Relationship Between Genetic Structure and Yield Characteristics of Some Libyan and Egyptian Wheat Cultivars

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ABSTRACT

Wheat (*Triticum aestivum* L.) is a major crop for human consumption and consider one of the world's major cereal crops. The present study conducted at the Farm and Labs of the Faculty of Agriculture, Saba Basha, Alexandria University, Egypt to study the different morphological, biochemical and molecular characteristics between some Egyptian and Libyan wheat cultivars during 2015/2016 and 2016/2017 seasons to estimate the genetic relationship. Based on morphological data the general genetic similirity between all wheat cultivars was 76%, the culster divided into two main groups e.g. the first group includes four Libyan wheat cultivars and the next group from the main culster includes all the Egyptian wheat with one cltivar from Libya (Sohag), The same results were found to Gemmeiza 9 was the onlyest cultivar in one custer by shareing the other Egyptian wheat. In total of 8 enzyme loci, three anodal and five cathodal loci were detected. One anodal (Pex.2a) was found as common band for all the cultivars for the positive charge. While three cathodal (pex 1c, pex 1c and pex 1c) were found to the negative charge. Pex.1a was found as unique for Egyptian wheat and (pex c4) was unique for Libyan and Egyptian wheat cultivars i.e. Sohag, Kofra, Kazeno, Kerem, Shakha 93 and Gemmeiza 9. Pex.5 was found in all wheat cultivars except Shakha 94. For the RAPD markers a total of 75 bands were detected among the studied cultivars. 53 bands showed polymorphism by 70.66 % genetic polymorphism. Out of these polymorphic bands, 13 unique bands were scored and the number of monomorphic bands was 9. These results could be helpful in study the genetic relationship between Egyptian and Libyan wheat cultivars.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is a major crop for human consumption. Its importance hinges upon unique rheological properties of wheat flour which allow for the production baked goods (Chiran *et al.,* 2012). Wheat is one of the world's major cereal crops (Collard *et al.,* 2005). Wheat (*Triticum aestivum* L) ranks second crop after rice, providing 23.0 % of dietary energy and serving as a staple food for 40.0 % of the total human population of the world (FAO, 2009). In accordance with its demand, it is the most widely grown crop throughout the world, accounting for 17.0 % of the total cultivated area and production of 605.0 million tons/year. Wheat (*Triticum* spp.) is a monocot and belongs to tribe *Triticeae* of family Poaceae (previously called Gramineae). Other important crops like rice (*Oryza sativa L.*), maize (*Zea mays L.*) and bamboo also belong to this family (Shitsukawa *et al.*, 2006). Wheat is an annual grass with inflorescence called spike. Several different types of DNA markers are currently available for genetic analysis and new marker types are being developed continuously. Markers vary from each other in respects: the initial workload and costs for building up the marker system, running costs and ease of use, level of polymorphisms, inheritance, number of loci analyzed per assay, reproducibility and distribution on the chromosomes. Detection of polymorphism at the DNA level is usually based either on restriction patterns or differential amplification of DNA. To select from several markers that have now become easy to use, it is necessary to make a comparison between different types of molecular markers (RFLPs, RAPDs, DAF, SSRs, AFLPs, etc.). Genetic markers reveal essential differences between detached organisms and species. While, they do not detail the propose genes themselves but approach as 'signs' or 'flags'. Genetic markers that are by genes (i.e. tightly linked) manage be involve out as gene 'tags'. Like markers themselves do not collect a friction to the phenotype of the kind of accomplishment because they are located only by or 'linked' to genes assigned to the trait. All genetic markers keep specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus') (Hammer *et al.,* 2000). While, Jones *et al.* (1997) indicated that there are three kinds of genetic markers: (1) morphological markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA markers, which reveal sites of variation in DNA. Morphological markers are usually characterized phenotypic parameters as flower color, seed shape, growth habits and pigmentation. Isozyme markers are differences in enzymes that are detected by electrophoresis and specific staining. The main disadvantages of morphological and biochemical markers are that they may be limited in number and are affected by environmental factors and developmental stage of plant. Molecular markers have become increasingly important in plant molecular biology in its relationship to plant breeding, plant systematic and plant evolution. Most molecular marker systems are presently based on PCR technology, molecular markers of various types have helped to increase understanding and elucidate new aspects of plant evaluation. DNA markers, reveal sites of variation at the DNA level. These markers have the advantage of being numerous in the nature and not affected by the environment as in the case of morphological markers. The expression of most genes is quantitative in segregating populations and is confounded by the environment. The development of molecular markers in wheat and their application in breeding and related research programmes, poses several significant challenges compared with some other crop plants (Marshall *et al.,* 2001, and Gupta *et al.,* 1999). RAPD analysis is applied to many different plant species, developed by (Welsh and McClelland, 1990).

The present study aims to study the different morphological characteristics between some Egyptian and Libyan wheat cultivars, calculate the genetic relationship of agronomic, yield and yield components between the current cultivars, assay the enzyme activity,

calculate the genetic polymorphism based on RAPD, detect the genetic distance and similarity between the Egyptian and Libyan wheat cultivars based on different markers.

MATERIALS AND METHODS

The present research was carried out at the Faculty of Agriculture, Saba Basha, Alexandria University, Egypt, during 2015/2016 and 2016/2017 seasons. A total of ten wheat (*Triticum aestivum* L.) cultivars were used in the present study, five Egyptian wheat namely, Sakha 93, Giza 168, Gemmiza 9; Shakha 94 and Egypt 1 and five Libyan wheat i.e. Mergawey, Sohag, Kofra, Kazeno and Kerem. Grain samples were obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt and Agricultural Researches center in Libya.

Filed experiment:

At 15th of November 2015 in the first season, the experimental area was 10.5 m^2 (3 x 3.5 m) and seeding rate was 60 kg grains/fed., first irrigation was applied at 25 days after sowing and plants were irrigated every 25 days till the dough stage at the Farm of faculty of Agriculture, Saba Basha, Alexandria University. At harvest, one square meter was taken randomly from each plot to determine yield and its components i.e. plant height (cm), tillers number/plant, spikelets number/plant, grains number/spike, 1000- grains weight, straw yield (kg/fed.), biological yield (kg/fed.), grain yield (kg/fed.), Harvest index (%), and grain protein content (%).

