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Cytomolecular Genetic Diversity Assessments of Two Wheat Species Grows in Egypt.

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ABSTRACT

Genetic diversity among three genotypes of tetraploid wheat and four of hexaploid wheat, it was estimated using inter simple sequence repeats (ISSR) and cytogenetic studies. Three genotypes of *Triticum dicoccum* (Sohag-1, Beni-suef-1 and Beni-suef-3 belonging to Tetraploid wheat and four hexaploid genotypes; Sids-8, Sids-12, Sids-13 and Giza-171 (*Triticum aestivum*)) sampled from upper and lower Egypt regions; the three durum wheat cultivars and the four hexaploid wheat were assessed. Genetic diversity within wheat genotypes was evaluated using 10 ISSR primers. Of the approximately 431 detected ISSR markers, 117 (27%) were polymorphic with 27 bands per utilized primer pair. Cluster analysis of seven genotypes belonging to the two species by UPGMA cluster analysis based on Jaccard's similarity estimates for ISSR data separated all genotypes into two major clusters depend nearly on their genome makeup. The first one include wheat species possesses AB genomes, while second cluster included wheat genotypes ABD genomes. The genetic similarity coefficients ranged from 0.05 between Beni-suef 3 (*Triticum dicoccum*) and Sids-8 and Sids-12 of *T. aestivum*. Concerning chromosome morphology analysis of the three durum wheat genotypes of, the highest value for chromosome length was observed in Sohag-1 (14.84 μ m for chromosome 2 A) and the smaller value in Beni-suef 3 (5.16 μ m for the chromosome 7B). The CI values (centromere index) obtained for durum wheat ranged from 0.48 for 5B to 0.97 for 7A. Thus, according to the CI values for karyotypes of Sohag 1, all chromosomes are metacentric except 5B chromosome. The CI values (centromere index) obtained for durum wheat ranged from 0.50 for 5B to 1.00 for 7A

Keywords: Inter simple sequence repeats (ISSR), genetic diversity, wheat, *Triticum* spp., karyotyping, chromosomes.



INTRODUCTION

Posterior corn wheat is the world's second most produced grain cereal. Bread wheat or common wheat which contain large quantity of starch moreover durum wheat which featured by containing greater protein portion are the most commonly cultured genotypes with superior potential of commerce as mentioned by (Felicio *et al.* 1999; Awika 2011). *Triticum aestivum* L. is the most distributed implanted flora in the planet, because of its physiological traits which enable different wheat genotypes for production in a wide range of geographic-ecological conditions. Moreover the chemical and physical properties of the wheat gluten that contributes to the wide use of wheat grain for many different food products. It is the staple nourishment for 35% of the total populace. To fulfill the need for growing high yielding and stress-safe wheat cultivars, it is attractive to build the genetic base of this plant. There is a developing concern about the rest of the fluctuation in the bread wheat gene pool which is lacking to address present and future breeding goals (Rejesus *et al.*, 1996). In decades ago, the limited genetic basis of neoteric wheat genotypes is well visible; breeders choose employ either improved cultivars as parents or advanced breeding

materials to quicken the advancement of new cultivars. Whereas, initially selection was employed to obtain pure lines from heterogeneous landraces or natural populations, nowadays improved cultivars were used as parents in wheat breeding programs. It is in this way important to widen the genetic base of wheat. Thusly, investigation of the genetic diversity of the genetic resources of such species may give critical data in regards to their potential for breeding objective. Genetic erosion brought about by present day development technique has been limited genetic base of numerous harvests, including common wheat. Egypt is extremely wealthy in living space decent variety because of the assorted variety in its atmosphere.

This has assisted the endurance of a various plant animal varieties in nature. Common wheat and its own taxonomic group considered a very remarkable portion of Iranian flora. These species exemplify an enormous supply of helpful qualities that can be abused for wheat improvement. Numerous economical remarkable traits, covering biotic and abiotic stress tolerance have been transmitted to wheat from such species as pointed out by (Jiang *et al.*, 1993 and Friebe *et al.*, 1996).

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(Huang *et al.*, 2002 and Arzani *et al.*, 2005) pointed out that Iran is main source of genetic diversity in wheat germplasm in comparison with wide range of world areas these investigation based on microsatellite technique. Lately molecular markers have been commonly employed to describe germplasm otherwise conventional agronomical and morphological investigations.

Diversification in DNA sequences within different cultivars can be explored by means of DNA molecular markers. Nowadays it became easy and routine to calculate relationship level between populations and lines of different flora species via inter simple sequence repeats, "ISSRs" employment. Because of ISSR considered so much efficient and reproducible technique, it has been widely applied to differentiate between various genotypes many plant species inclusive *T. aestivum* L. (Barrett and Kidwell 1998; Barrett *et al.*, 1998; Bohn *et al.*, 1999; Ridout and Donini, 1999; Soleimani *et al.*, 2002; Almanza-Pinzon *et al.*, 2003 and Lage *et al.*, 2003). Cytogenetic mapping mandatorily precondition karyotyping or awareness of chromosome complement. It is potentiality to identify genes or DNA sequences on specific chromosomes, especially in genotypes featured with well-known karyotype. Mapping, also enable identifying, and checking the existence of chromosomes or chromosome

sections during introgression in breeding projects. Evaluation of genetic variances in economic, implanted flora has significant impact for breeding programs and for the preservation of genetic resources.

The essential target of this investigation was to understand the extent and pattern of genetic diversity among tetraploid and hexaploid species of wheat using ISSR marker and karyotyping the three genotypes of *Triticum durum*. The objective of this investigation was also to characterize three durum wheat genotypes via karyotyping for longitudinal characterization of chromosomes. Knowledge of such karyotypes will allow mapping sequences affect biotic and abiotic stress tolerance within investigated wheat chromosome complement. This will empower checking the introgression of explicit chromosomes bearing sequences identified with sickness resistance through introgression lines in breeding programs.

MATERIALS AND METHODS

Plant materials:-

A collection of four bread wheat cultivars (*Triticum aestivum* L.) and three durum wheat cultivars (*T. dicoccum* L.) genotypes was used in this study (Table 1).

