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Taxonomic and molecular study on some Asian cultivars of *Triticum aestivum* L.

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Abstract

Wheat (*Triticum aestivum* L.) is one of the most vital food crops in the world. The differentiation of wheat cultivars is essential for farmers and breeders to evaluate the best quality and yield. Eight *T. aestivum* Asian cultivars were dealt with. The objectives of the present study are to extract data from the grain surface structures by aid of SEM and to determine genetic similarity/relatedness using Start Codon Targeted (SCoT) markers. The obtained exomorphic characters of the grains were diagnostic as they facilitated the differentiation between the studied Asian cultivars. Thirty primers were used and generated a total of 156 polymorphic loci with an average of 13 amplicons per primer. The mean of PIC and Rp have scored 0.24 and 4.6, respectively, which were at par of optimal values. Clustering and principal coordinate analyses were performed and revealed useful genetic similarities/relatedness between the studied cultivars. Trait-marker association through F-test analysis was also accomplished in this study. SEM and SCoT approaches were integral and efficient in assessing the taxonomic and genetic diversity among the cultivars under investigation. This study also investigated some Asian cultivars of the economic importance as resources for genetic improvement of *T. aestivum* L. in future breeding program between the developing countries.

***Key words*:** Exomorphic characters, Genetic Discrimination, Poaceae, SCoT Polymorphism, SEM, *Triticum aestivum* L.

1. *Introduction*

Wheat (*Triticum aestivum* L.) belons to the family Gramineae (Poaceae) (Soreng *et al*., 2003). Wheat is the most vital food crop of the world and tops the list of cereal crops. Wheat is able to grow in many different topographic regions and adapt to extreme climate conditions. Grouping of wheat grain has significant rank in taxonomic studies. Cultivars are classified according to the horticultural demand and the season of planting (spring against winter wheat), food usages, and texture (for pastry and food vs. hard one containing more gluten) (USDA-NRCS, 2005). Therefore, the discrimination and characterization of wheat cultivars are essential for farmers and breeders to predict quality and yield (Shouche *et al.,* 2001). Vaughan (1968) proposed that the structure of the mature seed, especially the coat structure is considered a useful source of taxonomic information. The great differences in the morphology and the different ornamentations of the seed coat support the study of taxa delimitation and may solve or simplify many taxonomic problems. Netolizky (1926) confirmed that the

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morphology of seed coat surface should be a fundamental principle for classification of the seeded plants. El-Khanagry *et al.* (2006) suggested a key to identify nine species of grasses by using grains and spikes features using SEM. El-Sgai (2006) also constructed a key to identify 11 species of grasses belonging to six genera by using SEM on grains. Mohamed *et al.* (2017) clarified that SEM exhibited diagnostic characteristics among some *Triticum aestivum* L. cultivars from different Northern African countries. Their SEM analysis revealed six grain surface sculpture for the ventral surface and four for the dorsal side of 14 studied cultivars.

Assessment of genetic variation and evaluation of genetic relationships between individuals and/or populations is of crucial consideration for an efficient conservation and utilization of plant genetic resources (Henry, 1998; and Semagn *et al.,* 2006). The using and developing of molecular markers for the discrimination and exploitation of DNA polymorphism is one of the potential development in the field of molecular genetics. Specifically, genetic markers (dominat/codominat) have been used in cultivars characterization and phylogenetic analysis (Fang et al., 1998; Bornet & Branchard 2001; Adawy et al., 2002; 2004 and Semagn, et al., 2006). These markers include; RAPD markers (Javouhey et al., 2000; and Badr et al., 2012), SSR markers (Aranzana, et al., 2001 and Ma et al., 2015), and ISSR markers (Bornet & Branchard 2001; 2004). Recently, Start Codon Targeted (SCoT) markers concerned with the conserved flanking short region of the ATG or start codon in plant genes (Collard and Mackill, 2009). Because of the lower recombination levels between its markers and the gene/trait, SCoT markers are directly used in designing marker-assisted breeding programs than RAPDs, ISSRs, and SSRs (Mulpuri et al., 2013). SCoT markers have been efficiently used for DNA fingerprinting of *Tritordeum bergrothii* L. - Poaceae (Cabo et al., 2014).

Moreover, the efficacy of highly reproducible SCoT markers in accessing genetic diversity and relationships between Chinese *Elymus sibricus* L. - Poaceae accessions was investigated (Zhang et al., 2015). SCot markers were employed to genetically characterize 14 cultivars of *T. aestivum* L. related to different Northern African countries (Mohamed *et al*., 2017). However, knowledge on the genetic diversity and relatedness between *T. aestivum* L. cultivars localized in Asian countries is so limited, but necessary for maintenance and breeding programs that focusing on harboring of high quality and productivity.

Since there is no recent report emphasizing the surface sculpture of wheat grains by SEM to address the taxonomic relatedness of *T. aestivum* L. Asian cultivars from different Asian countries so far.

The specific objective of the present study is to investigate some of the grain exomorphic characters and to assess genetic relatedness/characteristics among some of Asian *T. aestivum* cultivars. For that, high resolution SEM imaging was executed to monitor and to investigate the grain surface sculpture of dorsal and ventral views. Furthermore, the short-conserved flanking locus at the translation ATG start codon was studied using SCoT markers to unveil the genetic diversity between *T. aestivum* cultivars provided, to access and to attain how application of concomitant taxonomic and molecular approaches most likely could help in the development of comprehensive programs regarding *T. aestivum* conservation and sustainable utilization in and among developing countries.

