GENETICAL AND PRODUCTIVITY EVALUATION OF SOME TRITICALE GENOTYPES UNDER NORTH SINAI CONDITIONS

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olecular markers are important tools in plant genome analysis and became as a useful tool in crop improvement. It facilitates the identification of markers for agronomical important traits and for genetic and genomic characterization. The current study aims at the identification of the genetic diversity and relationship, as well as the evaluation of the productively of five Triticale genotypes, namely; ICBA 2, Triticale 8, 11, 7 and 6, imported from ICBA "International Center for Biosaline Agriculture" Dubai, Arab Emirates to perform genetic and productivity evaluation under North Sinai conditions. The study was performed at Baloza Research Station, Desert Research Center, in 2016-2017 and 2017-2018 seasons. Two molecular markers; ISSR and SCoT target different regions of DNA were used for genetic studies. Twelve ISSR and SCoT molecular markers were selected from 25 markers, which produced clear bands were selected. The six ISSR primers produced 78 amplified fragments, 30 of them were polymorphic, representing around 38.5%. The ISSR markers similarity matrix ranged from 81 to 92%, where the highest similarity matrix was between Triticale 8, 7 and 8, 6. The lowest similarity was between Triticale 11 and 7. The SCoT primers produced a total of 89 amplified fragments, where 28 of them were polymorphic, representing about 31.5% of polymorphism. The SCoT markers similarity matrix ranged from 94 to 85%. The highest similarity matrix was between Triticale 8, 7 and 8, 6, too. The dendrograms based on ISSR, SCoT and both of them were attended, the total polymorphism was 34.7% with PIC value of 0.9415. Triticale 11 and ICBA 2 were the most distinguished genotypes. The results showed that both ISSR and SCoT markers were efficient for analyzing genotypes. The productivity results revealed that Triticale 11 had the highest values in yield

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components as well as the other studied characters, while the lowest values were restricted to Triticale 6 in both studied seasons. According to the studied yield component characters (grain, straw and biological yield as kg/feddan), Triticale genotypes can be arranged in descending order as T. 11, ICBA 2, T. 8, T. 7 and T. 6, that have the highest value to the lowest in the two seasons.

Keywords: Triticale, ISSR, SCoT, molecular marker, yield component

Triticale (x Triticosecale Wittmack) is a hybrid between wheat (Triticum spp.) and rye (Secale cereale), so it has the properties of its parent cereals (Tams et al., 2004 and Jonnala et al., 2010) particularly those related to biotic and abiotic stresses (Vaillancourt et al., 2007). Triticale is a crop has broad genetic potential, so it is widely adapted to abiotic stress conditions such as aluminum toxicity, drought, salinity, and acidic soils (Kuleung et al., 2004). It is resistant to different diseases of wheat (Leonova et al., 2005). It has become an important grain and forage crop in many areas of the world (Avalew et al., 2018). Triticale gain is a good source of vitamin B, essential amino acids, proteins and lysine, which is considered more than wheat (Glamočlija et al., 2018). Medicinal importance of Triticale includes improving digestive efficiency, heart health, increasing healing and metabolic rate, improving energy levels, protecting infants in the womb, preventing and managing diabetes, increasing blood circulation, protecting against asthma, reducing various skin conditions, and contributing to strong bones (John Staughton, 2019). Information of germplasm diversity has a significant role in the improvement of crop plants. Molecular markers give an effective data for good selection of desired agronomic traits because they depend on the plant genotypes and thus, are independent of environmental conditions. Molecular markers are effective tools for plant genome analysis, and therefore has an essential role in crop improvement. Besides, the molecular markers are used for the detection and exploitation of DNA polymorphism. The genetic diversity helps breeders to select economical genotypes and thus multiply the productivity of crops (Randhawa et al., 2013). Determining genetic diversity can be dependent on agronomic, morphological, biochemical, and molecular information (Goncalves et al., 2009). Molecular markers have been employed for genetic diversity evaluation, genetic fingerprint, genetic mapping, and quantitative trait locus analysis. SCoT and ISSR markers have been used efficiently for studying genetic diversity of plants (Ma et al., 2008; Collard and Mackill, 2009 and Etminan et al., 2016). ISSR-PCR marker is a technique that requires primer based on SSR (microsatellites) markers. SSRs are common in the genome, the changes of which by deletion or insertion, lead to ISSR polymorphism (Wang, 2010). Possibility of ISSR markers to construct genetic maps, fingerprinting and phylogenetic analyses have been confirmed by many authors in connection with different crops such as; Gradzielewska et