Table 1. Plant materials (*Triticum durum* L. and *Triticum aestivum* L.) and characteristics collection regions

S.N.	Species	Genome	Origin
1	Sids-8	AABBDD	Maya "S"/Mon "S" // Cmh 74A.592/3 Sakha 8*2
2	Sids-12	AABBDD	BUC// 7c/ Ald/5/ Maya 74/ On/ 1160. 147/3/ BB/ G11/4/ Chat "S" // 6/ Maya/ vul // Cmh 74A.630/4* sx, SD7096- 4SD - 1SD-0SD.
3	Sids-13	AABBDD	ALmaz 19= Kauz "S" // Tsi /snb "S" ICW 94-0375- 4AP- 2AP-030AP-) APS- 2AP-0APS- 050AP- 0AP- 0SD.
4	Giza-171	AABBDD	Gemmeiza-9 / Sakha-93
5	Beni-suef-1	AABB	Jo "S" / AA/ g "S"
6	Beni-suef-3	AABB	Corn "S" / Rufo "S" CD4893-10y-1M-1Y-0M
7	Sohag-1	AABB	GERARDO-VZ-469/3/JORI(SIB)/ND-61-130/LEEDS

ISSR analysis:-

The experiment was conducted in the Biotechnology Laboratory, Department of Genetics and Genetic Engineering, Faculty of Agriculture, Benha University. Seven wheat genotypes were used in this study (Table 1). Coding numbers are used according to the order of collection.

Total Genomic DNA Extraction:-

DNA was extracted from wheat young leaves (Saghai-Marouf *et al.*, 1984). The extracted DNA was diluted to obtain a final concentration of 25 ng/μL in order to use it in the PCR amplification.

PCR amplification:-

The ISSR amplification was carried out in a 25μL volume, according to Hoisington *et al.*, 1994. The amplifications were performed in a BioRad thermocycler. The PCR products were detected by 1.6% agarose gel electrophoresis that was stained with ethidium bromide. Then, the PCR products were visualized in a ultraviolet light using transilluminator. In order to better distinguish the bands were used the molecular ladder contained known fragments.

Cytological Studies:-

Seeds of three durum wheat genotypes (Beni-suef-1, Beni-suef-3 and Sohag-1) were used to obtain the meristems in cytological preparations.

Pretreatment, fixation and preparation of chromosomes:-

Chromosomes in metaphase were obtained via pretreatment of durum wheat root tips. Germinated as following, Durum wheat seeds had been sprouted on saturate channel paper in petri dishes and afterward kept in obscurity in room temperature of 25 °C for 48 h. After this period, the roots were gathered and submitted to a pretreatment in cool water (4 °C) for 24 h. Afterwards pretreating, establishes were fixed in Carnoy arrangement (supreme ethanol : chilly acidic corrosive in a proportion of 3:1, separately).

Analysis of chromosome morphology:-

The root tips washed in refined water before moving in 70% liquor for additional utilization. The slides were set up in 2% acetocarmine for the investigation of karyotyping in mitotic metaphase and examination of chromosomal morphology under microscopy; Reeves, (2001) and Levan, *et al.*, (1964).

Data analysis:-

The PCR item groups were scored as [1] for the existence and [0] for nonattendance. The acquired information was utilized for examinations of hereditary relationship in the analyzed wheat material. A similarity matrix was developed utilizing the NTSYS-pc (Numerical Taxonomy and Multivariate Analysis for personal

computers) software, version 2.1 (Rohlf, 2005). For all pairs, wise comparisons were done, according to Jaccard's similarity coefficient. A dendrogram was constructed from the similarity matrix using the UPGMA method (Unweighted Pair-Group Method with Arithmetical Averages) and the SAHN subprogram (Sequential, Agglomerative, and Hierarchical and Nested clustering).

RESULTS AND DISCUSSION

Results

ISSR analysis:-

Ten ISSR primers (Table2) had been used to investigate the genetic interconnection within the Egyptian wheat genotypes (Sids-8, Sids-12, Sids-13 and Giza-171 for bread wheat (*Triticum aestivum* L.) and Beni-suef-1, Beni-suef-3 and Sohag-1 for pasta wheat (*Triticum dicoccum* L.). PCR reactions had been produced a sum of 431 bands (Figure 1 and Table 3), 117 of these bands (68%) were polymorphic. The number of polymorphic bands ranged from 5 (ISSR 1 and ISSR 9) to 22 (ISSR 3) with mean equal 11.70. The maximum polymorphism value were recorded with primers ISSR 3 and ISSR 4, respectively. The similarity matrix revealed that the highest similarity percentage has been observed between the varieties (Beni-suef-3 and Giza-171 or beni-suef-1) with 0.20 and 0.17 while the lowest similarity percentage was recorded between the cultivars (Beni-suef 3 and Sids 8 or

Sids 12) with value 0.05 (Table 5). The cluster analysis was done using Jaccard's similarity coefficients to study the genetic relationship among the wheat genotypes of the two *Triticum* species (Reif *et al.*, 2005). The cluster divided the genotypes into two main groups (Figure 4), the first group contained only the Sids-8, Sids-12, Sids-13 and Giza-171, while the subsequent cluster have the rest of genotypes (Sohag-1, Beni-suef-1 and Beni-suef-3); These results were in agreement with the findings of Yildirim and Akkaya (2006), Randhawa *et al.*, (2013), Dawlah *et al.*, (2015), Sabbour *et al.*, (2015), Etminan *et al.*, (2016),.

Table 2. ISSR primers (and their sequences) which produced polymorphisms across three durum wheat cultivars, one bread wheat cultivar, two triticale cultivars and one rye cultivar, respectively.