Protein percentage was determined by estimating the total nitrogen in the grains and multiplied by 5.75 to obtain the percentage according to A.O.A.C. (1990).

At 15th of November 2016 in the second season, Parents were sown and the same previous morphological characters were detected. Data obtained was exposed to the proper method of statistical analysis of variance difference among mean of different treatments as described by Gomez and Gomez (1984). The treatments means were compared using the least significant differences (L.S.D.) test at 5% level of probability by using the RCBD model as obtained by CoStat computer software package (CoStat, Ver. 6.4, 2005).

Biochemical studies:

Agar-starch-polyvinyl pyrolidine (PVP) gel electrophoresis was carried out according to the procedures described by Shaw and Kaen (1967). The extracts were made by grinding from young leaves using of tissue in a mortar with 10 µl of electrode buffer and centrifuged for 15 Sec., a sample of 10 µl of the homogenate was then absorbed onto a small rectangle (about 4 mm X 2 mm) of filter paper that was placed on the original line of gel plates, and after storage at 4 °C for 30 minutes, it was removed. This buffer was prepared by dissolving 18.55 gm of 0.3M Boric acid and 2 gm sodium hydroxide in 1 Liters of distil- led water then the solution adjusted to pH 8.3. Agar-starch-polyvinyl pyrolidine (PVP) gel was prepared by dissolving 1.0 gm agar, 0.5 gm PVP and 0.5 gm of hydrolyzed starch with 10 ml electrode buffer and 90 ml distilled water (El-Metainy *et al.*, (1977). Electrophoresis experiment were conducted in an incubator refrigerator adjusted at 4°C using a 250 volts AC electrical current, with constant voltage throughout the 90 minutes of the running period. Dissolving 13.6 gm sodium acetate and 5.77 ml acetic acid in 500 ml distilled water, from this solution taken 7.5ml acetic acid, 17.5 ml sodium acetate with 0.05 gm benzidine and 100µl hydrogen peroxide dissolved in 25 ml distilled water for staining. Incubation was extended for thirty minutes at room temperature and complete darkness. Plates were than distained in distilled water until a clear background of gel plate (Youssef *et al.*, 1989).

Molecular analysis:

DNA Extraction:

DNA was extracted following (Murray and Thompson, 1980) with minor amendments. Ten seeds sample of about 1-2 g was ground with pestle and motor in 1ml 2X

CTAB (1M tris (ph8), 0.5M EDTA (ph8), 5M NaCl, 20 gm CTAB (Cetyl-tetramethyl ammonium bromide) in 1000 ml containing 25µl mercaptoethanol,10 mg polyvinyl pyrolidine (p.v.p). About 750 µl well ground paste was taken in to 1.5 ml labeled eppendorf tube. Tubes were inverted gently to mix the ingredients incubated at 65°C for 45 minutes in water bath and centrifuged 12,000 rpm for 10 minutes.Then in new eppendorf tube taken 700µl supernetant,350µl 1M tris (ph8), 350µl chloroform: isoamyle alcohol (24:1),5µl proteinase K. Tubes were inverted gently to mix the ingredients and centrifuged 12,000 rpm 10 minutes. About 400 µl supernatant were taken carefully in to new labeled eppendorf tube,
400µl chloroform: isoamyle alcohol (24:1) and centrifuged 12,000 rpm 10 minutes. 400 µl supernatant were taken carefully in to new labeled eppendorf tube 700 µl chilled isopropanol was added to each tube and mixed gently. Tubes were incubated at 4° C over night and centrifugation 12,000 rpm for 10 minutes. Supernatant was discarded and DNA pellet was rinsed with 70% ethanol. DNA pellet was air dried. The pellet was dissolved in 100 µl TE buffer (10 mM Tris, 1mM EDTA and PH: 8.0). RNAase-A was used to remove RNA from the DNA. For this purpose, RNAase-A (0.20 µl of RNAase-A procured from Gene Link, USA) 40 µg was used and DNA samples were kept at 37°C for 30 minutes in water bath. DNA quantification was carried out in spectrophotometer. A 20 ng dilution of DNA was made in deionized water to use in Polymerase Chain Reaction (PCR). DNA samples were stored at -20 °C**.**

Random amplified polymorphic DNAs (RAPD) analysis:

RAPD has been developed, in which DNA is amplified by the polymerase chain reaction (PCR) using arbitrary short (10 nucleotides) primers (Williames *et al.,* 1990). RAPD has become an important technique for population genetic studies since the amplified products provide random representation of both coding and non-coding regions across the whole genome. RAPD analyze was carried out using ten oligonucleotide primers (Table 1) that were selected from the Operon Kit (Operon Technologies Inc., Alabameda, CA). The polymerase chain reaction mixture (25µl) consisted of 13µl master mix (Promega) *Taq* DNA polymerase; 2µl of genomic DNA, 2µl primer,8µl deionized water. PCR amplification was performed in a Biometra T1 gradient thermal cycler for 35 cycles after initial denaturation for 5min at 94°C. Each cycle consisted of denaturation at 94°C for 1min; annealing at 36°C for 1min; extension at 72°C for 2min and final extension at 72°C for 5min (Williames *et al.,* 1990).

Table (1). Primers name and their oligonucleotide sequences used in the current study.

Code	Primer Code	Sequence
1	OPN-04	5`- GACCGACCCA -3`
2	OPD-05	5`- TGAGCGGACA -3`
3	OPC-05	5`-GATGACCGCC -3`
4	OPM-05	5`- GGGAACGTGT -3`
5	OPB-07	5`-GAAACGGGTG -3`
6	OPN-10	5`-ACAACTGGGG -3`
7	OPG-12	5`-CAGCTCACGA -3`
8	OPQ-12	5`- AGTAGGGCAC -3`
9	OPN-13	5`-AGCGTCACTC - 3`
10	OPQ-14	5`-GGACGCTTCA -3

Fragments scored as present/absent. Fragment scoring and lane matching performed automatically on digital images of the gels, using Phoretix 1D advanced Version 4.00 (Phoretix International, Newcastle upon Tyne, UK). All but the faintest bands scored, where necessary scores and matches corrected manually. Clustering methods and

similarity coefficients were tested using the procedures SIMQUAL, SAHN, and TREE from the program NTSYSpcversion 2.10 (Applied Biostatistics, Setauket, New York, USA). The clustering methods UPGMA, WPGMA, Complete-link, and Single-link were applied in all possible combinations with the similarity coefficients Dice, Jaccard and simple matching. (Rohlf, 2000) describes clustering methods and similarity coefficients. One Way ANOVA in completely randomized experiments was used to reveal the significant differences among the samples. The LSD (least significant differences) test was conducted to identify the significant differences among the means at 5% level of probability. Comparison of the mean values is usually calculated after an ANOVA. The latter specify the factors that have significant differences between treatments, while the means comparison display the treatments which are significantly different from the others (Siugh 1994).

