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Genetic Relationship between some Egyptian and Yemeni Wheat Based on Different Markers

Abdullah M. G. Dawlah, Ahmed E. Khalid and Nader R. Abdelsalam

Agricultural Botany Department, Faculty of Agriculture Saba-Bacha, Alexandria University

ABSTRACT

Ten wheat (*Triticum aestivum* L.) cultivars were used in the present study, five Egyptian wheat namely, (E1) Sakha 93, (E2) Giza 168, (E3) Gemmiza 9; (E4) Shakha 94 and (E5) Egypt1 and five Yemeni wheat, (Y1) Behoth14, (Y2) Sonalica, (Y3) Acsadgahran, (Y4) Kaaalhakl and (Y5) Local wheat. Grain samples were obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt and Agricultural Researches Extinction authority (The Regional Agricultural Researches for Central Highland) in Yemeni Morphological, biochemical and molecular markers were used to detect the genetic relationship between all cultivars, also we study the effect of different salt levels on seedling performance as tool for calculate the genetic diversity between the Egyptian and Yemeni wheat cultivars. The results indicated that there are high significant variations with and within all wheat cultivars.

Key words: Genetic relationship, wheat, Egypt and Yemen

Introduction

Wheat (*Triticum aestivum*) is one of the world's major cereal crops as the unique molecular makeup of its grain allows its use as a primary structural ingredient of breads, pastas, tortillas, and other products worldwide. To achieve the food production levels needed to supply worldwide demand, plant breeders have focused on the development of agricultural varieties possessing two characters: high yield potential and high end-use quality. In order to meet the demands of future populations, we will need to develop new methods not only for increasing wheat yield, but also for increasing the utility and reliability of the resultant grain. (Collard *et al.,* 2005).

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 the main cereal grain grown in Egypt for thousands of years, serving as the principal source of calories in Egyptian diet. The productivity of Egyptian wheat cultivars has increased dramatically from 2.4 Mg ha^{-1} in 1958 to 6.8 Mg ha^{-1} in 2004. Enrichment of genetic diversity plays a crucial role in wheat cultivar improvement (Chao *et al.,* 2007).

In Yemen, wheat has been the first strategic food crop for more than 7,000 years. It has maintained its position as the basic staple food in urban and rural areas for bread making. Ancient Yemen used to grow the tetraploid wheat species *Triticum pyramidale* until hexaploid bread wheat, *Triticum aestivum*, was introduced from India in the early 20th century (Abdel Ghaffar, 1994).

Genetic markers represent genetic differences between individual organisms or species. Generally, they do not represent the target genes themselves but act as 'signs' or 'flags'. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait. All genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus'). There are three major types of genetic markers: (1) morphological (also 'classical' or 'visible') markers which themselves are phenotypic traits or characters; (2) biochemical markers, which include allelic variants of enzymes called isozymes; and (3) DNA (or molecular) markers, which reveal sites of variation in DNA (Jones *et al*., 1997)

There are many common technologies used as molecular markers such as Restriction Fragment Length polymorphisms (RFLP), Simple Sequence Repeats (SSR), Random Amplified Polymorphic DNA (RAPD) and Single Nucleotide Polymorphisms (SNP) (Fu *et al*., 2002). During the last few decays, molecular markers such as SDS-protein, isozymes, and DNA sequences have shown excellent potentialities to assist selection of quantitative traits. Different markers might reveal different classes of variation (Powell *et al.,* 1996; Russell *et*

Corresponding Author: Nader R. Abdelsalam, Agricultural Botany Department, Faculty of Agriculture Saba-Bacha, Alexandria University
E-mail: nader.wheat@yahoo.com

al., 1997). These molecular markers had been used in wheat for detecting genetic diversity, genotype identification and genetic mapping.