Primer	Sequence
ISSR 1	5'-AGAGAGAGAGAGAGAGTC-3'
ISSR 2	5'-AGAGAGAGAGAGAGAGTG-3'
ISSR 3	5'-ACACACACACACACACYT-3'
ISSR 4	5'-ACACACACACACACACTG-3'
ISSR 5	5'-GTGTGTGTGTGTGTGTAG-3'
ISSR 6	5'-CGCGATAGATAGATAGATA-3'
ISSR 7	5'-GACGATAGATAGATAGATA-3'
ISSR 8	5'-AGACAGACAGACAGACGC-3'
ISSR 9	5'-GATAGATAGATAGATAGC-3'
ISSR 10	5'-GACAGACAGACAGACAAT-3'

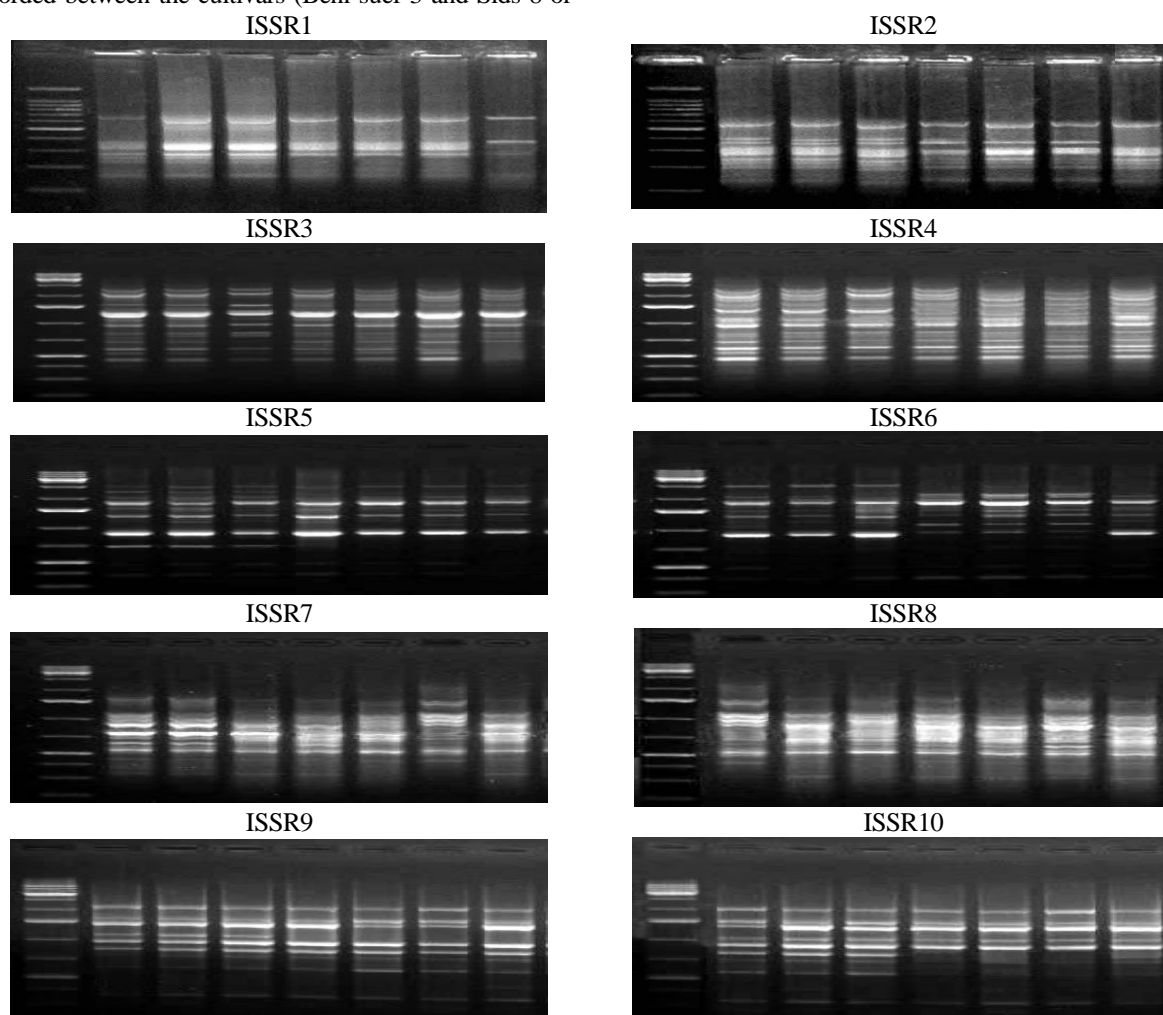


Figure 1. ISSR fingerprinting of wheat cultivars: M; DNA marker, lanes 1-7; Beni-suef-1, Beni-suef-3 and Sohag-1 for *Triticum dicoccum* L., and Sids-8, Sids-12, Sids-13 and Giza-171, for *Triticum aestivum* L., respectively.

Durum wheat karyotype:-

Mitotic analysis of durum wheat genotypes (Beni-suef-1, Beni-suef-3 and Sohag-1) allowed to observe complements with $2n = 4x = 28$ chromosomes and with karyotype formula of $26m + 2sm$. The bread wheat cultivars (Sids-8, Sids-12, Sids-13 and Giza-171) presented $2n = 6x = 42$ chromosomes (Tables 5, 6 and 7). Chromosomal complements with $2n = 28$ and $2n = 42$ chromosomes have been recorded previously in durum wheat and common wheat respectively by other authors such as Abd Abd El-Twab (2006) and Endo *et al.* (2014).

As indicated by the examination of the chromosome morphology of the three genotypes of durum wheat the most noteworthy incentive for chromosome length was seen in Sohag-1 (14.84 μ m for chromosome 2A) what's more, the littler incentive in Beni-suef-3 (5.16 μ m for the chromosome 7B). The CI values (centromere index) obtained for durum wheat ranged from 0.48 for 5B to 0.97 for 7A (Table 4). Thus, according to the CI values for karyotypes of Sohag-1, all chromosomes are metacentric except 5B chromosome which was considered asymmetrical or submetacentric chromosome. The CI values (centromere index) obtained for durum wheat ranged from 0.50 for 5B to 1.00 for 7A (Table 4). Thus, according to the CI values for karyotypes of both of Beni-suef-1 and Beni-suef-3, all chromosomes are metacentric and considered symmetrical or metacentric chromosome.