RESULTS AND DISCUSSION

1. Plant height (cm):
Data in Table (2) showed high significant variations between the Egyptian and Libyan wheat cultivars in relation to plant height (cm). The data showed high values for all Egyptian wheat in both harvest seasons (2016 and 2017) comparing with Libyan wheats cultivars. Cultivar Gemmeiza 9 showed the highest plant height values were 103.4 and 100.4 cm in both seasons, in respect. While Shakha 93 cultivar showed, the lowest values were 88.67 and 86.2 cm. On the other hand, for Libyan wheat cultivars showed huge gap in plant height (cm) comparing with Egyptian cultivars, the ranges in the first harvest season were 61.0 to 79.6 cm and from 63.0 to 77.6 cm in the next season. For Libyan wheat cultivars, the data showed that Sohag cultivar showed the highest plant height (cm) in both seasons by 79.6 and 77.6 cm and the lowest one was Kerem cultivar by 61.0 and 63.0 cm, in respect (Table 2). Analysis of variation showed high significant variations between Egyptian and Libyan wheat in the current character and the value started from 61.0 and 63.0 cm in Kerem to 103.0 and 100.4 cm in Gemmeiza 9 for both harvest seasons (Table 2).

2. Tillers number/plant:
Huge number of stem was observed to the Egyptian wheat comparing with the highest Libyan cultivar Sohag ~ from 2 to 4.67 tillers/plant were recorded to the Egyptian wheat, the highest cultivars were Gemmeiza 9, Egypt 1 and Geiza168 by 3.9, 3.6 and 3.2 tillers/ plant for the first season and 4.7, 3.7 and 3.7 tillers/ plant in the next season. While, for the Libyan wheat that showed range for number of tillers/plant from 1 to 2.7 and 1.2 to 2.4 tillers/plant in the two seasons, respectively. There were no significant variations between the Libyan wheat cultivars (Table 2), the tillers number/ plants ranged from 1.3 to 4.7 tillers/plant for all wheat cultivars during the first harvest season and were from 1.2 to 3.9 tillers/plant in the next season by general mean ~2 per plant.

3. Spikelets number/spike:
Egyptian wheat achieved the highest production in this character based on the spike length and number of spikelets/spike as reported in Table (2). Gemmeiza 9 record the highest number of spikelets/spike comparing with other wheat cultivars (22.3 and 21.2) for both harvest seasons, in respect. The lowest spikelets number/spike was observed in Kerem cultivar by 9.5 and 10.0 spikelets/spike. High significant variations were observed between the wheat cultivars in relation to number of spikelets/spike. The average of this character ranged from 9.5 to 22.2 in the first season and from 10.0 to 21.2 in the second season.

4. Grains number/spike:

Relationship was obtained between the number of spikelets and grain number/ spike, that was found between the tested cultivars (Table, 2). High significant variations were found between the Egyptian and Libyan wheat cultivars. The highest average was recorded to Gemmeiza 9 by 68.5 and 55.9 by general mean 62.2 grains/spike in both harvest seasons. And the lowest values were recorded to the cultivar Keram by 24.6 and 25.5 grains/spike by overall 25.0 grains/spike for both seasons. From the previous data, we can be found that Egyptian wheat in general achieved the highest grains number/spike comparing with Libyan wheat cultivars. Sohag cultivar showed the highest value in Libyan wheat in both seasons (Table 2).

5. 1000- grains weight:

Data in Table (2) showed that Gemmeiza 9 has the highest value in 1000 seed weight (g) by an average of (60.8 and 59.2 g) followed Egypt 1 (52.7 and 51.2 g) in both growing seasons. The results showed high significant variation between Egyptian and Libyan wheat cultivars for 1000- grains weight (g). The lowest mean values were recorded with Kofra and Mergawey cultivars by average ~ 32.0 g as showed in Table (2).

6. Grain yield (kg/fed.):

The final product of each process is the yield, grain yield (kg/fed.) is the main target for breeders and farmers. Data in Table (2) showed the difference between the Egyptian and Libyan wheat cultivars for Grain yield kg/fed. High significant variations were observed between the wheat cultivars in relation to this character especially between the Egyptian and Libyan wheat cultivars. The highest production/fed., was recorded to cultivar Gemmeiza 9 by 2972.8 kg followed by Sakha 94 by 2142.7 kg., in the first season and 2862.8, 2242.0 kg for the next season, respectively. While, the lowest cultivars were Kerem and Mergawey by 1185.0 ; 1164.0 kg in the first season then 1175.0 and 1140.8 kg for the second season with no significant variations.

7. Straw yield (kg/fed.):

Based on the previous data, straw yield (kg/fed.) were calculated as shown in Table 2 Analysis of variance showed high significant variations between the wheat cultivars and data was 184.50 for both harvest seasons. Also, Gemmeiza 9 showed the highest values in both seasons by 3900 and 3780 kg/fed., and the lowest one was Kerem by 1814.3 and 1810.0 kg/fed., in respect.

8. Biological yield (kg/fed.):

Results in Table 2 showed the same trend of the data, thus indicated that Gemmeiza 9 showed the highest values in both seasons by 6872.8 and 6642.8 by average was 6757.8 kg/fed., and the lowest values were recorded to Kerem by 2999.25 and 2985.25 kg/fed.