The study of genetic variation in plant populations was greatly facilitated by the development of protein based on markers i.e. allozymes over three decades ago (Hamrick and Godt, 1990). Allozyme markers remain the most useful tool for addressing many questions in plant population biology (Cruzan, 1998), although the development of DNA-based markers (Khandka *et al.,* 1997) has revolutionized this field in recent years owing to their higher levels of polymorphism and because they are thought to be less subject to selection (Jenczewski *et al.,* 1999).

The aims of the present research were to study the genetic relationship between ten Egyptian and Yemeni wheat cultivars based on morphological, biochemical and molecular markers and also, detect the effect of different salt levels on seedling performance as tool for calculate the genetic diversity between the Egyptian and Yemeni wheat cultivars.

Materials and Methods

The present research were carried out at the Faculty of Agriculture, Saba Basha, Alexandria University, Egypt during the seasons of 2013 up to 2015 to study the morphological, biochemical and molecular genetic markers of some selected wheat cultivars as a tool for genetic diversity. A total of ten wheat (*Triticum aestivum* L.) cultivars were used in the present study, five Egyptian wheat namely, E1: Sakha 93, E2: Giza 168, E3: Gemmiza 9; E4: Shakha 94 and E5: Egypt1 and five Yemeni wheat i.e. Y1: Behoth14, Y2: Sonalica, Y3: Acsadgahran, Y4: Kaaalhakl and Y5: Local wheat. Grain samples were obtained from Field Crops Research Institute, Agriculture Research Center, Giza, Egypt and Agricultural Researches Extinction authority (The Regional Agricultural Researches for Central Highland) in Yemen.

Ten seeds were sown in each pot from each cultivar; before sowing the silica jel was washed with adequate amount of distill water. Salt (NaCl) treatment was applied after 8 days of germination in four levels (0mM, 50mM, 150mM and 200mM) were applied as foliar spray after three weeks of germination. Completely Randomized Design (CRD) with four replicates was used.

Leaves from each cultivar were grounded separately, using a cooled mortar with a pestle, and adding 0.23 M Tris-acetate, pH 5.0. Homogenate was extracted by the solution containing Tris (27.7 g) and citric acid (11.0 g) in 1L volume adjusted with distilled water. Electrophoresis was carried out by the prescriptions recommending 1% agar-starch-polyvinyl-pyrrolidone gel and Tris-orate or Tris-acetate separation buffers. Electrophoresis was conducted at 270 v, 4ºC for 100 min. 100 ml of 0.01 M acetate buffer pH 5.0, containing 0.1% benzidine and 0.5% hydrogen peroxide (H_2O_2) were layered over the gel immediately before staining (Sabrah 1980). Proline was determined according to the method of Bates *et al.* (1973) by 3% Aqueous Sulfosalicylic Acid, Acid Ninhydrin:1.25 g Ninhydrin,30 ml glacial acetic acid,20 ml 6M phosphoric acid.

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 mastermix (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.

Table 1: Primers name and their oligonucleotide sequences used in the current study

Primer number	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

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). Amplification products were separated on 2% Agarose gels at 100 volts for 1.30 hrs with 1 x TBE buffer. To detect ethidium bromide/DNA complex, Agarose gels were examined on ultraviolet transilluminator (302nm wavelength) and photographed. Using 100pb Plus DNA ladder, ready-to-use (Gene Ruler, Fermentas, and Life Sciences), the lengths of the different DNA fragments were determined. For each sample, the reproducible DNA bands from two runs were scored for their presence or absence.

Simple Sequence Repeats (SSRs), also known as microsatellites, are repeating sequences of nucleotides, such as (AC)n. They have been found in abundance on the majority of eukaryotic chromosomes and are often highly polymorphic (Rafalski and Tingey 1993).Five SSR markers described (Kong *et al* 2000) were used for genotyping assays. Primers names, sequences and corresponding annealing temperatures are listed in Table (2).

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 (Siugh 1994).