Asymmetrical karyotype was also observed in hexaploid wheat by Arabbeigi *et al.* (2011). According to Stebbins (1971), the karyotypes of wheat genotypes examined right now be additionally viewed as deviated once they present chromosome matches very extraordinary long, that is, these karyotypes are viewed as heterogeneous with respect to the length of their chromosomes. the karyotypes of wheat genotypes dissected right now be likewise viewed as uneven once they present chromosome matches very unique long, that is, these karyotypes are viewed as heterogeneous with respect to the length of their chromosomes. This is clarified by that the littler

chromosomes is found in genome D, because, as revealed by Jahan and Vahidy (1989) and Gill *et al.* (1991), the bread wheat has the AABBDD genome while, durum wheat has the AABB genome, and the D genome chromosomes are littler when contrasted and chromosomes of A and B genomes. Every investigated genotype of tetraploid uncovered the existence of 2 sets of chromosomes conveying satellites (SAT) on the short arms. That is a typical among the *T. durum* species. Chromosomes that have satellites (SAT) typically are bearers of nucleolar organizing regions (NORs); Zhang, *et al.*, (2015).

Discussion

The dendrogram dependent on ISSR markers separated the wheat cultivars additionally into two principle bunches with certain distinctions. El-Assal and Gaber (2012) contemplated the capacities of RAPD, ISSR and SSR markers in inception of genetic relationship and contrasting among Egyptian and Saudi wheat genotypes.

They abridged the outcomes as the ISSR markers produce more repeat, polymorphism and can be utilized in cultivar recognition; Shoaib and Arabi (2006). Moreover, Abou-Deif *et al.* (2013) presumed that the ISSR markers were exceptionally proficient in recognition among 20 wheat genotypes that were diverse in their genetic background and origin.

Mitotic analysis of durum wheat genotypes (Beni suef-1, Beni suef-3 and Sohag-1) allowed to observe complements with $2n = 4x = 28$ chromosomes and with karyotype formula of $26m + 2sm$. The bread wheat cultivar (Sids-8, Sids-12, Sids-13 and Giza-171) presented $2n = 6x = 42$ chromosomes and karyotype formula of $34m + 8sm$ (de Oliveira and Pinto-Maglio, 2017). Chromosomal complements with $2n = 28$ and $2n = 42$ chromosomes have been recorded already in durum wheat and common wheat separately by different writers, for example, Abd Abd El-Twab (2006), Schubert, (2007), Endo *et al.* (2014), and Pang, *et al.*, (2014).

Table 3. ISSR analyses of wheat cultivars.

Primers	Total number of bands	Number of polymorphic bands	% of polymorphic bands	Unique bands
ISSR-1	21	5	24 %	16
ISSR-2	33	10	30 %	23
ISSR-3	55	22	40 %	33
ISSR-4	58	21	36 %	37
ISSR-5	45	9	20 %	36
ISSR-6	41	8	20 %	33
ISSR-7	46	13	28 %	33
ISSR-8	46	10	22 %	36
ISSR-9	49	5	10 %	44
ISSR-10	37	14	38 %	23
Total	431	117	27 %	314

Table 4. The dissimilarity matrix based on ISSR data between the seven wheat cultivars

Cultivars	Sids-8	Sids-12	Sids-13	Giza-171	Beni Swef-1	Beni Swef-3	Sohag-1
Sids-8	1						
Sids-12	0.16	1					
Sids-13	0.09	0.14	1				
Giza-171	0.07	0.07	0.11	1			
Beni-suef-1	0.06	0.07	0.08	0.10	1		
Beni-suef-3	0.05	0.05	0.10	0.20	0.17	1	
Sohag-1	0.06	0.10	0.06	0.08	0.09	0.12	1

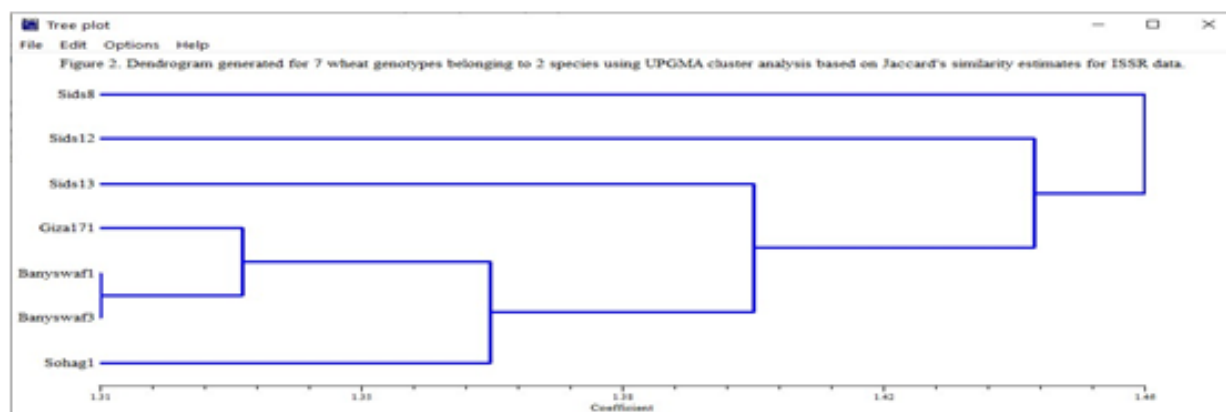


Figure 3. Dendrogram created for seven wheat genotypes having a place with two species utilizing UPGMA cluster analysis dependent on Jaccard's similarity estimates for ISSR information.