al. (2012) in Triticale, Idris et al. (2012), Žiarovská et al. (2013) in maize, Khaled et al. (2015) in wheat and Saad-Allah and Youssef (2018) in Quinoa. Lately, a DNA marker technique called Start Codon Targeted (SCoT) polymorphism was developed by Collard and Mackill (2009), their primers were designed from the conserved region surrounding the translation initiation codon, ATG (Joshi et al., 1997 and Sawant et al., 1999) and it is used for the construction of genetic maps, fingerprinting and phylogenetic studies. It has been proved by many authors in many crops, such as tomato (Shahlaei et al., 2014), citrus (Mahibi et al., 2015), date palm (Al-qurainy et al., 2015), castor (Kallamadi et al., 2015), mango (Gajera et al., 2014), barley (Aboulila and Mansour, 2017), maize (Vivodík et al., 2017 and Sadek and Ibrahim, 2018). This study aims at evaluation genetic diversity, relationships and productively of five Triticale genotypes, namely; ICBA 2, 8, 11, 7 and 6, which imported from ICBA, Dubai, Arab Emirates, for the identification of markers for the important agronomical traits and genetic and genomic characteristics at Baloza research station, Desert Research Center, under North Sinai conditions, North Sinai, Egypt.

MATERIALS AND METHODS

Five Triticale genotypes ICBA 2, 8, 11, 7 and 6, imported from ICBA "International Center for Bio saline Agriculture", Dubai, Arab United Emirates, were used in this study. Two field experiments were carried out during the two winter seasons of 2016-2017 and 2017-2018 at Baloza research station, Desert Research Center, under North Sinai conditions. Grains were tested in complete randomized block design in three replicates. The experimental site has been divided into plots, 10, 5 m² each. Each plot included 10 rows apart 30 cm. Triticale crop was sown in the first season (2016-2017) on the 16th of November and in the second season (2017-2018) was sown on the 20th of November. Young fresh leaves were taken for genetic studies. The physical and chemical properties of soil as well as irrigation waters were recorded according to El-Henawy et al. (2018) as follows (Table 1, 2 and 3):

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Loc	ations	Depth (cı	n) Sa	and (%)	Silt (%) Cla	ay (%)	Soil tex	ture			
Ba	loza	0-30		90	5		5	Sandy				
Tabl	Table (2). Chemical analysis of experiment soil.											
Location	рН	E.C. (ds/m)	O.M (%)		Soluble (me	anions q/l)		Soluble cations (meq/l)				
				CO3	HCO ₃ -	Cl	SO 4	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	\mathbf{K}^{+}	
Baloza	8.17	5.99	0.17	-	2.46	40.71	17.17	19.53	13.25	26.91	0.65	

Table (1). Mechanical analysis of experiment soil.

Location	рН	E.C. (ds/m)	Soluble anions (meq/l)				Soluble cations (meq/l)				
	-		CO3	HCO3 ⁻	Cl	SO4	Ca++	Mg ⁺⁺	Na ⁺	K ⁺	
Baloza	7.10	1512	0.50	3.81	3.69	8.20	3.25	3.05	9.50	0.40	

Table (3). Chemical analysis of irrigation water.

1. Extraction and Purification of Genomic DNA

DNA was extracted from young leaves of five Triticale genotypes by DNeasy Plant Mini Kit (Qiagen Santa Clarita, CA), Cat No./ID: 69104.

2. ISSR and SCoT Markers Reaction

Six ISSR primers were used in the detection of polymorphism among the studied Triticale genotypes. These primers were synthesized by Metabion Corp., Germany. The primers code and nucleotide sequences are presented in table (4).

Six SCoT primers (Table, 4) were designed by Collard and Mackill (2009) depending on the consensus sequences of translation initiation codon region in higher plants with ATG codon at locations +1, +2, +3; 'G' at location +4; and 'A,' 'C,' and 'C' at locations +7, +8, and +9, respectively.

The PCR amplification reactions were carried out as mentioned by Adawy et al. (2002) and Ibrahim et al. (2016). Reactions were prepared in 25 μ l volume composed of 1x reaction buffer, 0.2 mM of dNTPs, 1.5 mM MgCl₂, 0.2 μ M of primer, 0.5 unit of Taq polymerase (Qiagen Ltd., Germany) and 40 ng of template DNA, in sterile distilled water.