Table 2: Sequences of the SSR loci and annealing temperature for PCR reaction used in the current study

Locus	Sequence of forward and reveres primers	Annealing Tem.
Wmc661	F: CCACCATGGTGCTAATAGTGTC R: AGCTCGTAACGTAATGCAACTG	61
Xtxp-8	F: ACAT CTACT AC CCT CTCACC R: ACACATCGAGACCAGTTG	50
Xtxp-10	F: ATACTATCAAGAGGGGAGC R; AGTACTAGCCACACGTCAC	50
Xtxp-12	F: ATAT GGAAGGAAGAAGC C GG R: AACACAACAT GCAC GCAT G	55
Xtxp-19	F: ATACTATCAAGAGGGGAGC R: AGTACTAGCCACACGTCAC	55

Results and Discussion

A- Morphological markers

Results in Figure1 indicated high significant variations among the Egyptian and Yemeni wheat cultivars in the morphological characteristics. The Yemeni wheat cultivars were higher in seedling length(cm) with range 15.05 to 18.95 cm. Y4 was the highest one of by average 18.95cm, followed by Y5 in average 18.68 18.68 cm, while Y2was the shortest one (15.04 cm).Concerning to the Egyptian wheat data in Figure 1 indicated significant values between the E1, E2 and the other cultivars. The highest seedling length was recorded to E2 (15.67cm) followed by E1 (13.90cm). No significant variations were observed between E3, E4 and E5.The range between Egyptian and Yemeni wheat cultivars in seedling length ranged from 18.95 cm (Y4) to 13.28 cm (E3) by value = 5.67 ~ increasing 35% and between the shortest cultivars ranged from 15.05 (Y2) to 13.28 (E3) in increase by ~ 10% (Figure 1).

Results in Figure1 indicated high significant variations among all studied wheat cultivars in root length (cm). The E1 wheat cultivar recorded the highest value (4.29 cm) followed by Y1 in average 4.09 cm, while Y2 and E4 was the shortest in average 2.56 and 2.56 cm, in respect. Analysis of variance showed high significant variation between all the Yemeni wheat in relation to root length by $L.S.D._{.0.05}$= 0.163, and also data indicated that no significant variation was observed among Y3 and Y4. The highest seedling length was recorded to E1 in average 4.29 cm followed by E2 (3.50cm). No significant variations were observed between E3 and E5.The range between Egyptian and Yemeni wheat cultivars in root length ranged from 4.29 cm (E1) to 2.56 cm (Y3) by value = 1.9 ~ increasing 40%

Finally, all the wheat cultivars showed decrease in root length with increase the salt levels until 200 mM salt as shown in Figure 1 with 200 Mm salt E1 and Y1 showed the highest root length were 4.33 and 3.83 cm, in respect. While E5 showed the lowest value was 2.33 cm. Data in Table 4 showed significant variation between the salt levels with $L.S.D._{.0.05}$= 0.103. Results in Figure 1 indicated no significant variations were observed among all the studied wheat cultivars in number of leaves/seedling except with Y4. The Y4 wheat cultivar recorded the highest value (2.44).

Analysis of variance in Table 5 showed significant variation between the Yemeni wheat in relation to number of leaves/seedling by $L.S.D._{.0.05}$= 0.218, and also data indicated that no significant variation was observed among Y3 and Y4. Concerning to the Egyptian wheat data indicated no significant values between all the cultivars.

Results in Figure 1 indicated significant variations were observed among all the studied wheat cultivars in number of roots/seedling. The Y2 wheat cultivar recorded the highest value (7.01). Analysis of variance in Table 6 showed significant variation between the Yemeni and Egyptian wheat in relation to number of roots/seedling by $L.S.D._{.0.05}$= 0.199, and also data indicated that no significant variation was observed among Y1 and Y2. Concerning to the Egyptian wheat data in Table 6 indicated no significant values between E1and E2 also between E4 and E5 cultivars.