9. Harvest index (%) :

Egyptian wheat cultivars showed high harvest index in comparison with the Libyan wheat cultivars and the range was from 25.1 to 43.3 % in the first season and from 26.1 to 41.3 % in the next harvest season as shown in Table 3 & 4 and Figure 9. Gemmeiza 9 showed the highest values 43.3 and 41.3 %.

10. Grain protein (%) :

Finally, we calculated the percentage of protein of the tested cultivars and the data recorded in Table 2. All the tested cultivars showed less than 10% of protein content, but the Egyptian wheat showed high percentage of protein content comparing with other wheat cultivars. The highest protein content was recorded to Gemmeiza 9 by 8.2 and 9.5% and the lowest values was 5.2 and 5.1 for Kofra cultivar.

These results are in harmony with those obtained by Njuguna _et al._ (2010); Aown _et al._ (2012); Raisi _et al._ (2012); Abd El-Ghany _et al._ (2013); Kandil and Marie (2017) which cleared that three were significant difference among wheat cultivars on plant height and yield and its components.

Based on morphological and yield characters of the ten Egytian and libyan wheat cultivars, dendrograms showed the genetic relatioship beteen wheat cultivars. The data indicated that the general genetic similirity between all the wheat cultivars was 76% abs he culster divided into two main groups e.g. the first group includes four Libyan wheat cultivars sch as Mergawey, Kerem, Kazeno and Kofra, sepereed into three different sub groups i.e. Mergawey and Kerem were togeather by 97 %, Kerem was in sepertae group by 94% and Kofra (91%). The next group from the main culster includes all the Egyptian wheat with one cltivar from Libya (Sohag), within this cster, the results showed that Gemmeiza 9 was recoded in separate culster (86%) comparing with oher wheat cltivars, foewered by Sohag (90%) and the other four Egyptian wheat Sakha 94, 93, Egypt 1 and Giza 168 were in other culster by 97, 96 and 95% genetic simerity. The same results were also found in Figuer 1 which showed that Gemmeiza 9 was the onlyest cultivar in one custer by shareing he other Egyptian wheat.

Table (2). Plant attributes of ten Egyptian and Libyan wheat cultivars under field condition, during 2015/2016 and 2016/2017 seasons

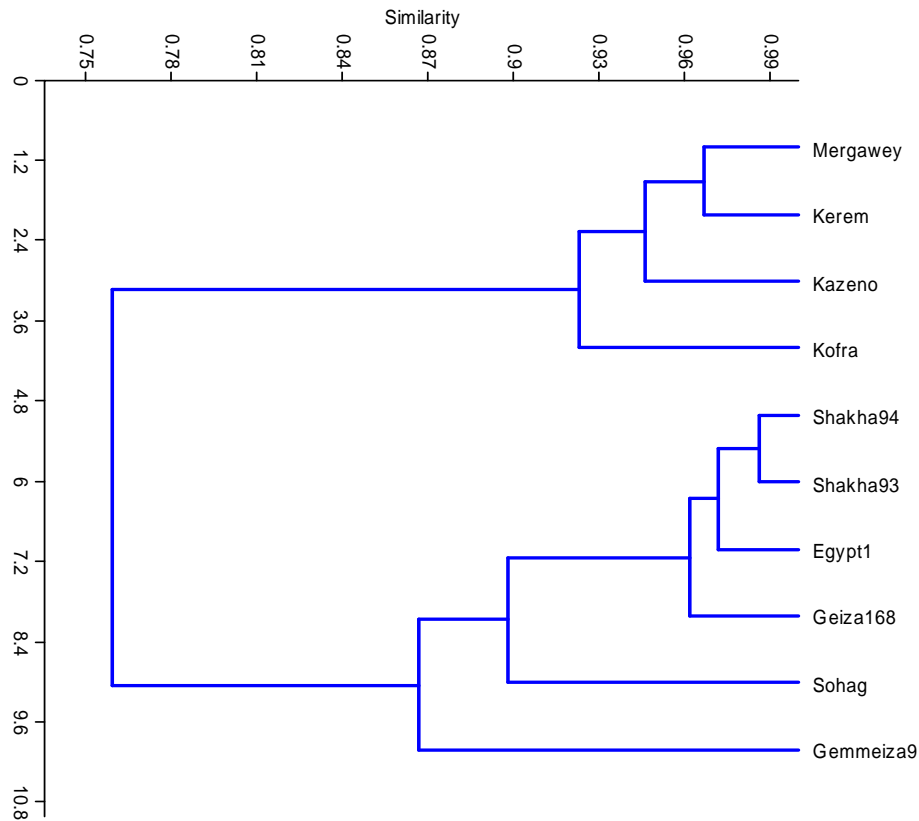| Cultivars	Plant height (cm)	Tillers No./ plant	Spikelets No./ spike	Grains No./spike	1000-grain Weight (g)	Grain yield kg/fed.	Straw yield kg/fed.	Biol. yield kg/fed.	H.I. %	Grain protein %
Season 2015/2016										
Shakha93	88.7	1.67	16.8	45.8	48.1	1863.8	3338.8	5202.5	35.8	8.6
Geiza168	90.0	3.67	18.0	50.0	47.3	2054.5	3615.0	5669.5	36.2	9.3
Gemmeiza9	103.4	4.67	22.2	68.5	60.8	2972.8	3900.0	6872.8	43.3	9.5
Shakha94	97.3	3.00	16.5	46.5	48.7	1948.8	3292.5	5241.3	37.2	7.9
Egypt1	94.3	3.66	19.5	63.5	52.7	2142.8	3237.5	5380.3	39.8	8.0
Mergawey	67.0	1.00	12.0	30.5	32.5	1164.8	2001.0	3165.8	21.8	5.1
Sohag	79.6	2.67	14.8	35.3	33.5	1606.8	2786.5	4393.3	28.9	6.2
Kofra	65.7	1.33	10.5	29.5	32.4	1369.5	2320.8	3690.3	25.7	5.1
Kazeno	77.0	2.00	11.5	28.0	36.6	1464.5	1899.8	3364.3	28.7	6.6
Kerem	61.0	1.67	9.5	24.6	36.3	1185.0	1814.3	2999.3	25.1	5.2
L.S. D$_{0.05}$	1·.03	0.829	1.62	3.16	2.97	111.88	244.50	278.60	2.91	0.494
Season 2016/2017										
Shakha93	86.2	2.0	15.7	44.7	50.2	1903.8	3238.1	5141.9	36.8	8.77
Geiza168	91.8	3.2	18.5	48.9	49.1	2154.5	3510.0	5664.5	35.9	10.0
Gemmeiza9	100.4	3.9	21.2	55.9	59.2	2862.8	3780.0	6642.8	41.3	9.5
Shakha94	95.3	3.2	17.0	49.5	49.0	1878.8	3432.5	5311.3	37.9	8.2
Egypt1	95.3	3.6	18.6	59.5	51.2	2242.0	3137.0	5379.0	40.0	8.2
Mergawey	65.0	1.2	11.0	31.5	31.5	1140.8	2021.0	3161.8	20.8	5.1
Sohag	77.6	2.4	13.8	33.3	34.5	1628.8	2740.5	4369.3	29.8	5.9
Kofra	66.7	1.0	10.1	29.0	33.0	1333.5	2300.8	3634.3	26.6	5.2
Kazeno	76.0	2.0	10.5	29.0	35.6	1484.5	1869.8	3354.3	27.5	6.4
Kerem	63.0	1.6	10.0	25.5	34.2	1175.0	1810.3	2985.3	26.1	5.3
L.S. D$_{0.05}$	12.0	0.640	1.72	2.78	2.45	99.12	184.50	198.60	2.66	0.590

