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MOLECULAR AND MORPHO-ANATOMICAL CHARACTERIZATION OF SOME EGYPTIAN DURUM WHEAT CULTIVARS/LINES

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Abstract

Grains of eight durum wheat cultivars were tested for identification of genetic relationship among molecular, anatomical and morphological levels. On the molecular level, two techniques have been used; Randomly Amplified Polymorphic DNA (RAPD) and Inter Simple Sequence Repeats (ISSR). Amplification of RAPD primers showed different numbers of fragments ranged from six to thirteen fragments. Percentage of polymorphism ranged from 0% to 100%. The highest similarity value recorded was 91%, while the lowest similarity value was 69%. Amplification of ISSR primers showed different numbers of fragments ranged from six to twelve fragments. The highest similarity value recorded was 91%, while the lowest similarity value was 68%. The grain's coat morphology was reticulated in all taxa. There were variations with regard to the alignment and the shape of network and architecture of interspaces enclosed by raised line. Reticulate surface patterns appeared some variations ranged from weakly reticulate such as G 413 to strongly reticulate such as G 203. Stem cuticles of all cultivars were thick except cultivar; Benisweif 1. For leaf anatomy, all cultivars had epidermis composed of one layer of thick wall cells except cultivars; G 203 and Benisweif 1.

Key words: Durum wheat, RAPD, ISSR, Morphology, Anatomy.

Introduction

Wheat is the most widely grown cereal in the world (Anon., 2003). It is the staple food for 35% of the world's population, and is becoming increasingly important in the developing world (Cimmyt, 2003).The estimation of genetic diversity at the DNA level improves the identification and characterization of primary and secondary centers of diversity (Serret *et al.,* 1997; Chowdhury & Slinkard, 2000). Knowledge of diversity patterns will also allow breeders to better understand the evolutionary relationships among accessions, to sample germplasm in a more systematic fashion, and to develop strategies to incorporate useful diversity in their breeding programs (Bretting & Widrlechner, 1995). Zafar *et al.* (2015) reported that study of genetic patternson soybean can help its breeders to make better plan for selecting germplasm from wide sources for a specific purposes. Using molecular marker in wheat cultivar characterization now became more stable and accurate than morphological and cytogenetic traits which at present are unstable, time-consuming, and affected by environmental conditions, (Boggini *et al.,* 1990). Using the RAPD method for detection of polymorphism among wild and cultivated tetraploid wheat and genetic diversity is very important in reducing genetic vulnerability during plant breeding efforts (Joshi & Nguyen, 1993). In order to estimate the genetic diversity, molecular markers provided excellent tools (Sofalian *et al.,* 2008). Inter simple sequence repeats (ISSRs) are one of the DNA-based markers that have become widely used in various areas of plant research (Karaca & Izbirak, 2008). ISSR technique exploits the abundant and random distribution of SSRs in plant genomes by amplifying DNA sequences between closely linked simple sequence repeats (SSRs). ISSR technique has been widely used in studies of cultivar identification, genetic mapping, genetic diversity, evolution and molecular ecology (Yang *et al.,* 1996). ISSR markers provided sufficient polymorphism and reproducible fingerprinting profiles for evaluating genetic diversity in combination with agronomical and morphological traits (Najaphy *et al*., 2012). El-Assal & Gaber, (2012) evaluated eleven wheat cultivars collected from Egypt and Saudi Arabia using RAPD, SSR and ISSR markers. They investigated the discriminating capacity and effectiveness of these markers in establishing genetic relationship and diversity of these landraces. The dendrogram cluster diagram classified the evaluated genotypes in three major clusters corresponding to the cultivation regions. ISSR markers could be efficiently used to evaluate genetic variations in the wheat germplasm, genetic similarity and dissimilarity among genotypes. ISSR is a useful technique for genetic differentiation of wheat accessions, selection strategies and genetic development of crop plants (Sofalian *et al.,* 2008, 2009). ISSRs markers used for genetic diversity analysis of some wheat varieties and no statistically significant differences were found between genetic diversity parameters of durum and bread wheat, most cultivars belonging to the same botanical variety were clustered in the same main group, however intra-variety ISSR polymorphism was also observed, (Carvalho *et al*., 2008a, 2008b, 2009). The cluster analysis tree using ISSR markers placed tested genotypes in groups. The known origin and the genetic relationships estimated by this polymorphism revealed greater level of genetic variability in Indian bread wheat varieties of wide adaptability and applicability (Chowdhury *et al*., 2008). The efficiency of ISSR markers is very high and two primers were sufficient to distinguish some examined durum wheat cultivars (Pasqualone *et al*., 2000). The genetic relationships of wheat accessions estimated by the polymorphism of ISSR markers were identical with those inferred by restriction fragment length polymorphism (RFLP) and RAPD markers, indicating the reliability of ISSR markers for estimation of genotypes (Nagaoka & Ogihara, 1997). ISSR markers succeeded in distinguishing most of 20 hexaploid, tetraploid and diploid genotypes of wheat (Abou-Deif *et al.,* 2013).