Our data are consonant to the results of Hamada (1996) which assessed 13 *Aegilops* and 3 wild *Triticum* originally Turkish species by using morphological, pathological, qualitative and agricultural traits. As it was determined by the author, plant height might vary from 16.6 (*Aegilops juvenalis*) to 112.0 cm (*Aegilops mutica*), while spike length - from 2.4 (*Aegilops ovata*) to 23.3 cm (*Aegilops mutica*). Our result is agreed with Singh (1994) used 12 yield parameters and 5 morphological traits of spring wheat to evaluate genetic divergence

among 19 durum wheat genotypes. These genotypes were subsequently classified into 7 separate clusters revealing high level of genetic divergence independent of original harvesting place. The present work is agreement with Hu and Schmidhalter (1998) showed that wheat growing in 120 mM NaCl reacted with a 25% reduction in growth rate, Na^+ in the growing cells of leaves was at maximum only 20 mM, and Cl^- only 60 mM. However, Ball (1988) found that the common decrease in leaf expansion is not related to a loss in turgor pressure and is most likely a result of a change in hormonal signaling from roots to leaves In the salt-sensitive genotypes, in which salt is not effectively excluded from the transpiration stream, salt will build up to toxic levels in the leaves, resulting in death of old leaves and new leaves becoming injured and succulent (Munns and James, 2003).