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Fig.1: Genetic relationship between Egyptian and Libyan wheat cultivars based on morphological characters

Biochemical analysis:

Peroxidase iso-enzyme assay was applied as the most appropriate technique for the evaluation of wheat cultivars (*Triticum aestivum* L.), and classified peroxidase patterns were ascribed to different phenotypes. Isozyme is an important tool to detect the variation between different species or cultivars. Peroxidase activity were assed for the ten wheat cultivars collected from Egypt and Libya as tool calculate the genetic relationship among wheat cultivars. In contrast, as shown in Figure 2, Peroxidase isozymes exhibited a wide range of variability among the different species at different localities. In total of 8 loci, three anodal and five cathodal loci were detected. One anodal (Pex.2a) was found as common band for all the cultivars for the positive charge. While three cathodal (pex 1c, pex 1c and pex 1c) were found to the negative charge. Pex.1a was found as unique for Egyptian wheat and (pex c4) was unique for Libyan and Egyptian wheat cultivars i.e. Sohag, Kofra, Kazeno, Kerem, Shakha 93 and Gemmeiza 9. Pex.5 was found in all wheat cultivars except Shakha 94. The activities of antioxidant enzymes have been studied intensively and yet the significance of these enzymes in salt tolerance is still a matter of controversy, because high antioxidant enzymes activities have been associated with salt tolerance as well as salt sensitivity. This led to the suggestion that genetic differences in salt tolerance among plants are not necessarily due to differences in the ability to detoxify ROS4 despite the large number of studies that correlate efficient antioxidative defense to salt tolerance. Isozyme loci have been used as markers in many genetic studies, such as genetic diversity in *Brassica juncea* (Kumar and Gupta, 1985); (Persson *et al.,* 2001) and isozyme markers as seed coat color (Rahman, 2001). Peroxidases are enzymes related to polymer synthesis in cell wall (Welsh, 1990), as well as in the prevention of oxidative damage caused by environmental stress to the membrane lipids (Kalir *et al.,* 1984; Reda, *et*

al., 2011). It was found that salt stress increased peroxidase band sintensity. Salt tolerant cultivars Gemmieza 10, Sohag and Beni-Sweif showed higher band intensity compared with the other cultivars. These results agreed with those of (Gaspar *et al.* 1982) who reported an increase in peroxidase activity in cultivars sensitive to salt, which could be responsible for the ability of such cultivars to adapt to external stimulus. Hong *et al.* (2005) showed that peroxidases are common and important indices for evaluating wheat redox, its activity display higher antioxidative abilities, reflecting higher resistance to drought. Peroxidase are enzymes related to polymer synthesis in cell wall (Welsh, 1990), as well as in the prevention of oxidative damage caused by environmental stress to the membrane lipids (Kalir *et al.*, 1984). Plant peroxidase have been used as biochemical markers for various types of biotic and Abiotic stresses due to their role in very important physiological processes, like control of growth by lignification's, cross linking of pectin's and structural proteins in cell wall, catabolism of auxins (Gaspar, *et al.*, 1982). The same trend of non-regular relationship between water regime treatments and peroxidase isozymes 4th and 5th was detected. Peroxidase activity that was expressed in terms of variation in band-intensity and/or presence or absence score proved to be not accurate criteria for the band characterization of wheat genotypes at different water treatments, since the banding patterns differ extremely between water stress tolerance or susceptibility. In general, these results are in accordance with those obtained by (Sairam *et al.* 2001), who found that peroxidase isozymes activities increased significantly under water stress. The modifications of gene expression due to environmental stress are a common response in the metabolism of plant cells. Activation of gene due to environmental stimuli which plays an important role in the plants adaptation to unfavorable conditions and promotes the appearance of specific proteins (Naqvi *et al.* 1995). In addition, proteins and isozyme polymorphisms are good indicators of response to biotic and abiotic stresses (Doebley, 1989).
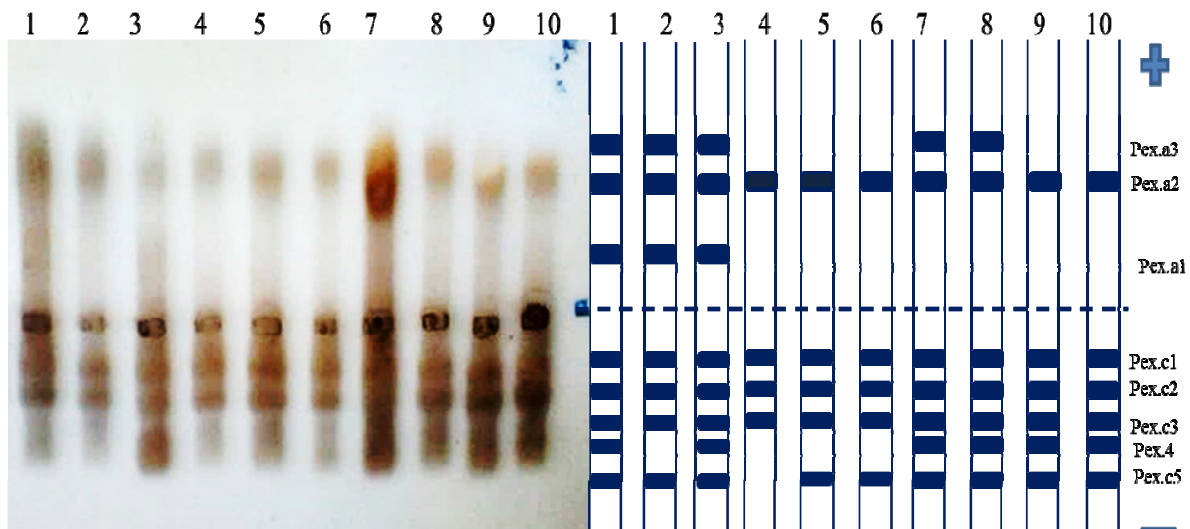