2. Material and Methods

2.1 Plant Material

Eight *Triticum aestivum* spring cultivars are dealt with, representing five Asian countries viz. Bangladesh, India, Iran, Pakistan and Syria. *Triticum* grains were provided and assessed by the International Center for Agricultural Research in the Dry Areas (ICARDA), research program of crop genetics that is established in the Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Giza, Egypt as listed in table 1.

Table 1. Geographical distribution of *T. aestivum* L. in some studied cultivars of Asian countries.

Geographic Origin	Code*: Cultivar name
Bangladesh (BGD)	BGD 1: Pavaon BGD 2: Sonalika
India (IND)	IND: Pbw-343
Iran (IRN)	IRN: Chamrran
Pakistan (PAK)	PAK1:Bakhtawar-94 PAK 2: Inqalab
Syria (SYR)	SYR 1: Cham-8 SYR 2: Cham-10
Total	8

***** Three digit codes used in this study were according to official country codes that were listed and stated through (http://www.nationsonline.org/oneworld/country_code_list.htm and http://www.worldatlas.com/aatlas/ctycodes.htm).

2.2 SEM Investigation

Surface sculpture of mature wheat grains was investigated by using SEM at different magnification levels at Regional Center of Mycology and Biotechnology, Al-Azhar University, Cairo, Egypt. The procedures of sample mounting, coating with gold sputter coater (SPI-module), and samples examination in a JEOL-JSM 5600 LV scanning electron microscope were performed as described previously (Mohamed et al., 2017). The SEM investigation emphasized the detailed views of three structural aspects/regions; ventral view, dorsal view near embryo, and dorsal view far from embryo. For the sake of consistency and uniformity of the studied exomorphic characters average 5-10 grains of each cultivar were examined. Grain sculpture was taxonomically characterized from the scheme adopted by Murley (1951).

2.3 Genomic DNA Extraction from *T. aestivum* L. cultivars

HMW (High Molecular Meight) plant genomic DNA was extracted from 50-100 mg of freeze-dried and powered grains of *T. aestivum* cultivars by as described previously (Mohamed et al., 2017). The quantity, integrity, and purity of the isolated DNA were checked by UV spectrophotometer using ND-1000 system (NanoDrop Technologies, Thermo Fisher Scientific Inc.) and agarose gel electrophoresis that were performed according to the Molecular Cloning Laboratory Manual (Sambrook et al., 1989).

2.4 SCoT-PCR technique

Genetic diversity between the studied *T. aestivum* cultivars was accessed by SCoT markers. The samples of *T. aestivum* were subjected to be differentiated using thirty SCoT primers (table 4). SCoT primers were designed as previously described by Collard and Mackill (2009), while primers 47-48 were designed according to Luo et al. (2010). Synthesis of SCoT primers is carried out by HVD Vertriebs-Ges. m.b.H. (Vienna, Austria). The underlined nucleotides sequence, representing ATG codon in the primers, was fixed (table 4). The polymerase chain reaction and cycling parameters were carried out as previously described (Mohamed et al., 2017). SCoT-PCR reactions were performed at least two times for the sake of accessing the same amplification profile. Fractionation of SCoT produced amplicons on a 1.5 % wt/vol agarose gel, staining procedure with EtBr, gel visualization, and finale documentation by gel documentation and image analysis system were carried out according to the Molecular Cloning Laboratory Manual (Sambrook et al., 1989).

2.5 Data analysis

Both of Grain exomorphic characters and the amplification profile generated by SCoT markers were analyzed to access the taxonomic and genetic relatedness of studied *T. aestivum* samples. Exomorphic characters SCoT amplified bands were scored as presence (1) or absence (0). Only clear distinct reproducible bands, in both SCoT reaction sets, were considered. SCoT primers capacity to discriminate between examined genotypes were analyzed by means of calculating the Polymorphic information content (PIC), Resolving power (Rp), and Marker Index (MI) values. PIC was calculated for each SCoT primer according to the formula: $PIC = 1 - \Sigma (P_{ith})^2$ as described

previously (Ghislain et al., 1999) where p is frequency of *ith* allele. MI was estimated according to Powell et al. (1996). Rp was calculated following the formula of Gilbert et al. (1999), Rp = Σ IB, where Ib represents band informativeness which was calculated using: Ib = 1 – (2 × | 0.5 - p |), where P is the frequency of accessions that harbor bands. Cluster analysis was constructed by Dice coefficient-based genetic similarity matrix to display the level of genetic relatedness using the unweighted pair group method with arithmetic mean (UPGMA) of NTSYS software version 2.10 (Rohlf, 2000). The principal coordinate analysis (PCoA) and population structure *T. aestivum* L. cultivars were constructed and analyzed using DCENTER module in NTSYS. Powermarker software V3.0 (Liu & Muse, 2005) was used for trait-marker association through F-test analysis. Binary matrix reveled from absence/presence of exomorphic characters (shown in Table 2) was blotted against the SCoT-based matrix of the thirty used primers. Markers with high p-value (>0.01) were selected.

