jakubz.) MacKey	*Triticum timopheevii* (Zhuk.) Zhuk.	ssp. *armeniacum* (Jakubz.) MacKey	*Triticum araraticum* Jakubz.
			Triticum militinae Zhuk. & Migush.
Section Triticum		Section *Speltoidea* Flaksb.	Section *Triticum*
Triticum aestivum L.		*Triticum aestivum* (L.) Thell.	*Triticum aestivum* L.
ssp. *aestivum*	*Triticum aestivum* L.	ssp. *aestivum*	
ssp. *compactum* (Host) MacKey	*Triticum aestivum* L.	ssp. *compactum* (Host) MacKey	*Triticum compactum* Host
ssp. *macha* (Dekapr. & Menabde) MacKey	*Triticum aestivum* L.	ssp. *macha* (Dekapr. et Menabde) MacKey	*Triticum macha* Dekapr. et Menabde
ssp. *spelta* (L.) Thell.	*Triticum aestivum* L.	ssp. *spelta* (L.) Thell.	*Triticum spelta* L.
ssp. *sphaerococcum* (Percival) MacKey	*Triticum aestivum* L.	ssp. *sphaerococcum* (Percival) MacKey	*Triticum sphaerococcum* Perciv.
			Triticum petropavlovskyi Udacz. et Migush.
			Triticum vavilovii (Thum.) Jakubz.
Triticum zhukovskyi Menabde & Ericz.	*Triticum zhukovskyi* Men. & Er.	*Triticum zhykovskyi* Menabde & Ericzjan	*Triticum zhukovskyi* Menabde et Ericzjan

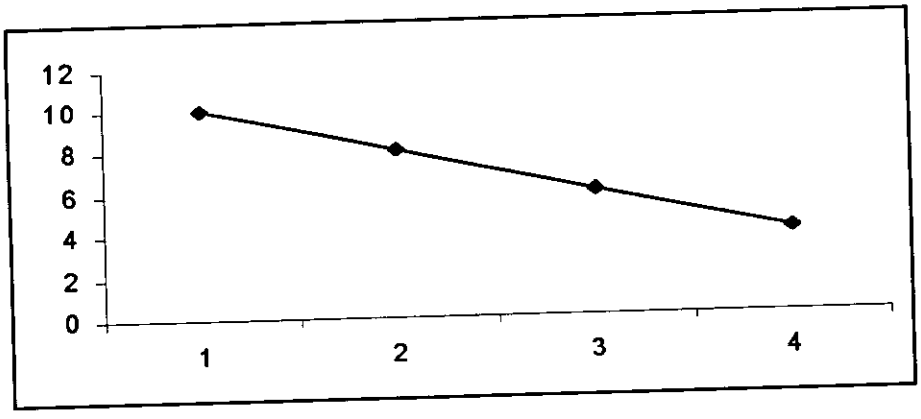
Appendix (B) standard curve for each primer for 11 wheat varieties used to determine DNA fragment size in base pair (Bp).



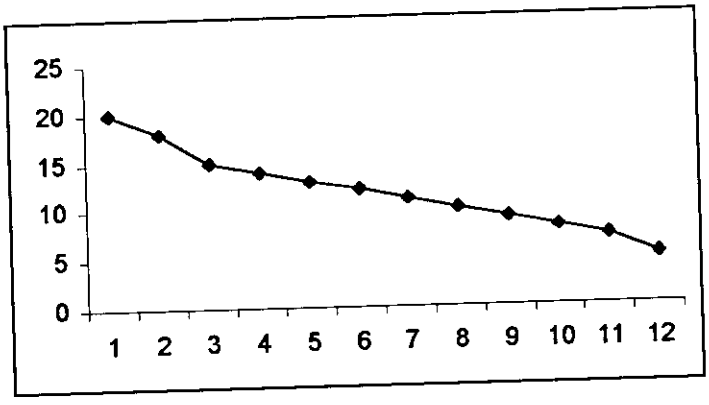
Fig(1) standard curve used to determine DNA fragment size in base pair (Bp) using OpA13 primer.



Fig(2) standard curve used to determine DNA fragment size in base pair (Bp) using OpT1 primer.



Fig(3) standard curve used to determine DNA fragment size in base pair (Bp) using OpN16 primer.