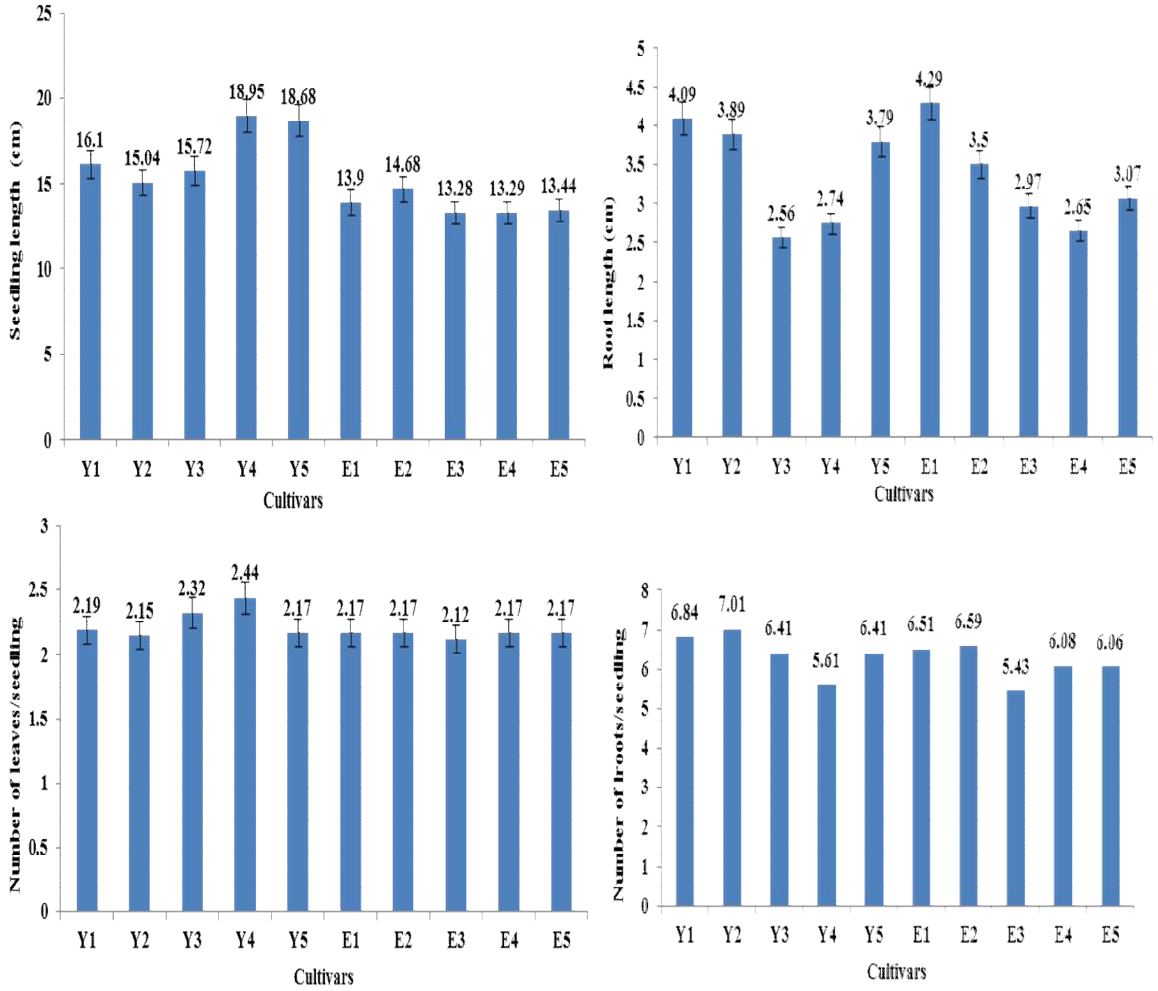

Fig. 1: General mean of morphological characters in Egyptian and Yemeni wheat varieties as affected by four different salinity concentrations and their interaction during season 2014 – 2015.

Our results are agreement with Ashraf *et al.* (1986) reported that root length can be used as selection criteria under salinity stress. Heritability estimates were low under 200 and 250 mM NaCl stress indicating that improvement in root length is difficult under stress conditions. Our results are agreement with Hasegawa *et al.,*(2000) reported that roots play a number of important roles during plant growth and development and are typically the first part of the plant to encounter salinity. In glycophytes, the root is the primary site of salt stress and the ability to maintain ion homeostasis and redox potential is critical for the normal root growth and function under saline stress.

Biochemical markers

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. In contrast, as shown in Figure 2, Peroxidase isozymes exhibited a wide range of variability among the different species at different localities. In control two cathodal (Pex.1cand pex 2a) was found as common band for all the samples. While (pex 2c) was unique for Yemeni wheat and (pex 3c) was unique for Egyptian wheat cultivars. The results detected one anodal (pex.a1) were found as common band for all the samples. In contrast, as shown in Figure 2 the treatment of 50Mm salt results detected that one cathodal (Pex.1c) were found as common band for all the samples. While the Egyptian wheat cultivars give two bands in cathodal (Pex.2c and Pex.4c), on the other hand Yemeni wheat cultivars give one band in cathodal (pex.3c),

In treatment 150Mm salt one cathodal (Pex.1c) were found as common band for all the samples. While Egyptian wheat cultivars gives two bands in cathodal (Pex.2c and pex.4c), while Yemeni wheat cultivars give one band in cathodal (pex.3c), the results detected tow anodal (pex.a1.pex.a2) were found as common band for all the samples.

In Figure 2 with 200 Mm salt two anodal (Pex.1Aand Pex.2A) were found as common band for all the wheat samples. While (pex.1c) at cathodal was as common band for all the wheat samples. The Egyptian wheat give two band in cathodal (Pex.2c and Pex.4c). Our results in a line with Hassanein, (1999) reported that the results showed that band number was exhibited in salt untreated and treated plants of all cultivars. This band was manifested higher densities and intensities in the salt treated cultivars than grown under control conditions. These results indicated that salt stress increased the accumulation of the esterase enzyme and that encoding gene(s) which accelerated in response to salt stress. Salinity increase esterase isozymes, the highest number of esterase isozymes were detected under the highest NaCl concentration.