Table (4). Primers used in ISSR and SCoT analysis.

Primer	Sequence
ISSR	
ISSR-1	5'-AGAGAGAGAGAGAGAGAGYC-3'
ISSR-2	5'-AGAGAGAGAGAGAGAGAGYG-3'
ISSR-3	5'-ACACACACACACACACYT-3'
ISSR- 5	5'-GTGTGTGTGTGTGTGTGTYG-3'
ISSR-6	5'-CGCGATAGATAGATAGATA-3'
ISSR-7	5'-GACGATAGATAGATAGATA-3'
SCoT	
SCoT-19	5'-CCATGGCTACCACCGGCG -3'
SCoT-20	5'-CAACAATGGCTACCACGC -3'
SCoT-23	5'-CATGGCTACCACCGGCCC-3'
SCoT-24	5'-CCATGGCTACCACCGCAG-3'
SCoT-25	5'-ACGACATGGCGACCGCGA-3'
SCoT-36	5'-CACCATGGCTACCACCAT-3'

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3. ISSR and SCoT Markers Thermocycling Profile and Detection of PCR Products

PCR amplification of the DNA was performed in a Perkin Elmer thermal cycler 9700. The temperature profile in the different cycles was as follows: an initial strand separation cycle at 94°C for 5 min, followed by 40 cycles comprised of a denaturation step at 94°C for 1 min, an annealing step at 45°C for 1 min and an extension step at 72°C for 1.5 min. The final cycle was a polymerization cycle for 7 min at 72°C.

The amplification products were separated in 1.5% agarose gels containing ethidium bromide (0.5 mg/ml) in 1 x TBE buffer at 120 volts. A 1kb DNA ladder was used as molecular size standard. PCR products were visualized under UV light and documented using a ^{TM}XR + Gel Documentation System (Bio-Rad).

4. Data Analysis

The banding patterns, generated by ISSR-PCR and SCoT-PCR markers, were compared to detect the genetic relations among the samples under study. Clear and distinct amplification products were scored as '1' for presence and '0' for absence of bands. Bands of the same mobility were scored as identical.

The polymorphism information content (PIC) was calculated according to the formula of Anderson et al. (1993), as follows:

PIC=1- $\Sigma pi2$

Where pi is the frequency of the *i* the allele of the locus in eight genotypes.

The genetic similarity coefficient (GS) between two genotypes was estimated according to Dice coefficient (Sneath and Sokal, 1973).

Dice formula: $GS_{ij} = 2a/(2a+b+c)$

Where GSij is the measure of genetic similarity between individuals i and j, a is the number of bands shared by i and j, b is the number of bands present in i and absent in j, and c is the number of bands present in j and absent in i.

The similarity matrix was analyzed in the cluster analysis. The cluster analysis was used to organize the observed data into meaningful structures to develop taxonomies. In the first step, when each accession represents its own cluster, the distances between these accessions are defined by the chosen distance measure (Dice coefficient). However, once several accessions have been linked together, the distance between two clusters is calculated as the average distance between all pairs of accessions in the two different clusters. It was named Unweighted Pair Group Method using Arithmetic Average (UPGMA) (Sokal and Michener, 1958).

5. Productivity Studies, Measurements and Calculations

Harvest has taken place after 160 days from sowing, for the first and second seasons. Sample of 2 square meters (2 m x 1 m) was taken for each plot random at the yield and yield component and another character harvest

dates. The following characters were measured: plant height (cm), no. of spikes per m², no. of spikelets per spike, grain yield, straw yield and biological yield kg per feddan.