Fig.2: Zymograme of peroxidase isozymes of Egyptian and Libyan wheat cultivars (1) Shakha 93, (2) Geiza 168, (3) Gammeiza 9, (4) Shakha 94, (5) Egypt 1, (6) Mergawey, (7) Sohag, (8) Kofra, (9) Kaa Kazeno, and (10) Kerem.

Molecular studies:

RAPDs are generated by applying the polymerase chain reaction (PCR) to genomic DNA samples, using randomly constructed oligonucleotides as primers. Since the technique is relatively easy to apply to a wide array of plant and animal taxa, and the number of loci that can be examined is essentially unlimited, RAPDs are viewed as having several advantages over RFLPs and DNA fingerprints. When the primers are of intermediate size (on the order of 10 base pairs), multiple amplifiable fragments (from different loci) are usually present for each set of primers in each genome. The fragments can be separated by size on a standard Agarose gel and visualized by ethidium bromide staining, eliminating the need for radio labeled probes. Since the primers consist of random sequences, and do not discriminate between coding and nonbonding regions, it is reasonable to expect the technique to sample the genome more randomly than conventional methods. In the present study, the genetic variability and relationships of different cultivars of wheat have been studied based on RAPD analysis. Initial screening of large number of RAPD primers with ten cultivars of wheat, resulted in ten RAPD primers that produced in formative and polymorphic products resolvable by agarose gel electrophoresis. In the present study ten random primers were used to differentiate through RAPD analysis among tested samples wheat (*Triticum aestivum* L.)

Primer OPN-04 :

The results of primer OPN-04 are illustrated in Table 3. The total fragments were 29 amplification products at the fragment lengths ranged between 138 bp. to 800 bp. Two of five fragments were monomorphic (616 and 222 bp) and three fragments were polymorphic (800, 388 and 138 bp). The percentage of the polymorphism between the tested cultivars ranged from 33 to 50% and the general genetic polymorphism was 60%. Results in Table 3 showed that Geiza168, Shakha 94, Mergawey, Sohag and Kofra detected the same number of fragments (3 fragments), while, the Kazeno and Kerem cultivars showed the highest number of DNA fragments (4 fragments). On the other hand, the Shakha 93, Gammeiza 9 and Egypt 1 amplified the lowest DNA fragments number (2 fragments). No unique fragment was detected for the current primer.

Primer OPD-05 :

The generated RAPD profile of DNA fragment bands with the primer OPN-05 is presented in Table 3. This primer gave a maximum of 35 amplification products at the fragment lengths ranged between 352 bp. to 1100 bp. and tasted samples were polymorphic and monomorphic fragments. The percentage of the polymorphism was 88%. One unique (specific) fragment (1096 bp.) was exhibited for Gammeiza 9 with frequency 0.4. The results in Table 3 showed that Shakha 93, Gammeiza 9, Mergawey, Sohag and Kerem have the same number of fragments (4 fragments), while, the Egypt 1 and Mergawey give the highest number of DNA fragments (5 fragments). On the other hand, the Shakha 94, Kofra and Kazeno gives the lowest DNA fragments number (2 fragments). The genetic polymorphism between wheat cultivars range from 50 to 80%.

Primer OPC-05 :

Concerning to the primer OPC-05 and the generated RAPD profile of DNA fragment bands is presented in Table 3. In a total of 36 amplification products at the fragment lengths ranged between 275 bp. to 1806 bp all were polymorphic fragments. The percentage of the polymorphism was 100%. Three unique fragments (582 bp, 492 bp and 275 bp) was exhibited for Gammeiza 9 and Shakha 93 with frequency 0.5. Results showed that Geiza 168, Shakha 94, Egypt 1, Mergawey, Sohag, Kofra, Kazeno and Kerem have the same number of fragments (4 fragments), On the other hand, the Shakha 93 and Gammeiza 9 gives the lowest DNA fragments number (2 fragments).

Primer OPM-05 :

The generated RAPD profile of DNA fragment bands with the primer OPM-05 is found in Table 3. This primer gives a maximum of 57 amplification products at the fragment lengths ranged between 236 bp to 1825 bp., and all tasted samples were polymorphic fragments. The percentage of the polymorphism was 100%. Two unique (specific) fragment (608 and 290 bp) were recorded to Gammeiza 9 and Kofra with frequency 0.4. The results showed that Geiza 168, Shakha 94, Geiza168, Mergawey, Sohag and Kerem have the same number of fragments (4, 5 and 7 fragments), while, the Kofra and Kazeno give the highest number of DNA fragments (8 fragments). On the other hand, the Shakha 93 gives the lowest DNA fragments number (1 fragments).

Primer OPB-07 :

The following data for generated RAPD profile of DNA fragment bands and the primer OPB-07 illustrated in Table 3. The current primer gave a maximum of 36 amplification products at the fragment lengths ranged between 317 bpto 1117 bp. One band (389 pb) was monomorphic and the other three bands were polymorphic. The percentage of the polymorphism was 80%. Results showed that Shakha 93, Gammeiza 9, Geiza 168, and Kazeno have the same number of fragments (2,4 fragments), while, the Egypt 1 Mergawey, Sohag and Kofra the highest number of DNA fragments (5 fragments). On the other hand, Local wheat gives the lowest DNA fragments number (1 fragments).