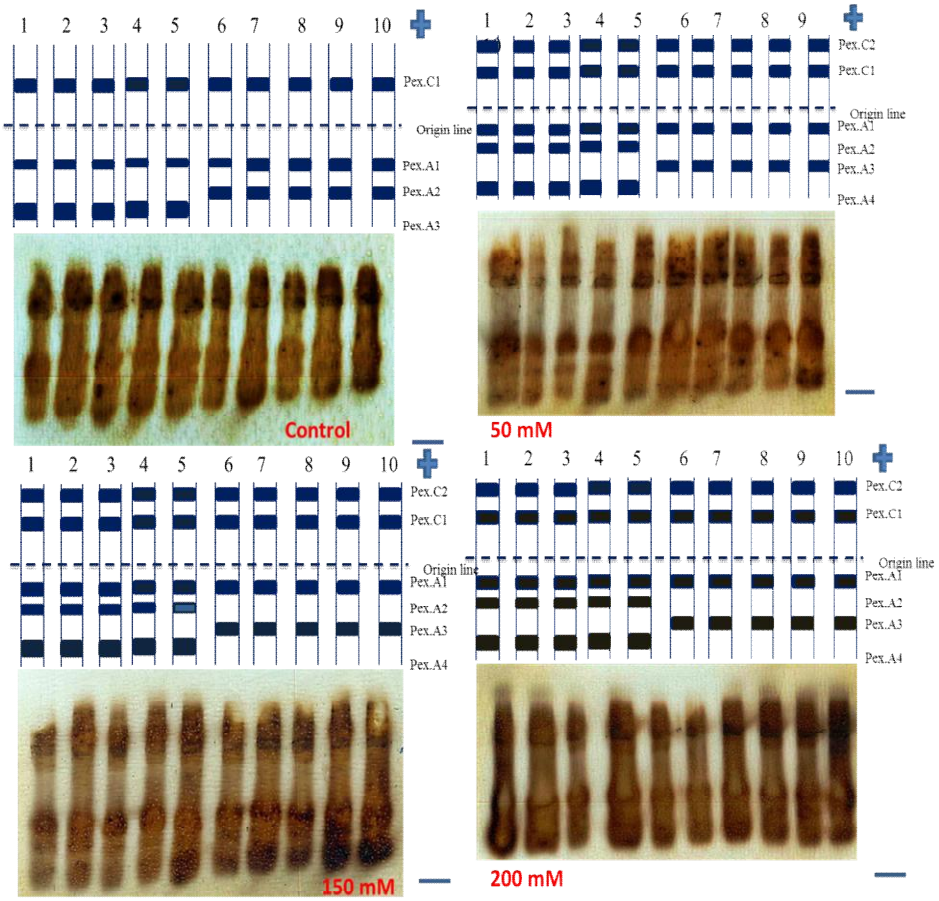

Fig. 2: Zymograme of peroxidase isozyme of Egyptian and Yemani wheat in control, 50, 150 and 200mM treatment (1) Shakha93, (2) Geiza168 (E2) , (3) Gammeiza9, (4) Shakha94, (5) Egypt1, (6) Behoth14, (7) Sonalica, (8) Acsadgahran, (9) Kaaalhakl and (10) Local wheat.

Molecular markers

In the present study ten random primers were used to differentiate through RAPD analysis among tested samples wheat (*Triticum aestivum* L.). Data in Table 3, 4 and Figure 3 showed the total unique fragments in all the 10 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. A total of 75 bands were detected among the studied genotypes. 53 bands showed polymorphism. Out of these polymorphic bands, 13 unique bands were scored and the number of monomorphic bands was 9 Table 37.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. The range of DNA size was between 138 and 1825 bp. In the present study, in Table 3, 4, ten wheat cultivars were studied using SSR and RAPD markers. 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 cored 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, 4 and Figure 3.