Morphological characterization and evaluation of the diversity of wheat resources & landraces has been extensively studied around the world (Buerkert *et al.*, 2006; Dotlacil *et al.*, 2002; Al-Maskri *et al.*, 2003). Anatomical parameters may play an important role in plant taxonomy (Metcalfe & Chalk, 1957). Anatomical characters have proved to be more useful for delimitation of higher taxonomic ranks, such as genera and families. There are a large number of examples where anatomical parameters have contributed in solving significant taxonomic problems within different taxonomic groups (Carlquist, 1996; Carlsward *et al.*, 1997; Colombo & Spadaro, 2003; Scatena *et al.*, 2005; Satil & Selvi, 2007; Matias *et al.*, 2007; Schweingruber, 2007; Erxu *et al.*, 2009; De la Estrella *et al.*, 2009; Zarrei *et al.*, 2010). Stem mechanical strength is an important characteristic of cereal breeding. The flattening of cereal crops, known as lodging, can cause large reductions in grain yield and quality (Berry *et al.*, 1998). The principal method to minimize growers lodging is through the use of high mechanical strength cultivars. Therefore, high mechanical stem strength is an object in wheat breeding. There are many reports concerning stem mechanical properties related to lodging resistance. Most of the studies have mainly focused on morphological and structural features of stem (Wang & Hu, 1991; Tripathi *et al.*, 2003; Wang & Li, 1997, 1998; Crook & Ennos, 1995), physiological and developmental mechanisms of stem strength (Tripathi *et al.*, 2003), and measurement technology (Berry *et al.*, 2003; Kashiwagi & Ishimaru, 2004). However, some information on the relationship of cell wall components and mechanical properties of stems is available (Li *et al.*, 2003). The importance of grain morphology for classification has long been recognized (Matias & Soares, 2009). Echlin, (1968) firstly used Scanning Electron Microscopy (SEM) photographs of grains of Arenaria, revealing the grain micro sculpturing without taxonomic comments. Since then, SEM pictures were used in systematic studies on different genera such as Sagina (Crow, 1979), Arenaria (Wofford, 1981; Wyatt, 1984), Silene (Melzheimer, 1977; Greuter, 1995; Oxelman, 1995; Hong *et al.*, 1999). To meet the demand for high yielding and stress-resistant wheat cultivars, it is desirable to increase the genetic base of this crop. Traditionally, germplasm has been characterized based on agronomic and morphological studies, but recently the use of molecular markers to study diversity within domesticated species has become common.

The objective of this study is to investigate the genetic differences between some genotypes of Egyptian durum wheat using molecular and traditional techniques.

Materials and Methods

Grains of 8 durum wheat (*Triticum durum* L.) cultivars; (Giza 203, Giza 409, Giza 413, Giza 823, Beniswif 1, Beniswif 2, Sohag 1 and Sohag 2) were obtained from the Agricultural Research Center (ARC), Giza, Egypt.

Molecular identification

Genomic DNA extractions: DNeasy™ Plant Mini Kit (Qiagen Inc., cat. no. 69104) was used for DNA isolation from the leaves of the eight wheat cultivars.

Randomly amplified polymorphic DNA analysis (RAPD): RAPD reactions were conducted according to the method of Michelmore *et al.* (1991) using ten random *10-mer* primers from Operon Technology (USA). Their codes, sequences and GC % are shown in Table 1.

The amplification was carried for as follows: 4 min at 94°C/ for denaturation; followed by 40 cycles of 1 min at 94°C/, 1 min at 36°C/, 2 min at 72°C/; finally; l0 min at 72°C/ for extension. PCR products were migrated on agarose (1.2%) according to Sambrook *et al*. (1989).

Inter-simple sequence repeats (ISSRs) amplification analysis: An alternative method to SSRs, called inter-SSR (ISSR), was used according to Zietkiewicz *et al.* (1994) using seven primers. The thermal cycler was programmed for three main steps as follows: 94°C /4 min. fordenaturation, followed by 40 cycles of 1 min at 94°C/, 1 min at 55°C/, 1 min at 72°C/; finally; l0 min at 72°C/ for extension. PCR products were migrated on agarose (1.2%) according to Sambrook *et al.* (1989).

Table 2 shows the codes of these primers, their sequences and GC%.

Data analysis: DNA fragments were detected and photographed using Gel-Documentation 2000, Bio-Rad ™ apparatus and analyzed by diversity database V.2.1.1. Cluster analysis based on RAPD and ISSRs were carried out using UPGMA computer program.

Table 2. Codes, sequences and GC% for the seven primers used in ISSRs analysis.

No.	Primer	Sequences (5'→3')	GC %
1.	HB-09	GTG TGT GTG TGT GC	57%
2.	HB10	GAGAGAGAGAGACC	57%
3.	HB11	GTGTGTGTGTGTCC	57%
4.	HB12	CAC CACCAC GC	73%
5.	HB13	GAG GAGGAG GC	73%
6.	HB14	CTCCTCCTCGC	73%
7.	HB15	GTGGTGGTGGC	73%

Table 1. Primer names, their sequences and GC% used for RAPD analysis.

No.	Primer name	Sequences (5'→3')	GC %	No.	Primer name	Sequences (5'→3')	GC %
1.	OP-A07	GTAACCAGCC	60%	6.	OP-C11	AAAGCTGCGG	60%
2.	OP-A09	GGGTAACGCC	70%	7.	OP-C18	TGAGTGGGTG	60%
3.	OP-A10	GTGATCGCAG	60%	8.	OP-D02	GGACCCAACC	70%
4.	OP-B07	GGTGACGCAG	70%	9.	OP-F04	GGTGATCAGG	70%
5.	OP-B12	CCTTGACGCA	60%	10.	OP-F08	GGGATATCGG	60%

Grains micro morphology and stem & leaf anatomical analyses

Grains micromorphology: Grains surface detailed scan attributes features were examined by Scanning Electronic Microscope (SEM) on copper stubs and coated with thin layer of gold in polaron E5000 sputter coater then examined by Joel-SMT330 SEM at an acceleration voltage of 20 kV. The magnification power was 50 µm for all photographs.

Anatomical analysis of stem and leaf: The segments of the organs from the middle part of fresh plants were separated, fixed and preserved in F.A.A (formalin 37% formaldehyde: 10m, glacial acetic acid: 5ml, ethanol 95%: 50 ml and distilled water: 35ml) for 48 hour then transfer to ethanol 70%. Stem and leaves sectioned at 10-20 µm using sliding microtome. Safranin, 2% and light green, 1% were used for double staining. Stem and leaves section examined under a light microscope (Olympus, BH2 REC, Tokyo, Japan) equipped with a digital camera (JVC, TK1280E, Japan) and an image analyzing system (Leica, Qwin, Cambridge, UK). The magnification power was 100 µm for all photographs.