RESULTS AND DISCUSSION

1. ISSR Analysis

Six ISSR primers from ten, showing distinct and reliable fragments, were selected for this study to investigate the genetic relationship, DNA fingerprint profile and assessed genetic markers for stress tolerance in Triticale genotypes (ICBA 8, 11, 7, 6 and 2). So, they were cultivated in harsh environment. The results were represented in table (5) and fig. (1). The total amplified fragments produced by all the six ISSR primers were 78 with an average of 13 fragments per primer, out of which there were 30 polymorphic fragments with an average of 5 bands per primer with 38.5% of total polymorphism. ISSR primer 6 gave the most polymorphic amplified fragments; 12 polymorphic bands from 22 total amplified fragments, with 54.6% of polymorphism. The primer that produced the lowest amplified fragments was ISSR 3, where 2 polymorphic bands were generated from 12 amplified fragments, with 16.7% of polymorphism. The percentage of polymorphism, which produced by the six primers ranged from 16.7 with ISSR primer 3 to 54.5 with ISSR 1. The number of monomorphic amplified fragments generated by all six ISSR primers ranged from 5 to 10 bands. ISSR primers 2, 3, 7 and 5 produced four unique positive markers, one for each primer was representive to Triticale 11 and 7 at 1050, 900, 1050 bp and 220 bp, respectively, and two unique positive markers with ISSR primer 1, which distinguished Triticale 11 at 670,460 bp. Triticale 11 has got two negative unique markers at 390 and 520 bp, while Triticale 7 has got two negative unique markers at 340 bp. So, Triticale 11 has got the highest number of unique markers (seven markers), while Triticale 7 has only two unique markers. In this regard, Abd El-Lateif and Hewedy (2018) used ISSR and SCoT markers to investigate the genetic diversity and relationships among eight cultivars of Egyptian wheat (Shandweel-1, Misr-2, Sakha-93, Sakha-94, Giza-168, Giza-171, Sids-1 and Gemmiza-9). They mentioned that SCoT primers produced a total of 32 bands, out of which 19 (59%) were polymorphic with a mean of 3.16. ISSR primers produced 34 bands and 23 of these bands (68%) were polymorphic with a mean of 4.6. Their results indicated good sources of diversity, which will help breeders to evaluate genetic diversity and potentially select economically important traits such as salinity tolerance. On the other hand, El-Assal and Gaber (2012) studied the abilities of RAPD, ISSR and SSR markers in establishing genetic relationship and distinguishing between Egyptian and Saudi wheat cultivars. They concluded that the ISSR markers gave more recurrence, polymorphism and can be used in cultivar discrimination. Furthermore, Abou-Deif et al. (2013)

showed that the ISSR markers were highly efficient analyze the genetic diversity and relationships among 20 wheat genotypes using ISSR markers. The produced ISSR markers succeeded in distinguishing most of the 20 varieties of in relation to their genetic background and geographical origin (Table 5).

Table (5). Total bands (TB), polymorphic bands (PB), positive unique markers (PUM), negative unique markers (NUM), monomorphic bands (MB), polymorphic percentage (PB %) and polymorphism information content (PIC) value as reveled by ISSR and SCoT markers.

Primer	No. of TB	No. of PB	PUM (bp)	NUM (bp)	No. of MB	PB (%)	Mean of frequency	PIC
ISSR								
ISSR1	11	5	670,460	390,340	6	54.5	0.8	0.9477
ISSR2	9	2	1050	-	7	22.2	0.8	0.9111
ISSR3	12	2	900	-	10	16.7	0.9	0.9277
ISSR5	9	4	220	340	5	44.4	0.8	0.9234
ISSR6	22	12	-	-	10	54.5	0.7	0.9721
ISSR7	15	5	1050	520	10	33.3	0.9	0.9473
Total	78	30			48	38.5		
Average	13	5			8	38.5		
SCoT								
SCoT36	18	6	-	-	12	33.3	0.8	0.9587
SCoT24	21	10	630	1600,770,	11	47.6	0.9	0.9631
SCoT25	11	3	-	-	8	27.3	0.9	0.9282
SCoT19	13	1	1300	-	12	7.7	0.9	0.9287
SCoT20	15	4	-	1350	11	26.7	0.9	0.9434
SCoT23	11	4	-	2300,1150,	7	36.4	0.9	0.9209
Total	89	28			61	31.5		0.9415
Average	14.83	2.66			10.2	31.5		0.9415