Primer OPN-10 :

The generated RAPD profile of DNA fragment bands with the primer OPN-10 is presented in Table 3. This primer gave a maximum of 29 amplification products at the fragment lengths ranged between 204 bp to 1040 bp and all tasted samples were polymorphic fragments. The percentage of the polymorphism was 100%. The results showed that Mergawey, Kazeno, and Kerem have the same number of fragments (3 fragments), while, the Gammeiza 9, Shakha 94, and Kazeno give the highest number of DNA fragments (4 fragments). On the other hand, the Shakha 93, Geiza168, Egypt 1and Sohag gives the lowest DNA fragments number (2 fragments).

Primer OPG-12 :

The generated RAPD profile of DNA fragment bands with the primer OPG-12 and its results of bands analysis are presented in Table 3. This primer gave a maximum of 35 amplification products at the fragment lengths ranged between 188 bp to 1089 bp and all tasted samples were polymorphic fragments. The percentage of the polymorphism was 100%. Two unique (specific) fragment (277 bp and 88 bp) was exhibited for Geiza168 with frequency 0.4. Results showed that Egypt 1, Mergawey, Sohag and Kofra have the same number of fragments (4 fragments), while, the Geiza 168 and Shakha 94 give the highest number of DNA fragments (5 fragments). On the other hand, the Kerem gives the lowest DNA fragments number (1 fragments).

Primer OPQ-12 :

The generated RAPD profile of DNA fragment bands with the primer OPQ-12 are presented in Table 3. This primer gave a maximum of 50 amplification products at the fragment lengths ranged between 199 bp to 1235 bp and tasted samples were polymorphic and monomorphic fragments. The percentage of the polymorphism was 75%. One unique (specific) fragments (199 bp) was exhibited for Gammeiza 9, with frequency 0.6. Results

showed that Gammeiza9, Mergawey, Kofra, Kazeno and Kerem have the same number of fragments (5 fragments), while, the Sohag give highest number of DNA fragments (7 fragments). While, Shakha 93 gives the lowest DNA fragments number (2 fragments).

Primer OPN-13 :

The generated RAPD profile of DNA fragment bands with the primer OPN-13 and bands analysis are presented in Table 3. This primer gave a maximum of 34 amplification products at the fragment lengths ranged between 214 bp to 544 bp and tasted samples were polymorphic and Monomorphic fragments. Two monomorphic fragments were detected (214 and 393 bp). The percentage of the polymorphism was 60%. One specific fragments (487 bp) was exhibited for Gammeiza 9, with frequency 0.7. Results showed that Gammeiza 9, Shakha 94, Mergawey and Sohag give highest number of DNA fragments (4 fragments). While the Shakha 93, Geiza168, Egypt1, Kofra, Kazeno and Kerem gives the lowest DNA fragments number (3 fragments).

Primer OPQ-14 :

The results of the primer OPQ-14 is illustrated in Table 3. The present primer amplified a maximum of 37 amplification products at the fragment lengths ranged between 213 bp to 912 bp and tasted samples were polymorphic and monomorphic fragments. The percentage of the polymorphism was 89%. Three unique (specific) fragments (417 bp, 306 bp, 213 bp) was exhibited for Gammeiza 9, with frequency 0.4. Results showed that Geiza 168, Shakha 94, Egypt 1, Sohag and Kofra have the same number of fragments (4 fragments), while the Mergawey give highest number of DNA fragments (6 fragments). On the other hand, Shakha 93, Kazeno and Kerem gives the lowest DNA fragments number (2 fragments).

Data in Table (3) showed the total unique fragments for all the ten RAPD-PCR primers which used in the current study and results indicated clearly that OPC-05 and OPQ-14 showed three unique fragments followed by OPM-05 and OPG-12 by two unique fragments Table 3. A total of 75 bands were detected among the studied cultivars. 53 bands showed polymorphism. Out of these polymorphic bands, 13 unique bands were scored and the number of monomorphic bands was 9. However, the primer OPC-05, OPM-05, and OPG-12 shows 100% polymorphism. While primer OPN-04, OPD-05, OPB-07, OPQ-12, OPN-13 and OPQ-14 showed 60, 88, 80,75,60 and 89% polymorphism, respectively (Table 3). The range of DNA size was between 138 bp and 1825 bp. Since the PCR techniques have been developed, a wealth of new DNA marker technologies has arisen enabling the generation of high-density molecular maps for all the major crop species. Molecular markers have also been extensively used to analyze the genetic diversity in crop plants. Based on the data obtained by RAPD analysis, it was possible to discriminate between the ten wheat genotypes used. The genotype-specific markers indicated that the highest number of RAPD specific markers was scored for OPC-05 and OPQ-14 (3 markers), while both OPM-05, and OPG-12 scored two markers each. On the other hand, OPD-05, OPQ-12 and OPN-13 scored one marker each as Table (3).

Table (3). Polymorphism data as detected by RAPD markers, total number of amplicons, monomorphic and polymorphic amplicons and the percentage of polymorphism among the ten cultivars

Primer code	Total amplicons	Monomorphic	polymorphic	positive unique markers	PIC %
OPN-04	5	2	3	-	60
OPD-05	8	1	6	1096	88
OPC-05	7	0	4	582-292-275	100
OPM-05	14	0	12	608-290	100
OPB-07	5	1	4	-	80
OPN-10	6	0	6	-	100
OPG-12	8	0	6	277-188	100
OPQ-12	8	2	5	199	75
OPN-13	5	2	2	487	60
OPQ-14	9	1	5	417-306-213	89
Total	75	9	53	13	85.2
Polymorphism %					