Results

Molecular identification of durum wheat cultivars

Randomly amplified polymorphic DNA (RAPD) analysis: PCR-based methods using arbitrary primers have been widely used as fingerprinting techniques. Among these techniques, RAPD is a reliable and very useful method for cultivar identification and genomic analysis. In this study, ten arbitrary *10-mer*oligo-nucleotide primers were used to amplify the genomic DNA from the eight durum wheat cultivars.

Amplification of RAPD primers showed different numbers of fragments ranged from six fragments (primers; OP- A10 and OP- C11), seven fragments (primer; OP- C18), nine fragments (primers; OP-A07, OP- A09, and OP- D02), eleven fragments (primers; OP- B07 and OP- B12), twelve fragments (primer; OP- F08) and thirteen fragments (primer OP- F04). All these data are shown in Fig. 1.

Percentage of polymorphism ranged from 0% (primer; OP- A10), 36.4% (primer; OP- B07), 44.4% (primer; OP- A09), 50% (primer; OP- F08), 66.7% (primer; OP- D02), 69.2% (primer; OP- F04), 77.8% (primer; OP- A07), 81.8% (primer; OP- B12) and 100% (primers; OP- C11 and OP- C18).

The results of the amplified fragments using RAPD method with ten arbitrary *10-mer* primers for the eight durum wheat cultivars are presented in Table 3. The number of total amplified fragments (TFA), polymorphic fragments (PF) for each primer, amplified fragments (AF) and specific marker (SM) for each genotype are shown in Table 3.

Genetic similarity and cluster analysis based on RAPD markers: The results of cluster analysis (Similarity Index) based on RAPD analysis are shown in Table 4. The highest similarity value recorded was 91% which was observed between Beniswif 2 and Sohag 1, while the lowest similarity value (69%) was recorded between Giza 203 and Sohag 2. A dendrogram for the genetic relationships via RAPD analysis among the eight durum wheat cultivars results were carried out and are shown in Fig. 2. The eight durum wheat cultivars were separated into two clusters; cluster 1 included Beniswif 2, Sohag 1, Beniswif 1 and Sohag 2 respectively, while cluster 2 comprised included Giza 413, Giza 824, Giza 409 and Giza 203 respectively.

Table 3. Number of amplified fragments and specific markers of the 8 durum wheat cultivars based on RAPD analysis.

Primers			Giza 203		Giza 409		Giza 413		Giza 823		Beniswif 1		Beniswif 2		Sohag 1		Sohag 2		
	TAF	PF	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	TSM
OP-A07	9	7	6	1 (-)	7	-	7	-	6	-	7	-	5	-	7	1(+)	4	-	3
OP-A09	9	4	6	1 (-)	9	1(+)	8	-	8	-	8	-	7	1 (-)	8	-	7	1 (-)	3
OP-A10	6	0	6	-	6	-	6	-	6	-	6	-	6	-	6	-	6	-	0
OP-B07	11	4	10	-	8	-	8	-	9	-	10	-	10	-	11	-	6	-	3
OP-B12	11	9	3	-	7	-	4	-	9	2(+)	2	-	5	-	9	-	5	-	2
OP-C11	6	6	3	-	3	-	4	-	4	-	3	-	2	1 (-)	3	-	2	-	1
OP-C18	7	7	4	1(+)	3	1(+) 1(-)	5	-	3	-	4	-	3	1 (-)	5	-	4	-	4
OP-D02	9	6	5	-	8	-	9	1(+)	7	-	6	-	5	-	6	-	4	-	1
OP-F04	13	9	6	-	7	-	8	-	10	-	10	1(+)	9	-	10	-	10	-	1
OP-F08	12	6	8	-	9	1 (+)	8	-	6	1 (-)	11	-	11	-	11	-	8	-	2
Total	93	58	57	3	67	4	67	1	68	3	67	1	63	3	76	1	56	1	20

TAF = Total amplified fragments, PF = Polymorphic fragment for each primer. AF = Amplified fragments. SM = Specific markers including either the presence or absence of a fragment. TSM = Total number of specific markers.

Table 4. Similarity matrix among the 8 durum wheat cultivars based on RAPD analysis.

	Giza 203	Giza 409	Giza413	Giza823	Beniswif 1	Beniswif 2	Sohag1
Giza 409	0.790						
Giza 413	0.780	0.857					
Giza 823	0.720	0.844	0.866				
Beniswif 1	0.790	0.791	0.842	0.815			
Beniswif 2	0.750	0.754	0.791	0.794	0.846		
Sohag 1	0.752	0.797	0.831	0.847	0.867	0.906	
Sohag 2	0.690	0.715	0.770	0.832	0.797	0.824	0.833