2. SCoT Analysis

A set of 15 SCoT primers were used to study the genetic relationships, DNA fingerprint profile and assessed genetic markers for stress tolerance in Triticale genotypes (Triticale 8, 11, 7, 6 and ICBA2). Only six primers were selected in band scoring, which produced distinct and reproducible band patterns. A total of 89 amplified fragments were scored with six SCoT primers among the studied Triticale genotypes, 28 of them were polymorphic with an average of 4.6 per primer (Table 5 and Fig. 2). The number of amplified fragments were ranged from 11 for SCoT (primer 23 and 25) to 21 for SCoT

primer 24 with an average of 14.8 per primer. The percentage of polymorphism ranged from 7.7 to 36.4 with an average of 31.5 in all genotypes. The monomorphic bands ranged from 7 to 12 bands with all primers. Triticale ICBA 2 has two positive unique markers at 1300 bp and 630 bp with SCoT primer 19 and 24, respectively, and four negative unique markers at 2300, 1150, 610 and 390 bp with SCoT primer 23, which distinguished it from all other genotypes. While Triticale 8 has got two negative unique markers at 1600 bp (SCoT primer 24) and 1350 bp (SCoT primer 20). Triticale 6, 7 and 11 have got only one negative unique marker. Therefore, ICBA 2 has got the highest unique markers (six markers).

Depending on the results of all markers, the total polymorphic bands was 85 and the non-polymorphic was 109 amplified fragments, total PIC value was 0.9415 and ranged from 0.9111 (minimum) to 0.972 (maximum). SCoT markers are useful in diversity analysis and diagnostic fingerprinting; it has been successfully demonstrated by many authors in many crops, such as tomato (Shahlaei et al., 2014), citrus (Mahjbi et al., 2015), date palm (Al-qurainy et al., 2015), castor (Kallamadi et al., 2015), mango (Gajera et al., 2014), barley (Aboulila and Mansour, 2017), *Triticum astevium* (Mohamed et al., 2017) and in maize (Vivodík et al., 2017 and Sadek and Ibrahim, 2018). Their results indicated that SCoT markers would be useful for further studies in population genetics, conservation genetics and genotypes improvement.

3. Genetic Relationship, Similarity Matrix and Cluster Analysis Depending on ISSR Makers

Genetic relationships among the Triticale genotypes were studied by using the Dice coefficient and UPGMA methods to produce the similarity matrices and generate dendrogram tree (Table 6 and Fig. 3). The highest similarity matrices were scored between the Triticale 8, 7 and 8, 6 genotypes and was 92%, while the lowest genetic similarity was between Triticale 11 and 7 of 81% and the percentage of similarity matrix ranged from 81 to 92 among Triticale genotypes. The dendrogram was separated into two main clusters, the first one involved only Triticale 11, that has got the highest number of unique markers. The second cluster included the other tested genotypes. Triticale 8, 7, 6 were separated in a subcluster, so they are closely related. Triticale 8, and 7 were in the same group and ICBA 2 was separated alone in a subcluster. In virtue of that, ISSR markers were effective in distinguishing and differentiating the studied Triticale genotypes. Therefore, it is a useful selection for breeding studies. The different genotypes are the most desirable in hybridizations.



Fig. (1). ISSR electrophoresis profile of Triticale genotypes. where No. 1 is ICBA 2, No. 2 is Triticale 8, No. 3 is Triticale 11, No. 4 is Triticale 7 and No. 5 is Triticale 6.



Fig. (2). SCoT electrophoresis profile of Triticale genotypes, where No. 1 is ICBA 2, No. 2 is Triticale 8, No. 3 is Triticale 11, No. 4 is Triticale 7 and No. 5 is Triticale 6.

		ICBA2	Triticale 8	Triticale 11	Triticale 7	Triticale 6
ICBA 2		100				
Triticale 8		90	100			
Triticale 11	_	89	83	100		
Triticale 7		90	92	81	100	
Triticale 6		88	92	84	90	100
0.88	0.896 -	0.912 -	0.9 0.9 228 	0.96-	0.992 -	
		ſ				Triticael 8
Γ						Triticale 11
						Tritical 7
			L			Triticale 6
						Tritical ICBA

Table (6). Similarity matrix of ISSR markers among Triticale genotypes.

Fig. (3). Dendrogram of five Triticale genotypes based on data from six ISSR markers.

4. Genetic Relationship, Similarity Matrix and Cluster Analysis Depending on SCoT Markers

Dice coefficient and UPGMA methods were used to study the similarity matrices and generate the dendrogram (Table 7 and Fig. 2). The highest similarity matrix was 94%, between Triticale 8, 7 and 8, 6. While, the lowest one was 85% between ICBA 2 and Triticale 8. Triticale ICBA 2 has got the highest unique markers that were six, whereas Triticale 8 has got only two unique markers, so depending on SCoT markers, Triticale 8, 7 and 6 are closely related to each other. Therefore, Triticale ICBA 2 can be regarded as the most different to others, where the dendrogram which generated by similarity matrix was separated into two main clusters, one of which represented ICBA 2 alone and the other one included the other Triticale genotypes, that were again divided into two sub-clusters, one of them for Triticale 11 only and the other one included Triticale 7, 8 and 6, where Triticale 7 and 8 were separated in a single group.