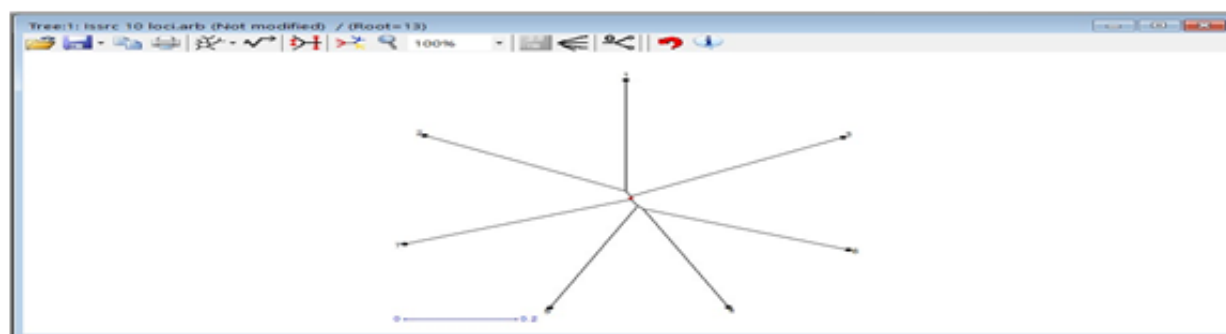


Figure 4. Radial tree created by DARwin from ISSR data utilizing Jaccard's similarity coefficient and UPGMA technique for seven genotypes of wheat dependent on 10 polymorphic fragments ISSR markers.

Table 5. Karyotypic characteristics of Beni-suef-1 (*Triticum durum* L.) genotype. Long arms (P) and short arms (q) for the genome A and Genome B for durum wheat at metaphase.

Morphological position	Chromosome No	type	P + q μ m	P μ m	Q μ m	CIP/q μ m	Area μ m ²	CL
1	2	1A	10.84	3.68	7.16	0.51	17.78	M
2	1	1A	11.26	3.89	7.37	0.53	18.59	M
3	4	2A	10.32	4.42	5.89	0.75	15.86	M
4	3	2A	10.00	4.32	5.68	0.76	17.84	M
5	6	3A	10.42	4.63	5.79	0.80	15.21	M
6	5	3A	10.32	4.53	5.79	0.78	17.65	M
7	8	4A	8.53	3.16	5.37	0.59	14.37	M
8	7	4A	8.95	3.26	5.68	0.57	15.52	M
9	10	5A	7.58	2.74	4.84	0.57	12.62	M
10	9	5A	8.21	2.95	5.26	0.56	14.34	M
11	12	6A	8.00	3.79	4.21	0.90	14.04	M
12	11	6A	8.95	4.21	4.74	0.89	14.2	M
13	14	7A	7.89	3.89	4.00	0.97	11.96	M
14	13	7A	8.32	4.11	4.21	0.97	13.8	M
15	16	1B	9.05	3.47	5.58	0.62	13.15	M
16	15	1B	9.58	3.68	5.89	0.63	15.89	M
17	18	2B	7.47	3.37	4.11	0.82	11.71	M
18	17	2B	8.00	3.58	4.42	0.81	11.91	M
19	20	3B	6.84	2.95	3.89	0.76	10.63	M
20	19	3B	8.11	3.47	4.63	0.75	11.67	M
21	22	4B	7.16	3.37	3.79	0.89	11.21	M
22	21	4B	7.26	3.37	3.89	0.86	11.27	M
23	24	5B	6.74	2.21	4.53	0.49	10.24	S M
24	23	5B	6.53	2.11	4.42	0.48	10.39	S M
25	26	6B	6.11	2.84	3.26	0.87	11.58	M
26	25	6B	8.42	3.89	4.53	0.86	11.7	17.79
27	28	7B	6.11	2.42	3.68	0.66	8.75	11.68
28	27	7B	6	2.42	3.58	0.68	9.63	12.95

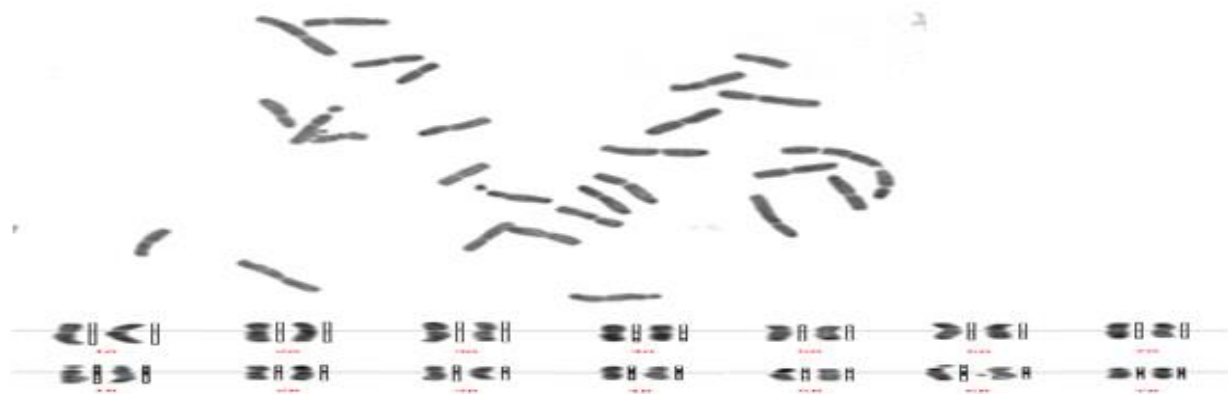


Fig. 5. Mitotic chromosomes of genotypes of Tetraploid wheat.

Table 6. Karyotypic characteristics of Beni-suef-3 (*Triticum durum* L.) genotype. Long arms (P) and short arms (q) for the genome A and Genome B for durum wheat at metaphase.