3. Results and Discussion

3.1 Exomorphic characters (SEM),

The obtained grain exomorphic characters are summarized in table 2. The grain surface sculpture in the present cultivars was the same on both dorsal and ventral views in BGD2, the surface sculpture is scalariform -reticulate foveate, while in PAK2 is scalariform -reticulate (fig. 1). Four patterns of grain sculpture for ventral view viz. scalariform as in BGD1 and SYR1. Scalariform -reticulate foveate as in BGD2 and IND1. Scalariform -foveate as in IRN and SYR2. Scalariform- reticulate as in PAK1 and PAK2. The grain sculpture of the dorsal region near or far from the embryo showed four patterns viz. scalariform-reticulate as in BGD1, IND, IRN, and PAK2 cultivars. Scalariform -reticulate foveate as in BGD2; Scalariform- foveate as in PAK1 or Scalariform in SYR2.

The anticlinal wall in all the studied grain cultivars was thin, smooth and elevated. In BGD2, the anticlinal walls surface striated at dorsal and shallow depressed at ventral one. In PAK1 and PAK2, the anticlinal walls surface shallow depressed at dorsal and ventral side. In IND1 and SYR1 the anticlinal walls thickness thick at ventral side. In PAK2, the anticlinal walls thickness thick at dorsal and ventral side.

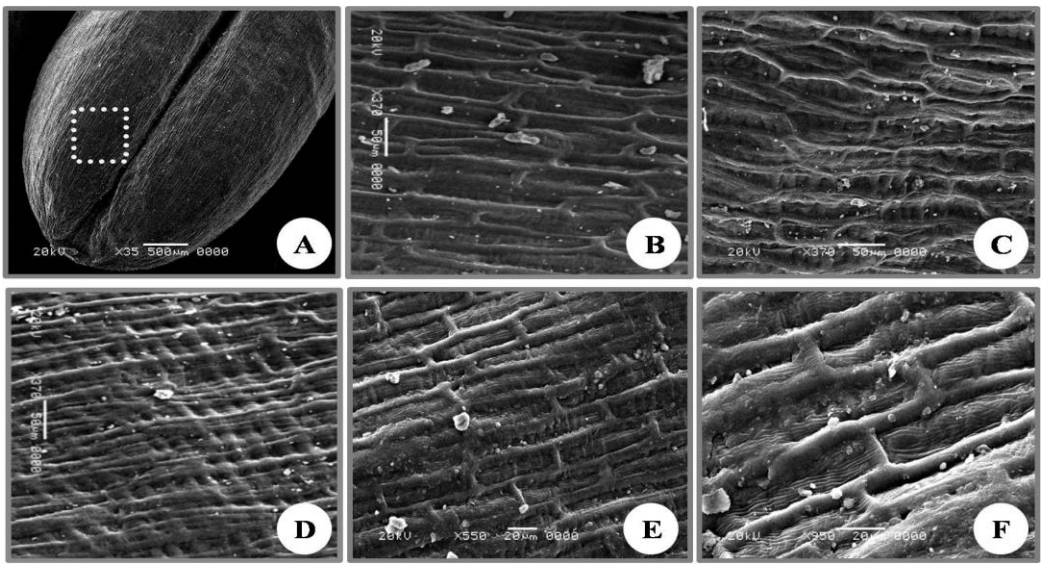

Figure.1. SEM microphotographs showing examples of four types of grain surface sculpture (ventral and dorsal side). A. whole ventral side of wheat grain; B. Scalariform reticulate (PAK1); C. Scalariform reticulate foveate (BGD2) showing faoveate stria of periclinal wall; D. Scalariform foveate (SYR2); E. Scalariform (SYR1); F. Smooth broad anticlinal wall and longitudinal striation (PAK2). The white dotted rectangle in panel (A) emphasizes the region that was analyzed by SEM in the ventral side of studied grains.

Table 2. Grain exomorphic characters of the studied Asian *T. aestivum* (SEM)

Cultivar code		Surface pattern		Anticlinal wall				Periclinal wall			
		Ventral	Dorsal	Thickness		Surface		Surface		Elevation	
				Ventral	Dorsl	Ventral	Dorsal	Ventral	Dorsal	Ventral	Dorsal
Bangladesh	**BGD1:** Pavaon	Scalariform	Scalariform - reticulate	Thin	Thin	Striated	Smooth	Longitudinal Stria	Transverse stria	Shallow elevated	Shallow elevated
	BGD2: Sonalika	Scalariform - reticulate foveate	Salariform - reticulate foveate	//	//	//	Striated	Stria foveate	Stria foveate	Shallow depressed	//
India	**IND1:** Pbw-343	Scalariform - reticulate foveate	Scalariform-reticulate	Thick	//	Smooth	Smooth	//	Transverse stria	Shallow elevated	//
Iran	**IRN1:** Chamrran	Scalariform - foveate	Scalariform-reticulate	Thin	//	//	//	//	Transverse stria	//	//
Pakistan	**PAK1:** Bakhtawar-94	Scalariform - reticulate	Scalariform-foveate	//	//	//	//	//	Stria foveate	Shallow depressed	//
	PAK2: Inqalab	Scalariform-reticulate	Scalariform - reticulate	Thick	Thick	//	//	Longitudinal Stria	Transverse stria	//	Shallow depressed
Syria	**SYR1:** Cham-8	Scalariform	Scalariform - foveate	Thick	Thin	//	//	//	Stria foveate	Shallow elevated	Shallow elevated
	SYR2: Cham-10	Scalariform - foveate	Salariform	Thin	//	//	//	Stria foveate	Longitudinal Stria	//	//

(//) = The same character was detected.