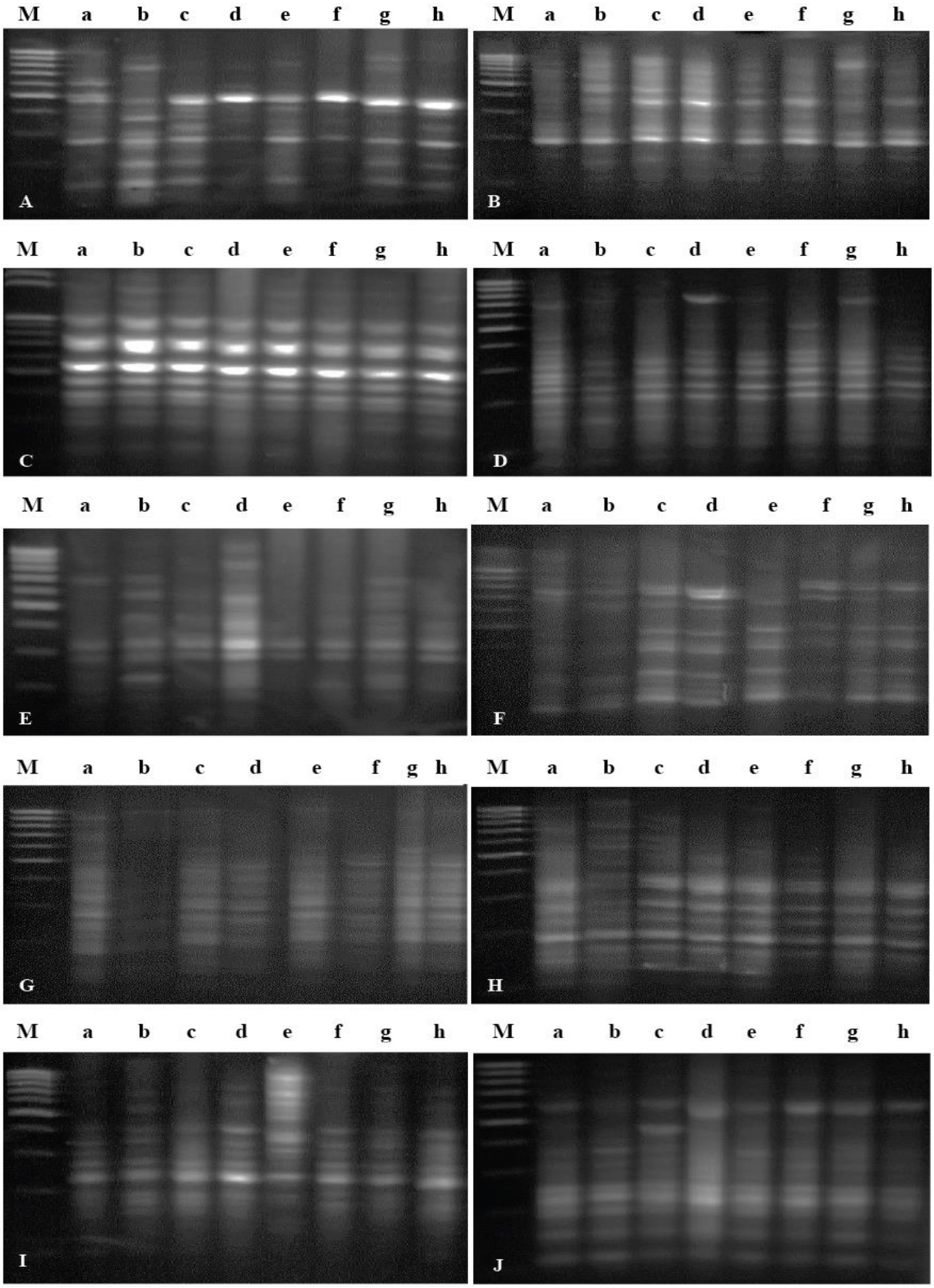

Fig. 1. DNA polymorphism using 10 primers for RAPD - PCR technique with the 8 durum wheat cultivars. Lanes a to h represent cultivars; a; Giza203, b; Giza409, c; Giza413, d; Giza823, e; Beniswif1, f; Beniswif2, g; Sohag1, h; Sohag2 and M = 100 bp DNA ladder. A= Primer; OP- A07, B= Primer; OP- A09, C= Primer; OP- A10, D= Primer; OP- B07, E= Primer; OP- B12, F= Primer; OP- C11, G= Primer; OP- C18, H= Primer; OP- D02, I= Primer; OP- F04, J= Primer; OP- F08.

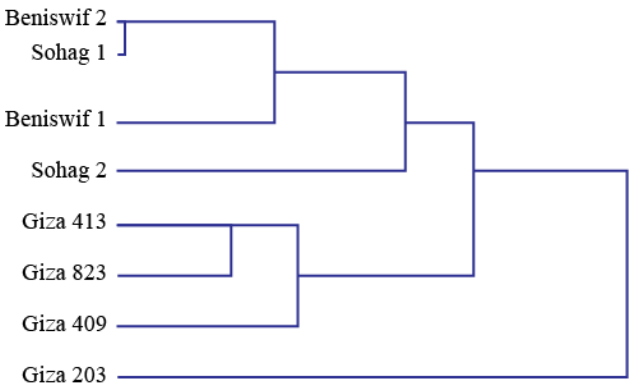

Fig. 2. Dendrogram for the genetic distances among the eight durum wheat cultivars based on similarity index data of RAPD analysis.

ISSR amplification analysis: Inter-simple sequence repeats (ISSRs) amplification is a technique which could be effectively used to quickly differentiate closely related individuals (Zietkiewicz *et al*., 1994). Seven ISSR primers were used in this study to characterize and identify the eight durum wheat cultivars (Fig. 3).

Amplification of ISSR primers showed different numbers of fragments ranged from six fragments (primer; HB- 13), eight fragments (primers; HB- 10 and HB- 14), nine fragments (primers; HB- 11and HB- 12) ten fragments (primer; HB- 15) and twelve fragments (primer; HB- 09).

The results of the amplified fragments using ISSR method with seven primers for the eight durum wheat cultivars are presented in Table 5. The number of total amplified fragments (TFA), polymorphic fragments (PF) for each primer, amplified fragments (AF) and specific marker (SM) for each genotype are shown in Table 5.

Genetic similarity and cluster analysis based on ISSRs markers: The results of the amplified fragments using ISSR method for eight durum wheat cultivars showed some specific markers. The results of cluster analysis (Similarity Index) based on ISSR analysis are shown in Table 6. The highest similarity value recorded was 92% which was observed between Sohag 1 and Sohag 2, while the lowest similarity value (68%) was recorded between Giza 409 and Sohag 2 & between Giza 203 and Sohag 1. A dendrogram for the genetic relationships via ISSR analysis among the eight durum wheat cultivars results were carried out and are shown in Fig. 4. The eight durum wheat cultivars were separated into two clusters; cluster 1 included Sohag 1, Sohag 2, Beniswif 2 and Beniswif 1 respectively, while cluster 2 comprised included Giza 824, Giza 409, Giza 413, and Giza 203 respectively.