Morphological position	Chromosome No	type	P + q μm	P μm	Q μm	CI P/q μm	Area μm^2	CL
1	2	1A	10.11	3.47	6.63	0.52	15.66	M
2	1	1A	11.26	3.89	7.37	0.53	18.76	M
3	4	2A	9.89	4.21	5.68	0.74	16.71	M
4	3	2A	10.21	4.42	5.79	0.76	17.07	M
5	5	3A	8.74	3.89	4.84	0.8	12.51	M
6	6	3A	10.32	4.53	5.79	0.78	15.9	M
7	8	4A	8.63	3.16	5.47	0.58	12.72	M
8	7	4A	8.84	3.26	5.58	0.58	14.13	M
9	9	5A	7.68	2.74	4.95	0.55	11.69	M
10	10	5A	8	2.84	5.16	0.55	11.77	M
11	11	6A	7.68	3.58	4.11	0.87	11.31	M
12	12	6A	7.68	3.58	4.11	0.87	12.62	M
13	14	7A	6.32	3.16	3.16	1	9.68	M
14	13	7A	6.84	3.37	3.47	0.97	9.88	M
15	15	1B	10.63	4.11	6.53	0.63	15.43	M
16	16	1B	9.89	3.79	6.11	0.62	15.79	M
17	17	2B	7.79	3.47	4.32	0.8	12.29	M
18	18	2B	8.11	3.68	4.42	0.83	13.39	M
19	20	3B	6.53	2.84	3.68	0.77	10.32	M
20	19	3B	6.95	2.95	4	0.74	11.1	M
21	22	4B	6.74	3.16	3.58	0.88	10.49	M
22	21	4B	6.63	3.05	3.58	0.85	10.86	M
23	23	5B	6.32	2.11	4.21	0.5	9.24	M
24	24	5B	6	2	4	0.5	9.3	M
25	25	6B	8.63	4	4.63	0.86	11.76	M
26	26	6B	8.21	3.79	4.42	0.86	13	M
27	28	7B	5.47	2.21	3.26	0.68	8.01	M
28	27	7B	5.16	2	3.16	0.63	8.58	M

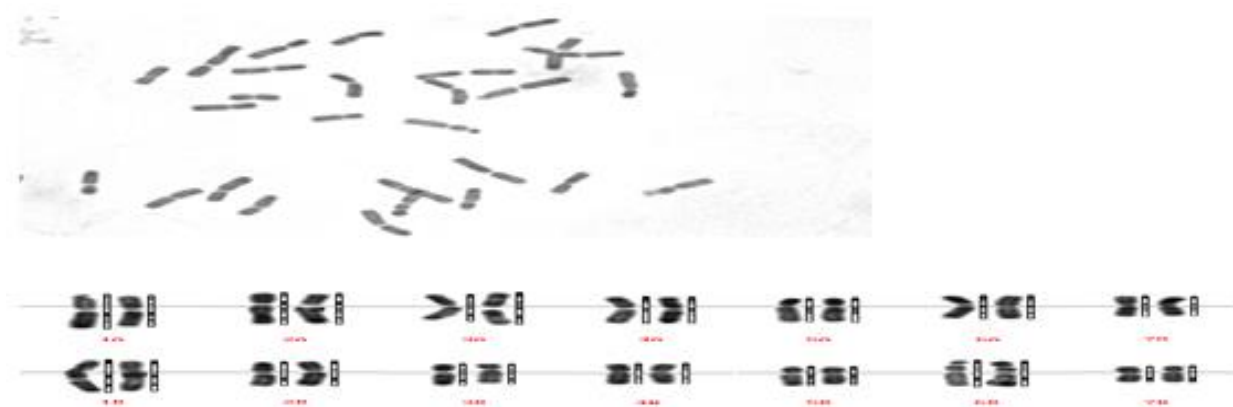


Figure 6. Mitotic chromosomes of genotypes of Tetraploid wheat.

Table 7. Karyotypic characteristics of Sohag-1 (*Triticum durum* .) genotype. Long arms (P) and short arms (q) for the genome A and Genome B for durum wheat at metaphase.

Morphological position	Chromosome No	type	P + q μm	P μm	Q μm	CI P/q μm	Area μm^2	CL
1	2	1A	13.37	4.63	8.74	0.53	20.27	M
2	1	1A	14	4.84	9.16	0.53	23.09	M
3	3	2A	14.53	6.21	8.32	0.75	23.2	M
4	4	2A	14.84	6.42	8.42	0.76	23.69	M
5	5	3A	12.21	5.37	6.84	0.78	19.98	M
6	6	3A	13.47	6	7.47	0.8	20.78	M
7	8	4A	11.37	4.21	7.16	0.59	16.96	M
8	7	4A	10.53	3.89	6.63	0.59	17.4	M
9	9	5A	10.53	3.79	6.74	0.56	17.17	M
10	10	5A	11.05	4	7.05	0.57	18.46	M
11	12	6A	9.79	4.63	5.16	0.9	15.48	M
12	11	6A	10.74	5.05	5.68	0.89	17.86	M
13	14	7A	9.68	4.84	4.84	1	16.18	M
14	13	7A	9.58	4.74	4.84	0.98	16.28	M
15	16	1B	9.37	3.68	5.68	0.65	14.52	M
16	15	1B	11.68	4.53	7.16	0.63	18.77	M
17	17	2B	9.58	4.32	5.26	0.82	14.07	M
18	18	2B	9.89	4.53	5.37	0.84	14.3	M
19	20	3B	8.74	3.79	4.95	0.77	13.16	M
20	19	3B	9.26	4	5.26	0.76	14.34	M
21	21	4B	8	3.79	4.21	0.9	12.04	M
22	22	4B	8.95	4.21	4.74	0.89	13.16	M
23	24	5B	7.58	2.53	5.05	0.5	12.31	M
24	23	5B	7.58	2.53	5.05	0.5	12.55	M
25	26	6B	7.05	3.26	3.79	0.86	11.15	M
26	25	6B	7.47	3.47	4	0.87	11.94	M
27	28	7B	6.32	2.53	3.79	0.67	10.43	M
28	27	7B	6.74	2.63	4.11	0.64	10.99	M



Figure 7. Mitotic chromosomes of genotypes of Tetraploid wheat.

CONCLUSION

This investigation gave some light on the genetic diversity among significant Egyptian wheat implanted genotypes utilizing ISSR markers. ISSR markers indicated higher polymorphism. Recognition of new specific markers is significant for breeders to assess wheat germplasm for breeding projects. Chromosomal characterization acquired by numerical and morphological investigation permits the localization and relationship of gene regions responsible for particular characters to their chromosomes.