	ICBA2	Triticale	Triticale	Triticale	Triticale
		8	11	7	6
ICBA 2	100				
Triticale 8	85	100			
Triticale 11	91	92	100		
Triticale 7	87	94	92	100	
Triticale 6	92	90	93	94	100
	0.84-	0.88 -	Similarity 0.92 1 1	0.96	0
					Triticale 11
					Triticael 8

 Table (7). Similarity matrix of SCoT markers among Triticale genotypes.



5. Coloration Between the Similarity Matrixes Measured Using ISSR and SCoT Markers

The similarity matrix and dendrogram tree depending on both ISSR and SCoT markers were very similar to each one separately (Table 8 and Fig. 5). Triticale genotypes 8, 7, and 6 had the highest genetic similarity (93%), while Triticale ICBA 2 and 8 had the lowest genetic similarity (87%), and Triticale 11 and 7 also had the lowest genetic similarity, as they showed by SCoT and ISSR markers. Triticale ICBA 2 and 11 were considered the most distinguished genotypes, so they have the highest number of unique bands, thus they may be promising for breeding studies. ISSR markers separated Triticale 11 in single main cluster, whereas SCoT markers separated ICBA 2 in a single main cluster. Thus, SCoT and ISSR markers confirmed that results by separating Triticale 11 and ICBA 2 in a main cluster. So, ICBA 2 and Triticale 11 can be selected for further breeding studies. ISSR and SCoT markers differentiated the studied genotypes. In general, the similarity matrix among Triticale genotypes was high and it had low to moderate variance of 34.7% polymorphism in average. It may be backed to the parents of high similar genotypes were closely related. In the same regard, Tams et al. (2004), Kuleung et al. (2006), de Costa et al. (2007) and Trebichalský et al. (2013) evaluated genetic distances in Triticale using microsatellite markers,

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mentioned that Triticale germplasm has high similarity. Also, Kuleung et al. (2006) studied 80 Triticale accessions, which were separated into five clusters with the average similarity of 0.45. Also, similarity (0.56) was observed among Brazilian Triticale forms (de Costa et al., 2007). About 15.3% of the variation was reported depending on European accessions of 13 breeding companies among available materials (Tams et al., 2004), indicating the limited variability of the materials.

Molecular characterization is almost used by Triticale breeders as an alternative way for selecting more promising genotypes and decreasing the cost and time needed to produce hybrid combinations. Based upon this approach theoretically, the most differences between crossed forms, the better performance of the offspring could be predicted (Moll et al., 1965). But, there is no evidence indicating that crossing even in closely related lines (high genetic similarity) sometimes may lead to reasonable grain yield (Fernandes et al., 2015). Nevertheless, crossing between heterotic groups increased the performance of the hybrid population by 2.8 % compared to non heterotic groups (Fischer et al., 2010). Thus, the exploitation of genetic variation and genetic structure is required and useful for breeders.

 Table (8). Similarity matrix of ISSR and SCoT markers among Triticale genotypes.



Fig. (5). Dendrogram of five Triticale genotypes of ISSR and SCoT markers.

Data in Table (9) revel that, in terms of the studied characters, the Triticale genotypes were non-significantly different from each other. There is a low variation among the five genotypes.

11	ve influent	e genotype						
Genotypes	Plant height (cm)		Spike length (cm)		No. of sj	oikes/m ²	No. of spikelets/spike	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
Triticale 11	138.70	130.50	16.13	15.33	490.83	452.30	38.58	36.65
ICBA 2	134.20	125.68	15.60	14.53	475.20	442.70	38.03	36.20
Triticale 8	130.76	121.23	15.03	13.50	463.35	434.03	37.45	35.63
Triticale 7	127.20	117.80	14.73	13.30	452.08	421.38	36.58	35.23
Triticale 6	122.90	114.60	14.38	12.78	438.60	410.85	36.08	34.65
LSD5%	NS	NS	NS	NS	NS	NS	NS	NS

Table (9). Analysis of variance of the productivity measurements of plant height, spike length, No. of spikes/m², No. of spikelets/spike of the five Triticale genotypes.