3.2 Taxonomic relationships between *T. aestivum* cultivars as revealed by SEM

The binary dataset resulted from SEM-characterized exomorphic characters were recorded as presence (1) or absence (0) and analyzed using the Dice's coefficient to construct the similarity matrix (table 3).

Table 3. Similarity matrix among studied *T. aestivum* L. cultivars as computed according to Dice coefficient as revealed by observed SEM -based exomorphic characters

Cultivar	Syria1	Syria2	Iran	India	Pakistan2	Pakistan1	Bangladesh2	Bangladesh1
Syria1	100							
Syria2	42	100						
Iran	67	27	100					
India	71	29	**93**	100				
Pakistan2	42	71	53	57	100			
Pakistan1	40	**13**	50	40	40	100		
Bangladesh2	63	38	70	63	62	59	100	
Bangladesh1	53	27	**75**	**80**	53	37	71	100

Estimated taxonomic similarities ranged from 13 to 93 % to record low to high levels of taxonomic relatedness. Highest detected similarity 93% was found between IND-IRN cultivars. Then, high similarity value of 80% was detected between BGD1-IND cultivars. This was followed by 75% between BGD1-IRN cultivars. On the other hand, the lowest taxonomic similarity pairs of 13% and 27% were detected between PAK1-SYR2 and BGD1-SYR2, respectively. It was observed that low similarity percentage 13-53% was investigated generally between Pakistanian/Bangladeshian/Indian and Syrian cultivars (table 3). These findings might be attributed to that Syrian cultivars might be regarded as the primary habits of wheat cultivars which differ in their grains characteristics from recent cultivation habits as previously discussed (Ozkan et al., 2005).

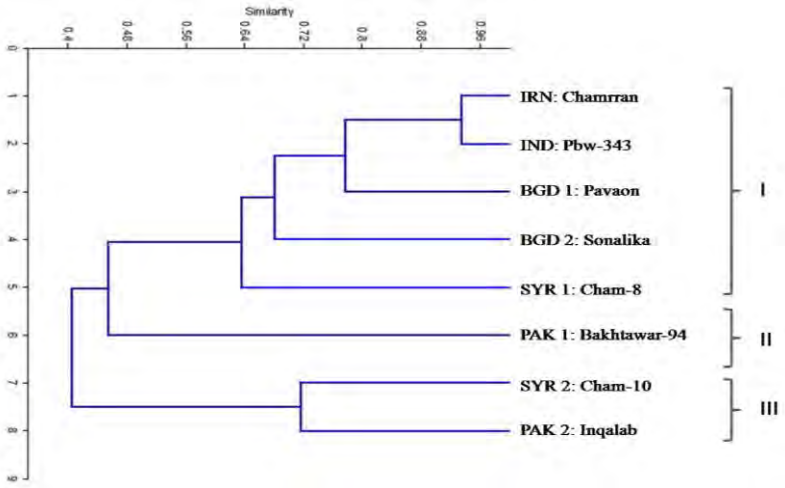

Figure.2. Cluster analysis resulted from SEM of some Asian *T. aestivum* L. cultivars. A dendrogram for the eight examined *T. aestivum* L. cultivars was constructed using scored exomorphic characters resulted by SEM data using Unweighed Pair-group Method of Arithmetic mean (UPGMA) and similarity matrices was computed according to Dice coefficient.

Clustering analysis was conducted using the UPGMA analysis (fig. 2) and resulted in grouping of *T. aestivum* cultivars into three main clusters. Cluster I includes IRN, IND, BGD1, BGD2, and SYR1 cultivars. Cluster II comprised only PAK1. Cluster III includes SYR2 and PAK2. Although the Dice's similarity matrix has revealed low levels of relatedness of Syrian cultivars and the rest of the studied cultivars, clustering analysis has grouped PAK2 and SYR2 together. These results might be explained by that exomorphic characters of examined grains may show a level of similarity, regardless of the extent of relatedness of these taxa on the genetic level as previously suggested by Ozkan et al (2005).

In conclusion, grouping of PAK1 cultivar in independent cluster and grouping the Syrian cultivars in two different clusters have prompted us to further analyze *T. aestivum* cultivars on the genetic level, by means of SCoT analysis, to proof/disproof unique characteristics of wheat cultivars and to decipher genetic diversity among *T. aestivum* Asian cultivars. The SEM information based on the aspects of the anticlinal walls and the sculpture of the ventral and dorsal sides delivers a vital tool for accurately distinguishing grain surfaces and serve as exceptional diagnostic criteria at cultivars level in the studied of wheat grain this is in harmony with the work of Barthlott (1981, 1984), Abou-Taleb et al. (2013), Mohamed et al. (2017).

3.3 SCoT-PCR Amplification profiling

Furthermore, the role of ecological conditions in determining the extent and distribution of genetic diversity has been well documented (Yan et al., 2006). The eight *T. aestivum* cultivars were subjected to be genetically distinguished after scoring of grain exomorphic characters that was carried out and revealed low levels of relatedness between some of Asian studied cultivars and high levels of similarity between the others. Hereby, genetic relatedness/diversity among the *T. aestivum* L. studied cultivars was studied by using SCoT markers. Amplification banding profiles revealed by some representative SCoT primers were shown (Fig. 3).