REFERENCES

Abou-Deif, M.H., Rashed, M.A., Sallam, M.A.A., Mostafa, E.A.H. and Ramadan, W.A., 2013. Characterization of twenty wheat varieties by ISSR markers. Middle East Journal of Scientific Research, 15(2), pp.168-175.

Almanza-Pinzon, M.I., Khairallah, M., Fox, P.N. and Warburton, M.L., 2003. Comparison of molecular markers and coefficients of parentage for the analysis of genetic diversity among spring bread wheat accessions. *Euphytica*, 130(1), pp.77-86.

Arabbeigi, M., Arzani, A. and Saeidi, G., 2011. Study of karyotype and nucleolar organizer regions (NORs) in wild, synthetic and cultivated wheats. *Emirates Journal of Food and Agriculture*, pp.196-203. <http://dx.doi.org/10.9755/ejfa.v23i2.6457>.

Arzani, A., 2005. Evaluation of diversity in wild relatives of wheat. *Czech J. Genet. Plant Breed.*, 41, pp.110-116.

Awika, J.M., Piironen, V. and Bean, S. eds., 2011. *Advances in cereal science: implications to food processing and health promotion*. American Chemical Society.

- Barrett, B.A. and Kidwell, K.K., 1998. AFLP-based genetic diversity assessment among wheat cultivars from the Pacific Northwest. *Crop science*, 38(5), pp.1261-1271.
- Barrett, B.A., Kidwell, K.K. and Fox, P.N., 1998. Comparison of AFLP and pedigree-based genetic diversity assessment methods using wheat cultivars from the Pacific Northwest. *Crop Science*, 38(5), pp.1271-1278.
- Bohn, M., Utz, H.F. and Melchinger, A.E., 1999. Genetic similarities among winter wheat cultivars determined on the basis of RFLPs, AFLPs, and SSRs and their use for predicting progeny variance. *Crop science*, 39(1), pp.228-237.
- Dawlah, A.M., Khalid, A.E. and Abdelsalam, N.R., 2015. Genetic relationship between some Egyptian and Yemeni wheat based on different markers. *Mid. East. J. Sci. Res*, 5(2), pp.487-495.
- Oliveira, É.C.D. and Pinto-Maglio, C.A.F., 2017. Cytomolecular characterization of cultivars and landraces of wheat tolerant and sensitive to aluminum toxicity. *Bragantia*, 76(4), pp.456-469.
- El-Assal, S.E.D. and Gaber, A., 2012. Discrimination capacity of RAPD, ISSR and SSR markers and of their effectiveness in establishing genetic relationship and diversity among Egyptian and Saudi wheat cultivars. *American Journal of Applied Sciences*, 9(5), p.724.
- El-Twab, M.H.A., 2007. Physical mapping of the 45S rDNA on the chromosomes of *Triticum turgidum* and *T. aestivum* using fluorescence in situ hybridization for chromosome ancestors. *Arab J. Biotechnol*, 10, pp.69-80.
- Etminan, A., Pour-Aboughadareh, A., Mohammadi, R., Ahmadi-Rad, A., Noori, A., Mahdavian, Z. and Moradi, Z., 2016. Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. *Biotechnology & Biotechnological Equipment*, 30(6), pp.1075-1081.
- Endo, T.R., Kubaláková, M., Vrána, J. and Doležel, J., 2014. Hyperexpansion of wheat chromosomes sorted by flow cytometry. *Genes & genetic systems*, 89(4), pp.181-185.
- Friebe, B., Jiang, J., Raupp, W.J., McIntosh, R.A. and Gill, B.S., 1996. Characterization of wheat-alien translocations conferring resistance to diseases and pests: current status. *Euphytica*, 91(1), pp.59-87.
- Gill, B.S., Friebe, B. and Endo, T.R., 1991. Standard karyotype and nomenclature system for description of chromosome bands and structural aberrations in wheat (*Triticum aestivum*). *Genome*, 34(5), pp.830-839.
- Hoisington, D., M. Khairallah and D. Gonzalea-de-leon 1994. Laboratory protocols, CIMMYT Applied Molecular Genetics Laboratory. 2nd ed. (Available online at <http://www.Cimmyt.org/abc/protocols/manualabc.html> verified 14 nov. 2004). Cimmyt, Mexico.
- Huang, X., Börner, A., Röder, M. and Ganal, M., 2002. Assessing genetic diversity of wheat (*Triticum aestivum* L.) germplasm using microsatellite markers. *Theoretical and Applied Genetics*, 105(5), pp.699-707.
- Jahan, Q. and A Vahidy, A., 1989. Karyotype analysis of hexaploid wheat, *Triticum aestivum* L. cv.'Sarsabz'. *Medical Journal of Islamic World Academy of Sciences*, 2(3), pp.179-181.
- Jiang, J., Friebe, B. and Gill, B.S., 1993. Recent advances in alien gene transfer in wheat. *Euphytica*, 73(3), pp.199-212.
- Lage, J., Warburton, M.L., Crossa, J., Skovmand, B. and Andersen, S.B., 2003. Assessment of genetic diversity in synthetic hexaploid wheats and their *Triticum dicoccum* and *Aegilops tauschii* parents using AFLPs and agronomic traits. *Euphytica*, 134(3), pp.305-317.
- Levan, A., Fredga, K. and Sandberg, A.A., 1964. Nomenclature for centromeric position on chromosomes. *Hereditas*, 52(2), pp.201-220. <http://dx.doi.org/10.1111/j.1601-5223.1964.tb01953.x>.
- Pang, Y., Chen, X., Zhao, J., Du, W., Cheng, X., Wu, J., Li, Y., Wang, L., Wang, J. and Yang, Q., 2014. Molecular cytogenetic characterization of a wheat–*Leymus mollis* 3D (3Ns) substitution line with resistance to leaf rust. *Journal of Genetics and Genomics*, 41(4), pp.205-214.
- Randhawa, H.S., Asif, M., Pozniak, C., Clarke, J.M., Graf, R.J., Fox, S.L., Humphreys, D.G., Knox, R.E., DePauw, R.M., Singh, A.K. and Cuthbert, R.D., 2013. Application of molecular markers to wheat breeding in Canada. *Plant Breeding*, 132(5), pp.458-471.
- Reeves, A., 2001. MicroMeasure: a new computer program for the collection and analysis of cytogenetic data. *Genome*, 44(3), pp.439-443.
- Reif, J.C., Zhang, P., Dreisigacker, S., Warburton, M.L., van Ginkel, M., Hoisington, D., Bohn, M. and Melchinger, A.E., 2005. Wheat genetic diversity trends during domestication and breeding. *Theoretical and Applied Genetics*, 110(5), pp.859-864.
- Rejesus, R.M., Smale, M. and Ginkel, M.V., 1996. Wheat breeders' perspectives on genetic diversity and germplasm use: findings from an international survey. *Plant Varieties and Seeds*, 9(3), pp.129-147.
- Ridout, C.J., Donini, P., Ridout, C.J. and Donini, P., 1999. Use of AFLP in cereals research. *Trends in Plant Science*, 4(2), pp.76-79.
- Rohlf, F.J., 2005. Numerical Taxonomy and Multivariate Analysis System Version 2.2 Exeter Software. New York, USA.
- Sabbour AM, Gomaa EF, Sallam RA, Shaaban SA (2015). Genetic diversity among some wheat (*Triticum aestivum* L.) genotypes as revealed by RAPD and SSR Analyses. *Am-Eur J. Agric. Environ. Sci*. 15 (10): 2069-2075.