Grains micromorphology, stem and leaf anatomical study

Grains micromorphology: The grains coat micromorphology is reticulate in all taxa, but there are variations with regard to the alignment and shape of network and architecture of interspaces enclosed by raised line (Fig. 5). The reticulate shape has a longitudinal projection along the grain coat surface. There are some variations inreticulate surface patterns. It may be strongly reticulate with the anticlinal walls raised highly above the level of the periclinal wall as for instance in cultivars; Giza 203, 409, 823 and Beniswif 1,2 orit may be weakly reticulate with the anticlinal walls raised only slightly above the level of the periclinal wall as in cultivars; Giza 413 and Sohag 1,2. The facets may also have varioustypes of secondary ornamentations in the form of small, wart-like protuberances (Giza 203).The reticulum was compact with thick wall in cultivars; Giza 409, Beniswif 2 and Sohag 1 or with thin wall in cultivars; Giza 203 and Beniswif 1 and Sohag 2.

Stem anatomy: Stem is an important organ consisting of storage, transportation and mechanical tissues and is closely related to yield. There is an irregular cavity in the stems. Stem is circular to oval in cross-section, with flat or ribbed margin (Fig. 6). Cuticles are thick, except cultivar; Beniswif 1which was thin. In cultivars; Giza 413 and Sohag 1, cuticles are much thick. Results showed that epidermis is composed of small cells with thickened cell walls in all cultivars except Giza 203 and Beniswif 1, which had thin wall epidermis. In cortex; parenchyma or collenchymas cells are arranged in six to ten rows, the area of cortex ranged from 0.9 to 3.5 ml. Cortex is composed of parenchyma in four cultivars; Giza 203,409 and Beniswif 1, 2 but it is composed of collenchymas in the other four cultivars; Giza 413, 823, and Sohag 1, 2. Collateral vascular bundles surrounded by fiber are arranged into two rows; Giza 409, 413, Beniswif 2 and Sohag 2 or three rows; Giza 203, 823, Beniswif 1 and Sohag 1. The number of bundles ranged from 54 to 78 bundles and central stem portion has large, thin-walled parenchyma cells. Table 7 summarizes all data of stem anatomy of eight durum wheat cultivars.

Leaf anatomy: Fig. 7 shows leaf anatomy which revealed that upper and lower cuticles are thick in both cultivars; Giza 203 and Giza 409 but very thick in other studied cultivars except both cultivars; Beniswif 2 and Sohag 1. In these two cultivars, the upper cuticle was thin and the lower was very thick. The epidermis composed of one layer of cells with thick wall in all cultivars except Giza 203, and Beniswif 1, which they hada thin cell wall. Palisade tissue was composed of two layers of cylindrical cells, while spongy tissue has two to three layers of cells, irregular in shape. Spongy tissue has two layers in cultivars; Giza 409, Giza 413, Beniswif 2, and Sohag 2, the sponge tissue of the rest cultivars have three layers. Collateral vascular bundles are arranged in a single row and surrounded by parenchymatous sheath cells. Larger vascular bundles have groups of sclerenchyma tissue on adaxial and abaxial sides. In parenchyma sheath cells, especially in sclerenchyma groups on abaxial side solitary. Table 8 summarizes all data of leaf anatomy of eight durum wheat cultivars.