Table 3: Genotype specific RAPD markers of the ten cultivars

Species	Primer code	Positive unique markers	Negative unique markers	Total unique markers/species
1	OPN-04	-	-	-
2	OPD-05	1096	-	1
3	OPC-05	582-292-275	-	3
4	OPM-05	608-290	-	2
5	OPB-07	-	-	-
6	OPN-10	-	-	-
7	OPG-12	277-188	-	2
8	OPQ-12	199	-	1
9	OPN-13	487	-	1
10	OPQ-14	417-306-213	-	3
Total	-	13	-	13

Table 4: 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	Unique	Polymorphism %
OPN-04	5	2	3	0	60
OPD-05	8	1	6	1	88
OPC-05	7	0	4	3	100
OPM-05	14	0	12	2	100
OPB-07	5	1	4	0	80
OPN-10	6	0	6	0	100
OPG-12	8	0	6	2	100
OPQ-12	8	2	5	1	75
OPN-13	5	2	2	1	60
OPQ-14	9	1	5	3	89
Total	75	9	53	13	
Average polymorphism %					85.2

A total of 11 bands were detected among the studied genotypes. 8 bands showed polymorphism. Out of these polymorphic bands, 3 bands were monomorphic. However, the primer wmc 661and primer xtxp19 shows 100% polymorphism. While primer XTXP8, XTXP10, and XTXP12 showed 50 % polymorphism, respectively. The range of DNA size was between 84bp in primer wmc 661 to 254 bp in primer XTXP12 as Table 5

Table 5: Number of alleles, fragment size range and polymorphism detected by SSR loci in the ten wheat genotypes

Primer	Fragment size (bp)	Number of alleles	Monomorphic bands	Polymorphic bands	Polymorphic bands%
wmc661	84-101	2	0	2	100
xtxp8	140-244	2	1	1	50
xtxp10	272	1	1	0	0
xtxp12	221-254	2	1	1	50
xtxp19	143-253	3	0	3	100