6.1. Plant height

Data in table (9) show that Triticale 11 genotype has given the highest values of plant height, that were 138.7 and 130.5 cm in the first and second seasons, respectively. On the other hand, the genotype Triticale 6 has given the lowest value of plant height. The increase in percentage between Triticale 11 and 6 was 11.4 to 12%, Triticale 11 and 7 was 8 to 9.8%, Triticale 11 and 8 was 5.7 to 7% and Triticale 11 and ICBA 2 was 3.2 to 3.6%, in the first and second seasons, respectively. Results were in accordance with that obtained by Pondini et al. (1997).

6.2. Number of spikes

Data in table (9) indicate that the genotype Triticale 11 produced the highest number of spikes/m² that was ranged between 490.8 and 452.3/m², while Triticale 6 has given the lowest values of 458.6 and 410.8/m² in the first and second seasons, respectively. The results also show that there are non-significant differences among the different genotypes, where the increasing percentage was from 3 to 10.6%.

6.3. Straw yield

As shown in table (10), there is non-significant differences among the tested genotypes, where the genotype Triticale 11 recorded the highest straw yield (4716. 3 and 4470.6 kg/feddan) in the first and second seasons, respectively. However, the genotype Triticale 6 produced the lowest value of straw yield (4379.2 and 4227.7 kg/feddan) in the first and second seasons, respectively. The increasing percentage between the highest and lowest values in the first and second seasons fluctuated between 7.1 to 5.4%.

6.4. Biological yield

Table (10) shows that there are insignificant differences among the tested genotypes. The genotype Triticale 11 recorded the highest biological yield (7096.5 and 6732.03 kg/feddan) in the two seasons, however the genotype Triticale 6 had given the lowest biological yield (6606.73 and 6382.3 kg/feddan), representing about 6.9 to 4% in the first and second seasons, respectively. The results agree with El-Karamany et al. (2009).

6.5. Grain yield

Gain yield has shown non-significant differences among the tested genotypes as represented in table (10), where genotype Triticale 11 produced the highest yield of 2381.2 and 2261.4 kg/feddan in the first and second seasons, respectively. The increasing percentage among all genotypes ranged between 6.5 to 1.8% and 4.7 to 1.1% in the first and second seasons, respectively, demonstrating a low diversity among the tested genotypes. **6.6. Harvest Index**

The data presented in table (10) illustrate insignificant differences among the tested genotypes, but the results indicated the genotypes Triticale 11, then ICBA 2 are promising under conditions of the experiment.

 Table (10). Analysis of variance of the productivity measurements; straw yield, biological yield, grain yield and harvest index of the five Triticale genotypes.

Genotypes	Straw yield (kg/feddan)		Biological yield (kg/feddan)		Grain (kg/fe	ı yield ddan)	Harvest index	
	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18	2016/17	2017/18
Triticale 11	4716.30	4470.60	7096.5	6732.03	2381.18	2261.43	0.34	0.34
ICBA 2	4636.30	4419.70	6975.9	6656.16	2336.60	2236.46	0.33	0.34
Triticale 8	4580.20	4353.30	6882.4	6561.40	2302.20	2208.13	0.33	0.34
Triticale 7	4488.00	4272.60	6760.2	6455.20	2272.18	2182.58	0.34	0.34
Triticale 6	4379.20	4227.70	6606.7	6382.30	2227.53	2154.60	0.34	0.34
LSD5%	NS	NS	NS	NS	NS	NS	NS	NS

CONCLUSION

The present research indicated that, the ISSR and SCoT markers analyses were effective for evaluating the genetic relationships among the studied Triticale genotypes and fingerprinting them. The dendrogram, depending on Dice coefficient and UPGMA method, was successful in separating genotypes, were Triticale 11 and ICBA 2 were the most distinguished and different genotypes. In general, there is low to moderate genetic differences (dissimilarity) among genotypes, were the total polymorphic amplified fragments were 58, representing about 34.7%, with average PIC of 0.9415. The number of non-polymorphic fragments was 109

with 65.2% of 167 total amplified fragments. There is a general agreement of the results obtained, regarding the productivity aspect, where Triticale 11 followed by ICBA 2 are considered the most promising genotypes under North Sinai conditions.