Fig(4) standard curve used to determine DNA fragment size in base pair (Bp) using OpN14 primer.

Appendix (c)

Scoring of RAPD markers among 11 durum wheat varieties . The number of each marker represent size of DNA band in bp using the corresponding primer.0 is used for absent band and 1 when the band is present.

variety/ Frag.size	HEW	HE8	HEB	HEY	SH6	AC933	V870	DEB	ANB	DEW	SH5	Total
OpN18												
1870	0	0	0	0	1	0	1	0	0	0	0	
1500	0	0	1	1	1	0	1	1	0	0	0	
1070	0	0	0	0	1	0	1	0	0	0	0	
860	0	0	1	1	1	0	1	1	0	1	0	
690	1	0	1	1	1	0	1	1	0	1	1	
610	0	0	0	0	1	0	1	0	0	1	0	
Sub-total	1	0	3	3	6	0	6	3	0	3	1	26
OpN14												
1910	0	1	0	1	1	1	1	0	0	0	0	
1520	1	0	0	0	0	0	0	0	0	0	0	
1360	0	0	0	1	0	1	1	0	0	0	0	
1210	1	1	0	0	0	0	0	0	0	0	0	
1080	1	1	1	1	1	1	1	1	1	0	0	
970	1	1	0	0	1	0	0	0	0	0	0	
860	1	1	1	1	1	1	1	1	1	0	0	
770	0	0	0	0	0	1	0	0	0	0	0	
690	0	0	0	0	0	0	0	0	0	0	0	
610	0	0	0	0	1	1	0	0	0	0	0	
440	0	0	0	0	1	0	1	0	0	0	0	
350	0	0	0	0	1	0	0	0	0	0	0	
Sub-total	5	5	2	4	7	6	5	2	2	0	0	38
OpN16												
1730	0	0	0	0	0	0	0	0	0	1	0	
1100	0	1	1	1	0	0	0	1	0	1	0	
700	1	1	1	1	1	1	0	1	0	1	0	
440	1	1	1	1	0	0	0	0	0	1	0	
Sub-total	2	3	3	3	1	1	0	2	0	4	0	19
OpA13												
1620	1	1	1	0	1	1	1	0	1	0	0	
1390	1	1	1	0	1	1	1	0	1	0	0	
1270	0	0	0	0	1	1	1	0	1	0	0	
1260	1	0	1	1	1	1	1	0	1	0	0	
1200	1	0	1	1	0	0	0	0	0	0	0	
1150	0	0	0	0	1	1	1	0	1	0	0	
1100	1	1	0	1	0	0	0	0	0	0	0	
990	0	1	1	0	0	0	0	0	0	0	0	
Sub-total	5	4	5	3	5	5	5	0	5	0	0	37
OpT1												
1510	0	1	1	0	1	1	0	1	1	1	0	
1280	0	1	1	1	1	0	0	1	0	0	0	
1080	0	1	1	1	0	1	0	0	1	1	0	
910	0	1	1	1	0	0	0	0	0	0	0	
760	0	1	1	1	1	1	0	1	1	1	1	
640	0	0	0	0	1	1	0	1	0	0	0	
460	0	1	1	1	1	1	0	1	0	0	0	
Sub-total	0	6	6	5	5	5	0	5	3	3	1	39
Grand Total	13	18	19	18	24	17	16	12	10	10	2	159

OpN18=Operon N 18 $_{1870}$ primer with fragment size 1870 base pair
HEW= white Hiteyah, HE8= Hiteyah 8, HEB= black Hiteyah, HEY=Hiteyah yellow
SH6= Sham 6, AC933= ACSAD 933, V870 =Variety 870, DEB= black Dibeyah
ANB= Anber, DEW= white Dibeyah, SH5= Sham 5.

Appendix (D)

Solutions and Buffers:

- 0.7% agarose (0.7gm agarose /100 ml of 0.5 X TBE buffer).

- Loading buffer: 0.25% bromophenol blue and 40% (w/v) glycerol in water.

- Deionized water.

- 0.5X TBE buffer: 45m M Tris - borate, 1m MEDTA, and (pH 8.0). The concentrated stock solution (5X: 54 g Tris base, 27.5 g Boric acid, 20 ml 0.5 EDTA (pH 8.0).

- Ethidium bromide: (8µl / 100 ml gel buffer), stock solution (10 mg/ml water).