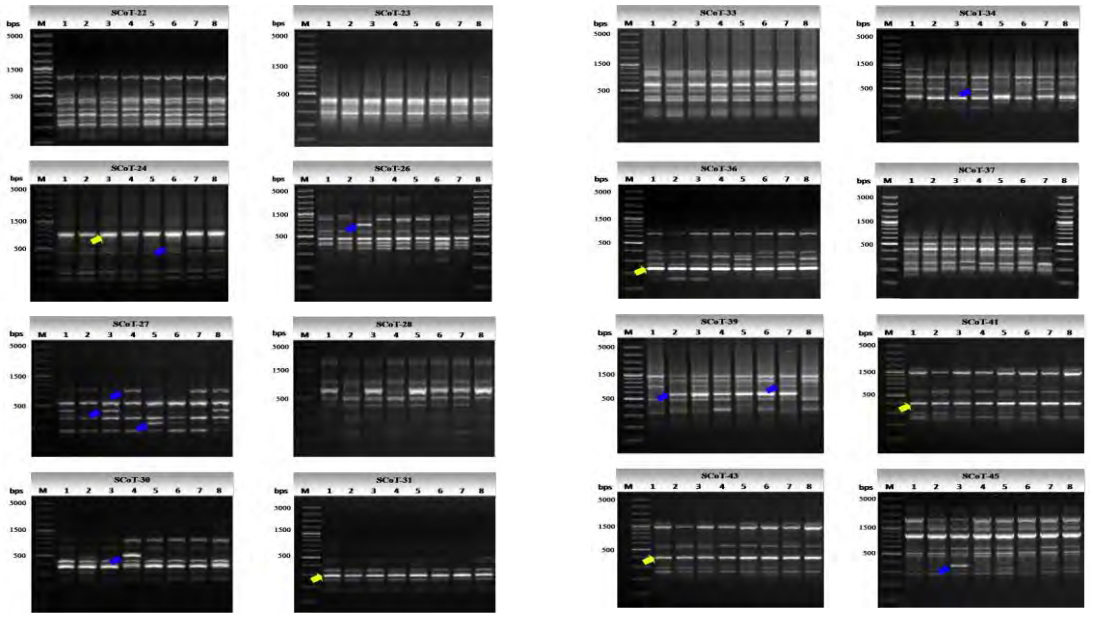

Figure. 3. SCoT markers Polymorphism. Agarose gel electrophoresis of PCR amplicons of some representative SCoT primers was shown. DNA size marker GeneRuler 1 Kb plus DNA ladder (lane M) was loaded and denoted by numbers left-handed of the figure indicating molecular size standards in bps. Yellow and blue arrows refer to unique mono- and polymorphic characterized SCoT amplicons, respectively.

SCoT-PCR amplified fragments have shown a significant polymorphism among the studied *T. aestivum* cultivars. Highly pronounced unique polymorphic SCoT amplicons (Fig. 3, denoted by blue arrowheads) were observed like SCoT26, SCoT27, SCoT30, SCoT34, SCoT39, and SCoT41. Also, unique monomorphic SCoT amplicons obtained with SCoT24, SCoT31, SCoT36, and SCoT41 were detected (Fig. 3, denoted by yellow arrowheads). These results most likely strengthened the implication of genetic diversity of *T. aestivum* genome to the level of cultivars as revealed by SCoT markers. On the other hand, the total number of monomorphic amplified fragments reflects the extent of genetic conservation of *T. aestivum* genome as previously shown (Mohamed et al., 2017).

Table 4. The list of primers' sequence, **T**otal **N**umber of **A**mplicons (TNAs), **M**onomorphic **A**mplicons (MAs), **P**olymorphic **A**mplicons (PAs), **P**ercentage of **P**olymorphism (%P), **P**olymorphism **I**nformation **C**ontent (PIC), **R**esolving **P**ower (RP), and **M**arker **I**ndex (MI) as revealed by SCoT analysis of some examined *T. aestivum* L. Asian cultivars