Efficient and effective crop improvement program depends on the extent of genetic diversity either existing or created (Naz *et al*., 2006 and Akbar *et al*., 2011). In the present study, it was observed that half of the RAPD primers were polymorphic that can be used for other wheat germplasm for investigation of genetic diversity between the Egyptian and Libyan wheat cultivars. To measure of diversity of genetic, the molecular markers also provide opportunity to go beyond indirect diversity based on agronomic traits or geographic origin (Mir *et al*., 2012). In the present study, RAPD analysis was found to be a valuable diagnostic tool to evaluate genetic diversity and in addition it could serve the purpose of genotyping if the number of primers are increased, and preferably the DNA markers be made specific either converting the reported fragments to SCAR (Gupta and Varshney, 2000). These results agree with those of Guadagnuolo *et al.* (2001) who reported that RAPD can produce a large set of markers, which can be used for the evaluation of both between- and within-species genetic variation; and Cao, *et al.* (2002) who indicated that the RAPD markers are useful in pedigree assessment in common wheat and for the identification of some wheat varieties. Maric *et al*. (2004) revealed that markers of RAPD recorded a high level of polymorphism among the cultivars and the breeding lines under study. Also, these results in the line with Bhutta *et al*. (2005) used RAPD analysis to estimate the degree of genetic divergence in 7 wheat genotypes from diverse locations of Pakistan. They found that 160 DNA fragments were amplified with 20 random decamer primers with an average of 8 bands per primer. In this study, high level of polymorphism was observed as compared to other previous RAPD marker studies on wheat, 1.7 polymorphic band per primer in China wheat polymorphic band per primer in Pakistani (Anwar *et al.,* 1998) cultivars (Zheng *et al.,* 2001), 11 polymorphic band per primer in Iranian wheat cultivars (Naghavi *et al*., 2004) and 10.4 bands per primer in Pakistani wheat cultivars (Nawaz *et al*., 2009). Joshi and Nguyen (1993) used 40 primers in studying wild and cultivated wheat and revealed 88% polymorphism among all accessions**.**

In the present study, the genetic relationships among the ten cultivars were determined by Dice Coefficient (Figure 3). The matrix of similarity index ranged from 0.26 recorded between cultivars Geiza168 and Gemmeiza 9 to 0.74 recorded between Egypt1 and Mergawey with an average of 0.50. The values similarity between the different cultivars of genotypes is specific as recorded in Figure 3. The dendrogram Figures 3 illustrating the distance among the examined wheat cultivars. The dendrogram in this case revealed two clusters by 33% similarity. The first one has Gemmeiza 9 in separate cluster and the next cluster divided into sub two cluster (39%), the first one had Sakha 93 and the second divided into two sub-sub clusters (50%). The data in the cluster showed that wheat cultivars Kazeno and Kerem were in one group by 70% and Egypt 1 and Sohag in the same cluster by 75%.
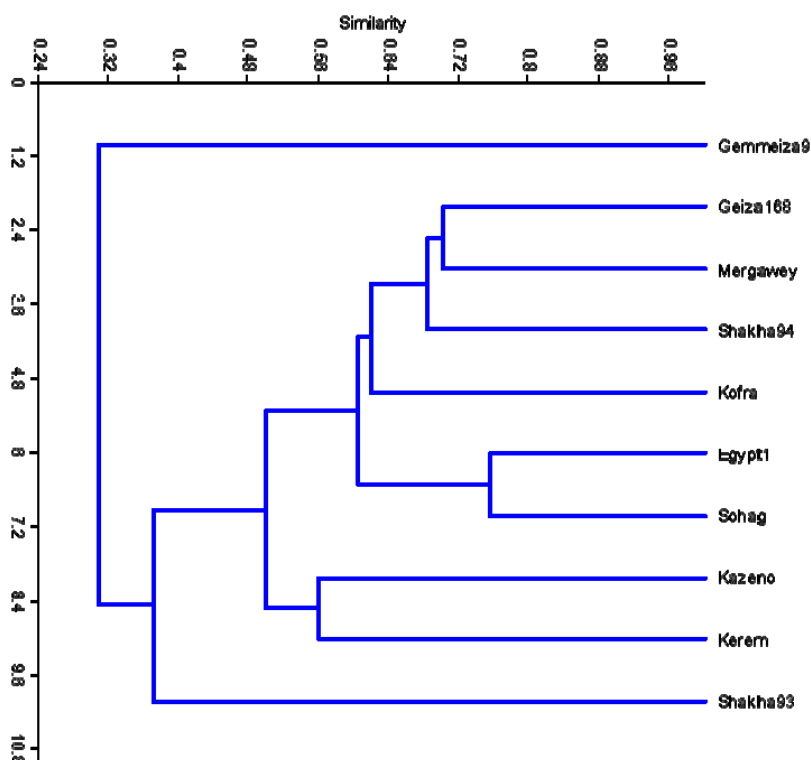

Fig.3: Genetic relationship between Egyptian and Libyan wheat cultivars based on RAPD-PCR markers.

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ARABIC SUMMARY

العلاقة بين التركيب الوراثى والخصائص المحصولية لبعض انواع القمح الليبية والمصرية

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يعتبر القمح من اهم المحاصيل الاستراتيجية الاستهلاكية على مستوى العالم لما يتمتع بة من قيمة اقتصادية وتغذوية عالية وتنتشر العديد من الأصناف والأنواع عالمياً مما يشكل تنوع وراثى لذلك الأصل النباتى. تهدف الدراسة الى تقدير العلاقة بين التركيب الوراثى والخصائص المحصولية لعشرة أصناف من القمح المصرى والليبيى عن طريق استخدام عديد من المعلمات التى منها المورفولوجية والبيوكيمائية والجزيئية. بناء على النتائج المحصولية أوضحت النتائج ان جميع الاصناف تتشابة بدرجة ٧٦% وهناك اختلاف وراثى قدرة ٢٤% ومن ثم تم تقدير النشاط الانزيمى للبروكسيذيز وأعطت النتائج ثمانية مواقع جينية فى المجموع والتى قسمت الى ثلاثة ناحية الانود وخمسة مواقع اتجاة الكاثود ووجد موقع جينى واحد كموقع ثابت للكل. اوضحت نتائج الوراثة الجزيئية الحصول على ٧٥ قطعة جينية حيث كان منها ٥٥ بها تعدد فى الشكل المظهرى بنسبة ٧٠.٦٦% وكان هناك ١٣ قطعة جينية متخصصة للاصناف. بينت هذة الدراسة مدى الاختلاف الوراثى بين الاقماح المصرية والليبة وكذلك مدى العلاقة بين التركيب المحصولى والتركيب الوراثى للاصناف موضع الدراسة
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