- Saghai-Marouf, M.A., Soliman, K.M., Jorgensen, R.A. and Allard, R.W.L., 1984. Ribosomal DNA spacer-length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proceedings of the National Academy of Sciences*, 81(24), pp.8014-8018.
- Schubert, I., 2007. Chromosome evolution. *Current opinion in plant biology*, 10(2), pp.109-115.
- Shoaib, A. and Arabi, M.I.E., 2006. Genetic diversity among Syrian cultivated and landraces wheat revealed by AFLP markers. *Genetic Resources and Crop Evolution*, 53(5), pp.901-906.
- Soleimani, V.D., Baum, B.R. and Johnson, D.A., 2002. AFLP and pedigree-based genetic diversity estimates in modern cultivars of durum wheat [*Triticum turgidum* L. subsp. durum (Desf.) Husn.]. *Theoretical and Applied Genetics*, 104(2-3), pp.350-357.
- Stebbins, G.L., 1971. Chromosomal evolution in higher plants. *Chromosomal evolution in higher plants*.
- Yildirim, F. and Akkaya, M.S., 2006. DNA fingerprinting and genetic characterization of Anatolian *Triticum* spp. using AFLP markers. *Genetic Resources and Crop Evolution*, 53(5), pp.1033-1042.
- Zhang, H., Li, G., Li, D., Gao, D., Zhang, J., Yang, E. and Yang, Z., 2015. Molecular and cytogenetic characterization of new wheat—*Dasypyrum breviaristatum* derivatives with post-harvest re-growth habit. *Genes*, 6(4), pp.1242-1255.

تقييمات جزيئية خلوية للتنوع الوراثي في نوعي القمح المنزرع في مصر.

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تم دراسة الاختلافات الوراثية بين سبعة تراكيب وراثية من القمح ثلاثة تراكيب رباعية وأربعة تراكيب سداسية المجموعة الكروموسومية. تم تقدير درجة التباعد الوراثي باستخدام تكنيك التتابعات البينية للتكرارات البسيطة المتسلسلة وأيضاً باستخدام تقنيات الوراثة الخلوية. وقد تم تقييم ثلاثة تراكيب وراثية من الـ *Triticum dicoccum* (سوهاج-1، وبني سويف-1، وبني سويف-3) أقماح الرباعية. وأربعة تراكيب وراثية من سداسي المجموعة الكروموسومية (*Triticum aestivum*) سدس-8، سدس-12، سدس-13، جيزة-171. تم تقييم التنوع الوراثي بين مختلف التراكيب الوراثية من القمح باستخدام 10 بادئات ISSR. تم الكشف عن 431 واسم جزيئي. كان 117 واسم جزيئي بنسبة (27%) متعدد الأشكال بمتوسط 27 حزمة تقريباً لكل زوج من أزواج البادئات. تم تحليل شجرة التفرع المتكونة من 7 تراكيب وراثية تنتمي إلى النوعين (الدندرجرام) ظهرت التراكيب الوراثية المستخدمة في مجموعتين رئيسيتين تعكس تكوين الجينوم. المجموعة الأولى تشمل القمح ذات الجينومات AB. في حين اشتملت المجموعة الثانية القمح صاحب الجينومات ABD. وتراوحت معاملات التشابه الوراثي بين 0.05 بين بني سويف-3 (تريتيكوم ديكوكوم) وسدس-8 وسدس-12 (تريتيكوم إساتيفوم). وفيما يتعلق بتحليل شكل الكروموسومات للتراكيب الوراثية الثلاثة لقمح الديوروم، لوحظت أعلى قيمة لطول الكروموسوم في سوهاج-1 (14.84 ميكرومتر للكروموسوم أ2) والقيمة الأصغر في بني سويف-3 (5.16 ميكرومتر للكروموسوم 7ب). وتراوحت قيم CI (مدلول السنتروميتر) التي تم الحصول عليها لقمح الديوروم ما بين 0.48 في 5ب إلى 0.97 في 7أ. وبالتالي، وفقاً لقيم CI الخاصة بطراز الهيئة الكروموسومية للتراكيب الوراثي سوهاج 1، فإن جميع الكروموسومات هي وسطية السنتروميتر ما عدا كروموسوم 5ب. وتراوحت قيم CI التي تم الحصول عليها لقمح الديوروم ما بين 0.50 في 5ب و 1.00 لـ 7أ.