Sl. No.	Name	Sequence (5′› 3′) %GC	% GC	TNAs	MAs	PAs	% P	PIC	RP	MI
1	ScoT1	CAACA**ATG**GCTACCACCA	50%	21	14	7	33	0.14	6.25	0.94
2	ScoT2	CAACA**ATG**GCTACCACCC	56%	17	8	9	53	0.1	6.75	0.92
3	**ScoT3**	CAACA**ATG**GCTACCACCG	56%	6	6	**0**	**0**	**0**	**0**	**0**
4	ScoT5	CAACA**ATG**GCTACCACGA	50%	17	8	9	53	0.10	6.75	0.93
5	ScoT8	CAACA**ATG**GCTACCACGT	50%	11	8	3	27	0.29	1.5	0.88
6	ScoT9	CAACA**ATG**GCTACCAGCA	50%	14	9	5	36	0.18	4.5	0.93
7	ScoT10	CAACA**ATG**GCTACCAGCC	56%	19	12	7	37	0.13	7	0.94
8	ScoT11	AAGCA**ATG**GCTACCACCA	50%	21	10	11	52	0.09	**8.75**	0.93
9	ScoT12	ACGAC**ATG**GCGACCAACG	61%	20	12	8	40	0.12	8.25	0.94
10	**ScoT13**	ACGAC**ATG**GCGACCATCG	61%	11	10	1	**9**	0.91	0.75	0.89
11	ScoT16	ACC**ATG**GCTACCACCGAC	56%	11	9	2	18	0.45	3	0.89
12	ScoT20	ACGAC**ATG**GCGACCCACA	67%	14	7	7	50	0.13	8.25	0.92
13	ScoT22	CC**ATG**GCTACCACCGCAC	67%	15	9	6	40	0.15	5	0.93
14	ScoT23	C**ATG**GCTACCACCGGCCC	78%	11	4	7	**63**	0.13	7.5	0.89
15	ScoT24	CC**ATG**GCTACCACCGCAG	67%	15	8	7	47	0.13	3.25	0.93
16	ScoT26	ACGAC**ATG**GCGACCACGT	61%	16	13	3	23	0.31	2.75	1.14
17	ScoT27	ACC**ATG**GCTACCACCGTC	61%	9	4	5	56	0.22	5	0.97
18	ScoT28	CAACA**ATG**GCTACCACCA	50%	13	7	6	46	0.11	7	0.68
19	ScoT30	CAACA**ATG**GCTACCACCT	50%	10	4	6	60	0.13	7.5	0.76
20	ScoT31	CAACA**ATG**GCTACCACGA	50%	9	6	3	33	0.29	2.25	0.86
21	ScoT33	AAGCA**ATG**GCTACCACCA	50%	10	8	2	20	0.45	2	0.89
22	ScoT34	ACGAC**ATG**GCGACCAACG	61%	11	6	5	45	0.18	4.5	0.88
23	ScoT36	CACC**ATG**GCTACCACCAT	56%	10	9	1	10	0.89	**0.5**	0.89
24	ScoT37	GCAACA**ATG**GCTACCACC	56%	13	6	7	54	0.13	6	0.91
25	ScoT39	CAACA**ATG**GCTACCACGG	56%	10	7	3	30	0.30	3	0.89
26	ScoT41	CAACA**ATG**GCTACCAGCA	50%	11	6	5	45	0.18	4.75	0.89
27	**ScoT43**	ACGAC**ATG**GCGACCATCG	61%	9	3	6	**67**	0.14	5	0.86
28	ScoT45	ACC**ATG**GCTACCACCGAG	61%	15	9	6	40	0.15	7.25	0.93
29	ScoT47	ACA**ATG**GCTACCACTGCC	56%	11	7	4	39	0.22	2.25	0.95
30	ScoT48	ACA**ATG**GCTACCACTGGC	56%	11	8	3	27	0.3	2	0.89
	Total			**391**	**235**	**156**	**-**	**-**	**-**	**-**
	Mean			**13**	**-**	**-**	**38.4**	**0.24**	**4.6**	**-**

3.4 Genetic discrimination of the studied *T. aestivum* L. cultivars as revealed by SCoT-PCR

Amplified SCoT-PCR fragments produced from thirty SCoT primers in terms of the percentage of PCR products appeared in the studied genotypes (Table 4). A total of 391 bands were generated, among which 156 bands were polymorphic. The percentage of polymorphism ranged between 9 % for SCoT13 and 67 % for SCoT43. Notably, one of the SCoT primers did not record and/or generate any polymorphism between studied samples, SCoT3.

The average number of SCoT produced amplicons was 13; the maximum was 21 with SCoT1 and the minimum was 6 with the SCOT3. The amplification profile, revealed by the three SCoT primers; SCoT43, SCoT23, and SCoT30, yielded highly informative patterns based on obtained percentage of generated polymorphic loci resulted from these primers (Table 4). The efficacy of SCoT markers in discriminating studied genotypes was estimated by obtaining the PIC, Rp, and MI values of used primers. PIC values varied from 0.09 to 0.91 with an average of 0.24. SCoT primers with the highest PIC values may have more potential for further study, allowing investigating more cultivars and/or sampling accessions. The Rp values ranged from 0.5 to 8.75 for SCoT36 and SCoT11, respectively with an average of 4.6 that represents, at par, with PIC mean value optimal and informative parameters in this study (Botstein et al., 1980; Smith et al., 1997; Prevost & Wilkinson, 1999). On the other hand, the genetic sequence of SCoT3 locus might be regarded as conserved genomic sequence between investigated Asian studied cultivars and most likely might be counted as unique specific marker of the Asian cultivars under study.

To characterize and access the genetic similarity/relatedness of *T. aestivum* Asian analyzed cultivars as revealed by SCoT markers, the scored binary dataset obtained from thirty primers was analyzed using the Dice coefficient to construct the similarity matrix (Table 5). The estimated genetic similarities ranged from 86% to 93% revealing high levels of genetic similarity among the studied cultivars. The highest recorded genetic similarity 93% was detected between PAK1-IND, PAK1-PAK2, and BGD1-IRN. This was followed by 92% among BGD1-BGD2 and BGD2-IND that represents same or near geographical regions. On the other hand, the lowest genetic similarity values were detected between Pakistanian/Bangladeshian/Indian and Syrian cultivars of distant geographical distributions. These results were in accordance with Ozkan et al (2005).