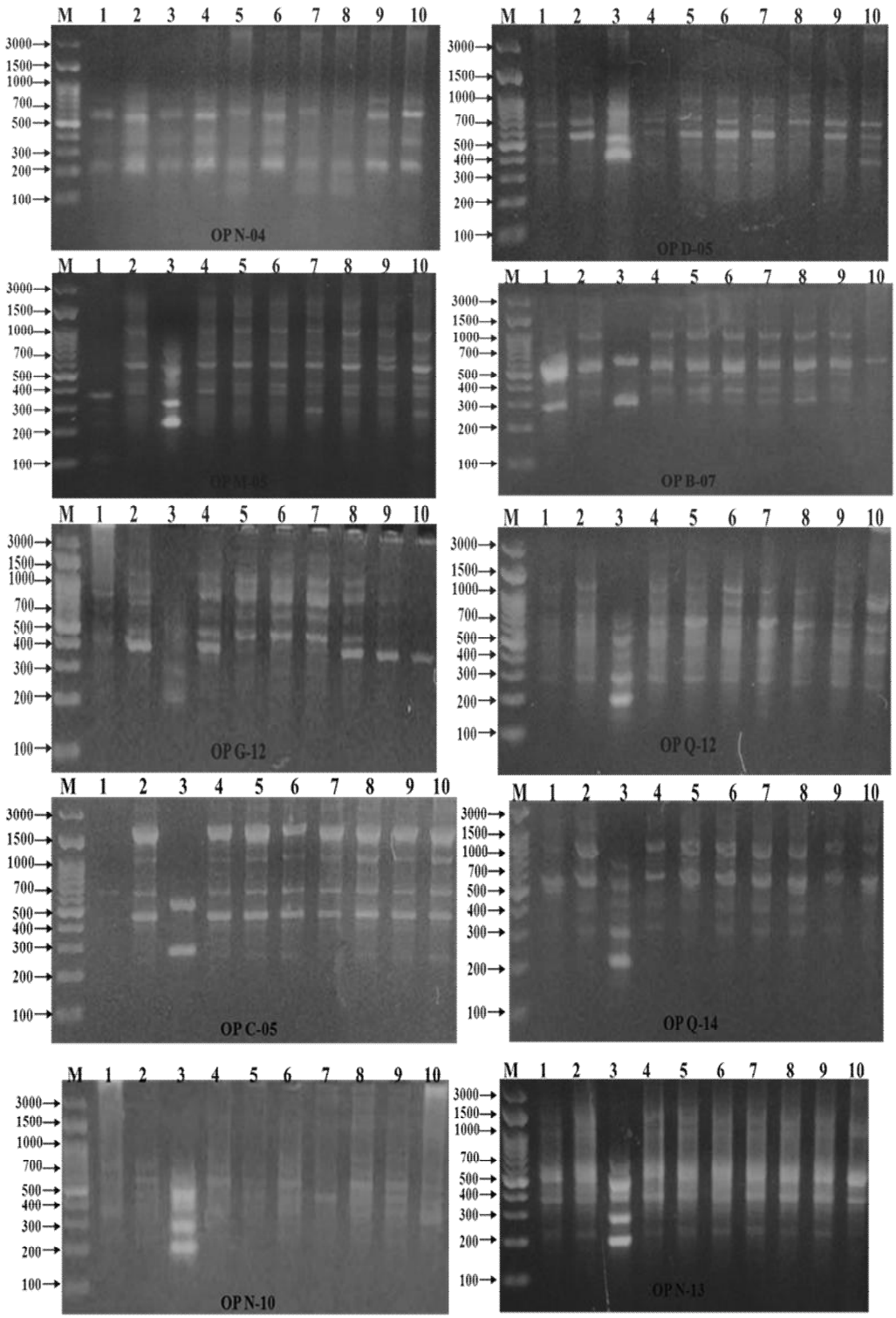

Fig. 3: DNA polymorphism of the Egyptian and Yemani wheat cultivars using RAPD DNA (1) Shakha93, (2) Geiza168 (E2), (3) Gammeiza9, (4) Shakha94, (5) Egypt1, (6) Behoth14, (7) Sonalica, (8) Acsadgahran, (9) Kaaalhakl, and (10) Local wheat.

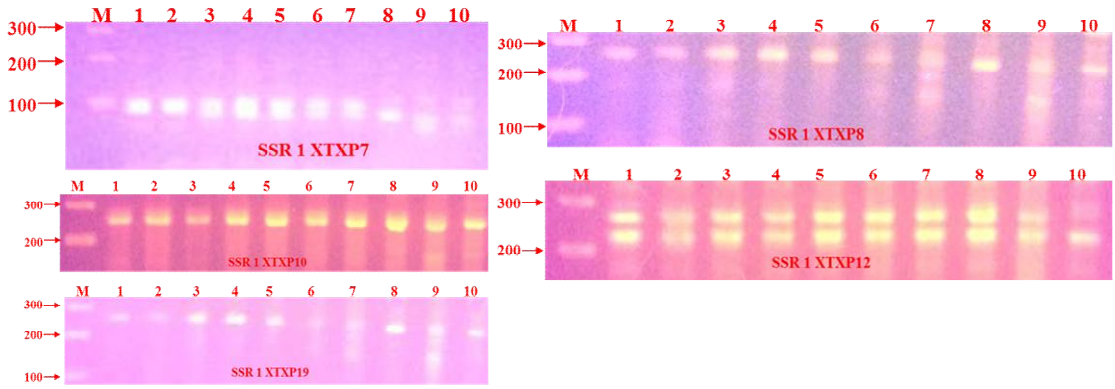

Fig. 4: DNA polymorphism of the Egyptian and Yemani wheat cultivars using SSR markers (1) Shakha93, (2) Geiza168 (E2), (3) Gammeiza9, (4) Shakha94, (5) Egypt1, (6) Behoth14, (7) Sonalica, (8) Acsadgahran, (9) Kaaalhakl, and (10) Local wheat.

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