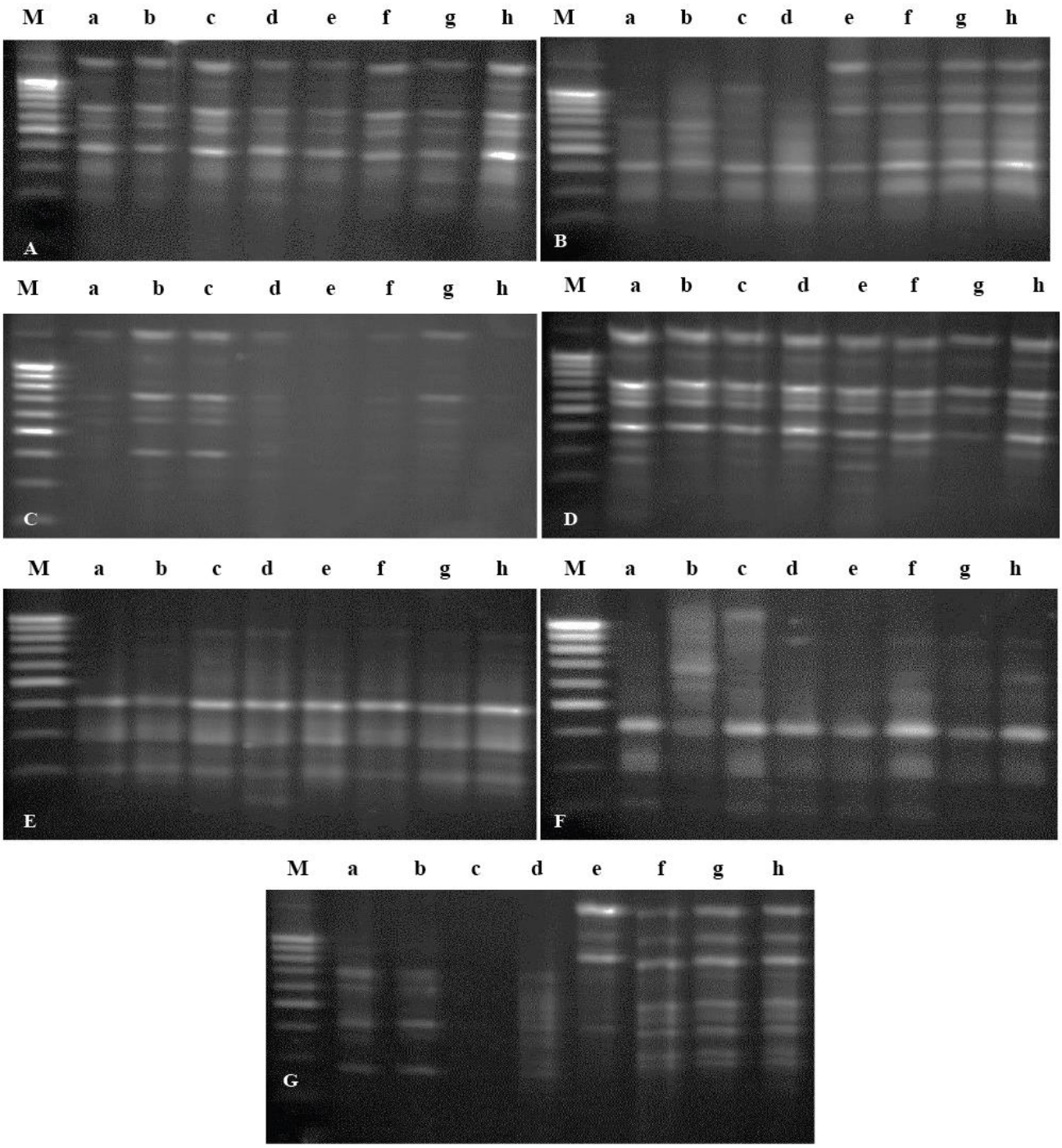

Fig. 3. DNA polymorphism using 7 primers for ISSR technique with the eight durum wheat cultivars. Lanes a to h represent cultivars; a; Giza 203, b; Giza 409, c; Giza 413, d; Giza 823, e; Beniswif 1, f; Beniswif 2, g; Sohag 1, h; Sohag 2 and M = 100 bp DNA ladder. A= Primer; HB09, B= Primer; HB10, C= Primer; HB11, D= Primer; HB12, E= Primer; HB13, F= Primer; HB14, G= Primer; HB15.

Table 5. Number of amplified fragments and specific markers of the 8 durum wheat cultivars based on ISSR analysis.

Primers			Giza 203		Giza 409		Giza 413		Giza 823		Beniswif 1		Beniswif 2		Sohag 1		Sohag 2		
	TAF	PF	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	TSM
HB-09	12	6	8	-	8	-	10	-	11	-	10	-	10	-	10	-	11	-	-
HB-10	8	6	4	1 (-)	5	-	5	-	5	-	7	-	6	-	6	-	7	-	1
HB-11	9	6	6	-	9	-	9	-	7	-	5	-	6	-	8	-	3	1 (-)	1
HB-12	9	3	9	-	8	-	9	-	8	-	9	-	7	-	7	1 (-)	8	-	1
HB-13	6	3	3	-	3	-	5	-	5	1(+)	4	-	4	-	5	-	5	-	1
HB-14	8	7	3	-	6	1 (-)	7	1(+)	5	-	4	-	6	1 (-)	4	-	4	-	2
HB-15	10	8	6	1(+)	5	1(+)	3	1 (-)	5	-	6	1 (-)	9	-	9	-	9	-	2
Total	62	39	39	2	44	2	48	2	46	1	45	1	48	1	49	1	47	1	8

TAF = Total amplified fragments. PF = Polymorphic fragment for each primer. AF = Amplified fragments. SM = Specific markers including either the presence or absence of a fragment. TSM = Total number of specific markers.

Table 6. Similarity matrix among the 8 durum wheat cultivars based on ISSR analysis.

	Giza 203	Giza 409	Giza 413	Giza 823	Beniswif 1	Beniswif 2	Sohag 1
Giza 409	0.867						
Giza 413	0.805	0.870					
Giza 823	0.824	0.822	0.830				
Beniswif 1	0.810	0.742	0.774	0.813			
Beniswif 2	0.736	0.739	0.771	0.851	0.860		
Sohag 1	0.682	0.710	0.784	0.821	0.830	0.907	
Sohag 2	0.721	0.681	0.737	0.839	0.891	0.905	0.917

Table 7. Stem anatomical structure ofeight durum wheat cultivars.

Characters / Cultivars	Cuticle		Epidermis		Cortex		Vascular bundles	
	Size(mm)	Structure	Size (mm)	Structure	Size (mm)	Structure	Number	Structure
Giza 203	0.1	Thick	0.1-0.3	Thin	0.9-2.0	Parenchyma	72	3 rows
Giza 409	0.15	Thick	0.2-0.4	Thick	2.0-3.5	Parenchyma	54	2 rows
Giza 413	0.1	Very thick	0.15-0.4	Thick	1.3-2.3	Chollenchyma	62	2 rows
Giza 823	0.15	Thick	0.15-0.5	Thick	1.5-2.5	Chollenchyma	56	3 rows
Beniswif 1	0.1	Thin	1.5-0.3	Thin	1.3-2.3	Parenchyma	62	3 rows
Beniswif 2	0.1	Thick	0.1-0.3	Thick	1.2-1.6	Parenchyma	58	2 rows
Sohag 1	0.1	Thick	0.15-0.5	Thick	1.7-3.0	Chollenchyma	78	3 rows
Sohag 2	0.15	Very thick	0.15-0.3	Thick	1.4-4.0	Chollenchyma	73	2 rows

Table 8. Leaf anatomical structure of eight durum wheat cultivars.