Table 5. Similarity matrix among studied *T. aestivum* L. cultivars as computed according to Dice coefficient as revealed by SCoT markers

Cult.	Syria1	Syria2	Iran	India	Pakistan2	Pakistan1	Bangladesh2	Bangladesh1
Syria1	100							
Syria2	89	100						
Iran	89	88	100					
India	88	88	89	100				
Pakistan2	89	**86**	89	93	100			
Pakistan1	88	89	89	**93**	**93**	100		
Bangladesh2	89	88	91	**92**	91	91	100	
Bangladesh1	89	87	**93**	89	88	90	**92**	100

Genetic similarities percentages, revealed by SCoT analysis, were concomitant to major extent with their corresponding percentages (revealed by SEM) and helped to dissect the genetic diversity between Asian studied samples. The higher investigated similarity percentages of 86-93% observed by the SCoT analysis might be speculated due to the conservation of *T. aestivum* genome organization regardless of the close/distant geographic distribution. Furthermore, there is no report that covered the use of SEM and SCoT markers for characterization of taxonomic and genetic diversity of *T. aestivum* cultivars studied from some Asian countries so far.

3.5 Assessment of genetic relationships

Previously generated similarity matrix was used to construct a phylogenetic tree using the UPGMA clustering. Based on 156 polymorphic SCoT generated binary matrix dataset, a dendrogram was constructed (Fig. 4). The eight studied genotypes were grouped into three main clusters. Cluster I included the genotypes from Iran and Bangladesh. Cluster II consisted of the genotypes from India and Pakistan, while Cluster III comprised only the Syrian cultivars. The clustering outcome of the eight Asian studied genotypes may refer to distinct geographical tendency in the distribution of the genetic diversity which accounts for isolation of Syrian cultivars in independent cluster from other studied genotypes.

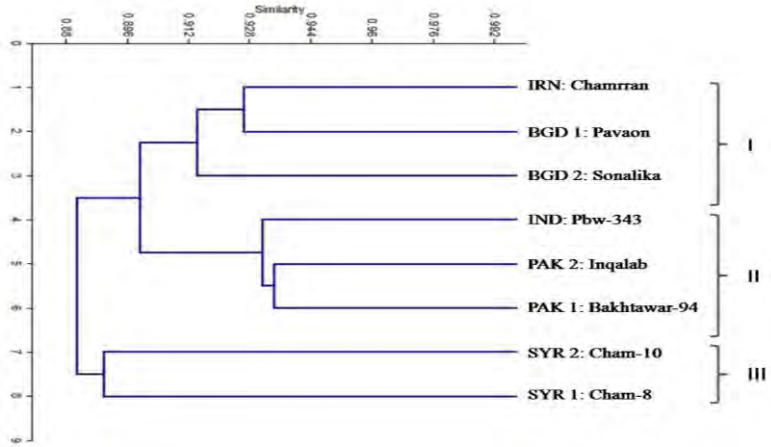

Figure.4. Cluster analysis resulted from SCOT markers of *T. aestivum* L. cultivars. Dendrogram for the eight examined *T. aestivum* L. cultivars constructed from the SCoT polymorphism data using Unweighed Pair-group owing the level of genetic material Arithmetic mean (UPGMA) and computed similarity matrices was according to Dice coefficient.

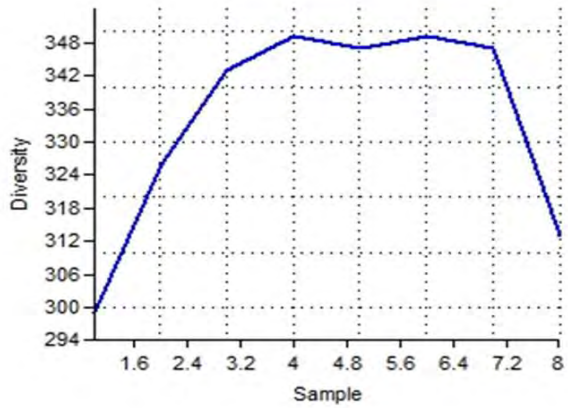

Figure.5. Genetic diversity curve showing the level of genetic material diversity between studied cultivars.

The same notion was found in clustering of Pakistanian and Bangladeshian genotypes each in isolated cluster. The same conclusion was revealed by showing of the genetic diversity curve (Fig. 5) and was in agreement with Ozkan et al (2005). To conclude, the SCoT-based dendrogram was almost in accordance with SEM-based results.

To manifest the genetic relatedness between *T. aestivum* studied cultivars, the principal coordinate analysis (PCoA) was conducted based on Dice's similarity matrix (Fig. 6). The PCoA multivariate approach was performed to complement the cluster analysis outcomes, because cluster analysis shows a higher resolution for analysis of closely related populations. On the other hand, the PCoA is more informative regarding distances among major group(s). On the same context, PCoA separated the eight genotypes by the first two PCoAs. The first PCoA explained about 48 % of the total variation, while the second PCoA resolved 23% of the total variation (Fig. 6). The results have demonstrated a marked overlap between Indian and Pakistanian genotypes. The relationships estimated from PCoA were in agreement with and confirmed the clustering analysis.