Characters / Cultivars	Cuticle		Epidermis		Palisade tissue		Sponge tissue	
	Size (mm)	Structure	Size (mm)	Structure	Size (mm)	Structure	Size (mm)	Structure
Giza 203	0.05-0.1	Thick	0.4-1.5	Thin	1.3-2	2 row	1.5-2	3 rows
Giza 409	0.05-0.1	Thick	0.2-0.4	Thick	1.5-2.5	2 row	2-3	2 rows
Giza 413	0.05-0.15	Very thick	0.3-1.5	Thick	1-2	2 row	2-3	2 rows
Giza 823	0.05-0.15	Very thick	0.2-1.1	Thick	1.5-2.3	2 row	2-2.5	3 rows
Beniswif 1	0.05-0.15	Very thick	0.3-0.5	Thin	1-2.5	2 row	3-4.5	3 rows
Beniswif 2	0.05-0.15	Upper thin, lower very thick	0.2-0.5	Thick	1-1.5	2 row	1-1.5	2 rows
Sohag 1	0.05-0.15	Upper thin, lower very thick	0.5-1.5	Thick	1.5-2.5	2 row	2-2.5	3 rows
Sohag 2	0.05-0.15	Very thick	0.2-1.1	Thick	2-2.5	2 row	2-2.5	2 rows

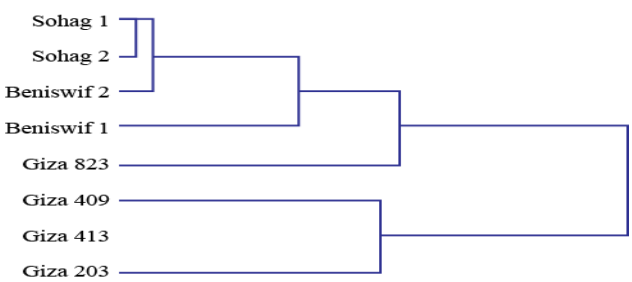

Fig. 4. Dendrogram for the genetic distances among the eight durum wheat cultivars based on similarity index data of ISSR analysis.

Discussion

The introduction of molecular markers in plant breeding has presented a valuable tool for the characterization of genetic materials (Aliyev *et al.,* 2007). However, there are genetic structure changes in the populations and genetic variations in the individuals at the molecular level (Yuhan *et al.,* 2015).The genetic similarity values calculated from RAPD markers were very similar to those calculated with RFLP markers for intra specific comparisons of 49 diploid wheat accessions (Castagna *et al.,* 1997). Knowledge of genetic diversity within as well as genetic relatedness among populations from different geographic areas is expected to have a significant impact on the conservation and utilization programs of emmer germplasm (Teklu *et al.,* 2007). Our data is in agreement with (Karaca & Izbirak, 2008) whose used 42 RAPD and 18 ISSR primers to characterize the genetic relationship among 25 durum wheat cultivars. Guasmi *et al.* (2012), reported that RAPD and ISSR techniques are very useful to assay the genetic diversity among 80 barley specimens and the percentage of polymorphism was 66.67%. In our data, similarity of ISSR ranged between 68% and 91% while it was 77% (Zamanianfard *et al.,* 2015) among 25 durum genotypes examined, 84.4% (Abou-Deif *et al.,* 2013) among 20 wheat cultivars and 83% (Shirnasabian *et al.,* 2014) via 18 durum wheat cultivars. The use of the Scanning Electron Microscopy (SEM) in the study of seeds has revealed a great variation in seed coat micromorphology and allowed the description of a number of morphological features, for these, comparison with seeds of closely related. The use of microscopic methods and anatomical characteristics can supply useful information to differentiate between cultivars. The importance of seed morphology for classification has long been recognized (Matias & Soares, 2009). Few SEM studies have been concerned with the fine structural differences in taxonomical and morphological features of closely related species, especially within groups of plants of the same species (Liu *et al.,* 2005; Joshi *et al.,* 2008).Also Minuto *et al.* (2006) used seed micromorphological characters to compare between taxa within the caryophyllaceae. While, Silva *et al.* (2012) used leaves epidermis to differentiate between ten *Solanum* species. Characters of the leaf, such as the epidermis, stomata and indumentums characters, have proved to be much more reliable for taxonomic considerations in many genera (Dickison, 2000; Yang & Lin, 2005; Strgulc-Krajsek *et al.,* 2006).The layers of each cross section of leaf mesophyll differed according to the taxa (Gowayed, 2003).

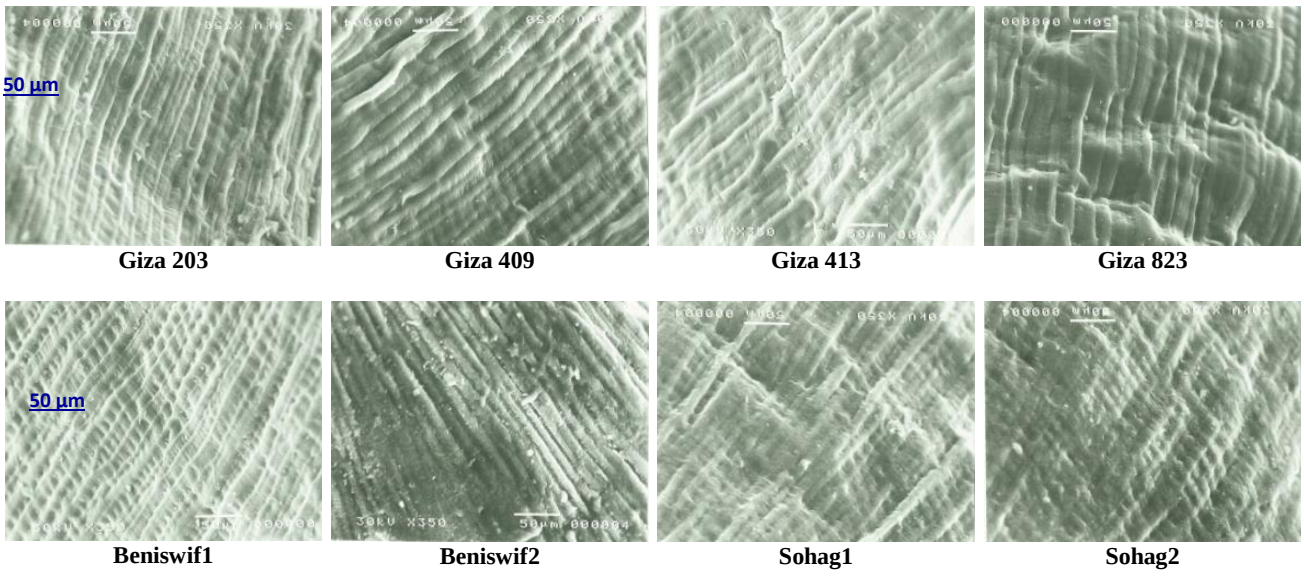

Fig. 5. SEM of grains surface micro morphology of eight durum wheat cultivars.

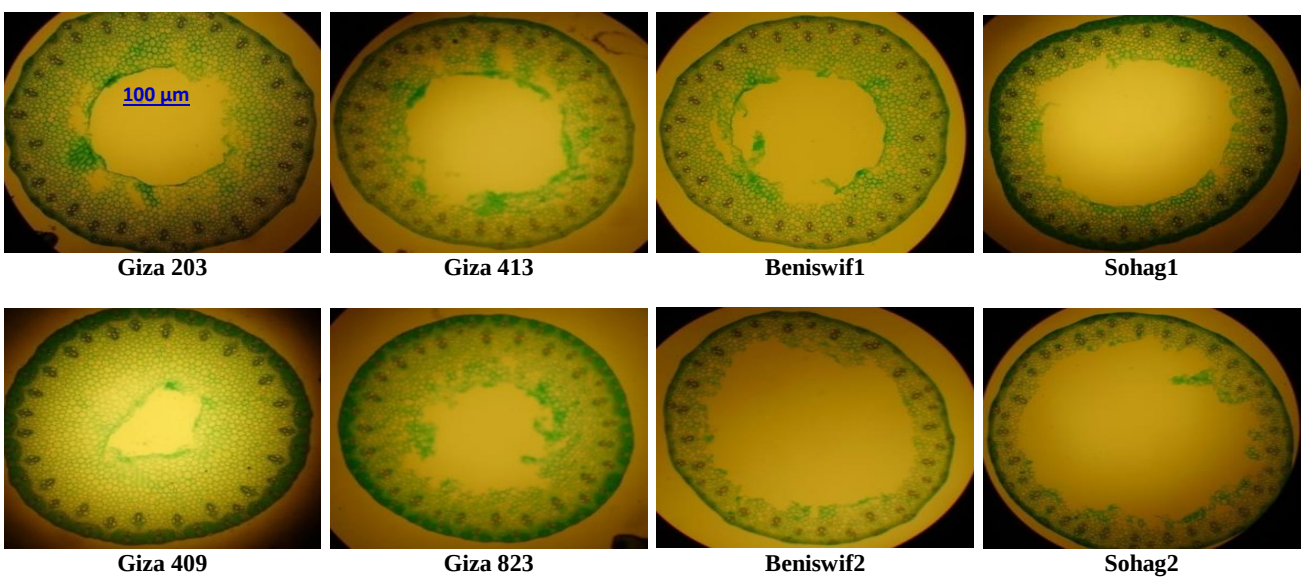

Fig. 6. Light micrographs of stem cross section of eight durum wheat cultivars.

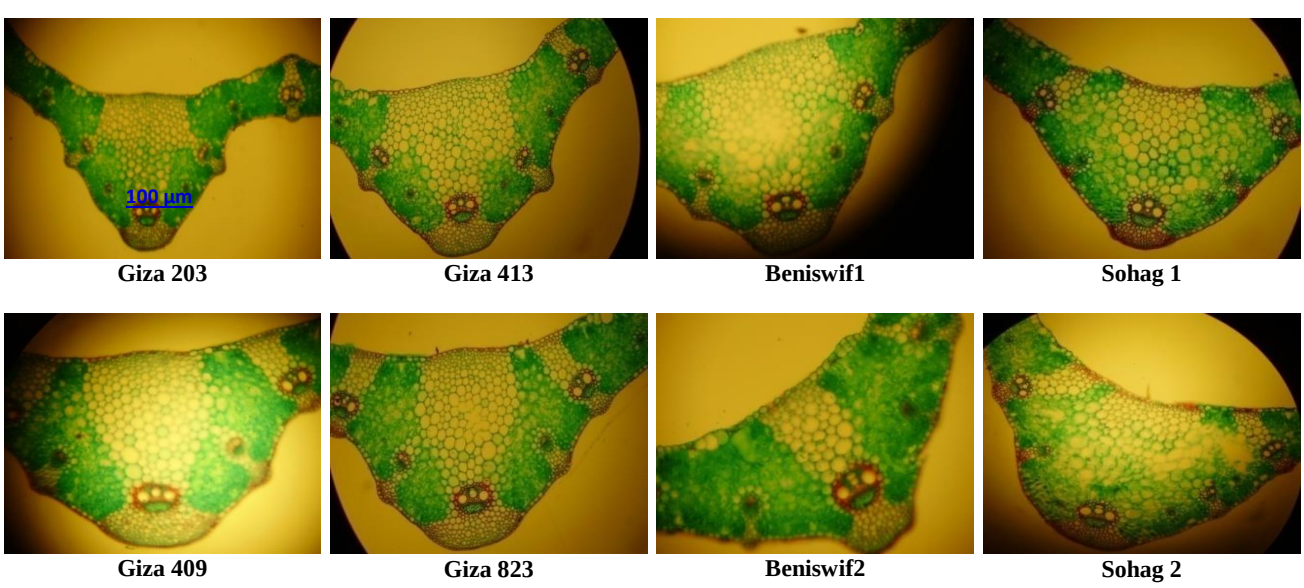

Fig. 7. Light micrographs of leaf anatomy of eight durum wheat cultivars.

Conclusion

Detection of genetic relationship is very useful for breeders to know the best relation between cultivars for breeding programs to obtain the best hybrid with improved characters specially yield to cover increased consumption all over the world. Phylogenetic relationships detected the percentage of similarity between cultivars and these molecular results supported by morphological and anatomical results. Data from phylogeny suggests the possible hybridizations may be done between different Egyptian durum cultivars.

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