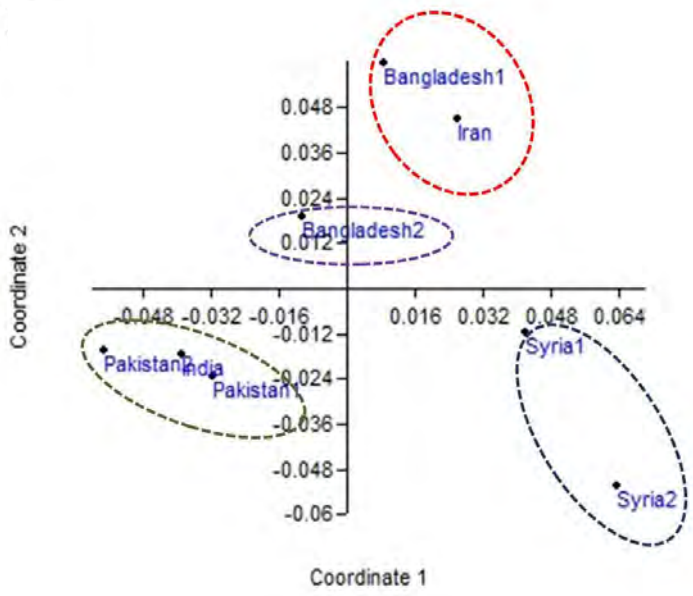

Figure.6. Population analysis. Principal coordinate analysis based on the calculation of the first three coordinates was performed according to analysis of SCoT markers of the studied eight *T. aestivum* L. Asian cultivars.

Trait-marker association analysis was achieved using single locus F-test module in Power maker software (Fig. 7). The results have shown that the property of one trait for one locus was characterized. Characterized locus generated by SCoT12 was found to be uniquely associated with anticlinal dorsal thickness trait of wheat grains. Moreover, several traits were found to be associated with one or more than one locus. Amplified loci of SCoT5 were found to be associated to the different characterized sculptures of surface patterns. Notably, SCoT47 loci were significantly found to be not only associated to sculpture trait of surface dorsal patterns, but also to anticlinal wall thickness on dorsal/ventral sides, and surface striation traits (Fig. 7). Previous results have suggested the high potential of using of molecular markers in the search for genes affecting crop productivity and defense mechanisms against biotic and abiotic stress factors by identifying statistical associations between the genetic markers and the traits of interest.

Characterized exomorphic characters of *T. aestivum* cultivars, as observed by SEM, have demonstrated that the grain sculpture(s) as well as aspects of anticlinal and periclinal walls were able to discriminate among studied cultivars. Moreover, SEM provided an important tool for reasonable characterization of grain surface. However, genetic characterization by means of high sensitive markers, like SCoT, is needed and indispensable to further dissect and closely discriminate the genetic similarity/diversity between studied genotypes.

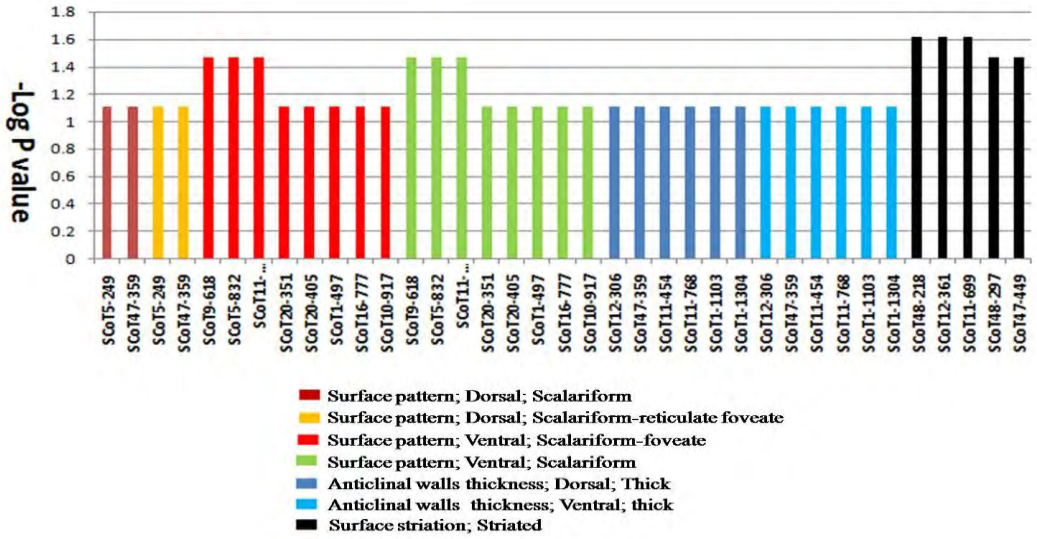

Figure.7. Association analysis. Trait-marker association was achieved through F-test analysis. Binary matrix reveled from absence/presence of exomorphic characters (shown in Table 2) was blotted against the SCoT-based matrix of the thirty used primers. Power marker software V3.0 was used and SCoT markers with high p-value (>0.01) were selected in the association analysis. X and Y axes refer to -Log P values and the name of SCoT primer associated with certain trait, respectively. Nomenclature of X-axis elements, for example SCoT5-249 refers to the primer SCoT5 locus with 249 bps is associated to X trait.

This study has emphasized the validity of notion that the genetic diversity of wheat cultivars related to primary habitats (i.e., Syrian cultivars) is obeyed by the rule of geographical distribution tendency. Moreover, SCoT analysis was significant to monitor the genetic discrimination and to generate unique genomic loci at *T. aestivum* cultivars. Hereby, breeding lines from *T. aestivum* Asian cultivars (presented in this study) along with Northern African cultivars (Mohamed et al., 2017) and their rational comparison may emerge novel insights and give a better understanding of the domestication and genetic diversity of *T. aestivum* genome. This study has also investigated some of Asian cultivars of important economic and genetic resources for genetic improvement of *T. aestivum* in future breeding program in the developed countries.

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