REFERENCES

- Abd El-Lateif, K.S. and O.A. Hewedy (2018). Genetic diversity among Egyptian wheat cultivars using scot and issr markers. Sabrao Journal of Breeding and Genetics, 50 (1): 36-45.
- Abou-Deif, M., M. Rashed, M.A. Sallam, E.A. Mostafa and W. Ramadan (2013). Characterization of twenty wheat varieties by ISSR markers. Mid. East. J. Sci. Res., 15 (2): 168-175.
- Aboulila, A.A. and M. Mansour (2017). Efficiency of triple-SCoT primer in characterization of genetic diversity and genotype-specific markers against SSR fingerprint in some Egyptian barley genotypes. Am. J. Mol. Biol., 7 (3): 123-137.
- Adawy, S., E.H.A. Hussein and D. El-Khishin (2002). Genetic variability studies and molecular fingerprinting of some Egyptian date palm (*Phoenix dactylifera* L.) cultivars, II -RAPD and ISSR profiling. Arab J. Biotech., 5: 225-236.
- Al-Qurainy, F., S. Khan, M. Nadeem and M. Tarroum (2015). SCoT marker for the assessment of genetic diversity in Saudi Arabian date palm cultivars. Pak. J. Bot., 47 (2): 637-643.
- Anderson, J.A., G.A. Churchill and J.E. Autrique (1993). Optimizing parental selection for genetic linkage maps. Genome, 36: 181-186.
- Ayalew, H., T.T. Kumssa, T.J. Butler and X. Ma (2018). Triticale improvement for forage and cover crop uses in the Southern Great Plains of the United States. Front Plant Sci., 9: 1130.
- De Costa, C.T., A.C.S. Albuquerque, A. do Nascimento Junior, F.C. Marcelino and J.F. Pereira (2007). Genetic diversity of Brazilian Triticales evaluated with genomic wheat microsatellites. Pesqui Agropecu. Bras., 42: 1577–1586.
- Collard, B.C. and D.J. Mackill (2009). Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep., 27 (1): 86-93.
- El-Karamany, M.F, O.M. Ibrahim, S.F. El Habbasha and N.I. Ashour (2009). Screening of 40 Triticale (x *Triticosecale* Wittmack) Genotypes under sandy soil Egypt conditions. J. Appl. Sci. Res., 5: 33-39.
- El-Assal, S.E.D. and A. Gaber (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. Am. J. Appl. Sci., 9 (5): 724-735.

- Etminan, A., A. Pour-Aboughadareh, R. Mohammadi, R. Ahmadi, A. Noori, Z. Mahdavian and Z. Moradi (2016). Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat genotypes. Biotechnol. Equip., 30 (6): 1075-1081.
- Fernandes, E.H., I. Schuster, C.A. Scapim, E.S.N. Vieira and M.M.D. Coan (2015). Genetic diversity in elite inbred lines of maize and its association with heterosis. Genet. Mol. Res., 14: 6509–6517.
- Fischer, S., H.P. Maurer, T. Würschum, J. Möhring, H.P. Piepho, C.C. Schön, E.M. Thiemt, B.S. Dhillon, E.A. Weissmann, A.E. Melchinger and J.C. Reif (2010). Development of heterotic groups in Triticale. Crop Sci., 50: 584–590.
- Gajera, H.P., R.P. Bambharolia, R.K. Domadiya, S.V. Patel and B.A. Golakiya (2014). Molecular characterization and genetic variability studies associated with fruit quality of indigenous mango (*Mangifera indica* L.) cultivars. Plant Syst. Evol., 300: 1011–1020.
- Glamočlija, N., M. Starčević, J. Ćirić, D. Šefer, M. Glišić, Ž.M. Baltić, R. Marković, M. Spasić and D. Glamočlija (2018). The importance of triticale in animal nutrition. Veterinary Journal of Republic of Srpska, XVIII (1): 73–94.
- Goncalves, L.S., R. Rodrigues, A.T. do Amaral Junior, M. Karasawa, C.P. Sudre (2009). Heirloom tomato gene bank: Assessing genetic divergence based on morphological, agronomic and molecular data using a Ward-modified location model. Genet. Mol. Res., 8: 364-374.
- Gradzielewska, A., D. Gruszecka, J. Leśniowska-Nowak and E. Paczos-Grzęda (2012). Identification of hybrids between Triticale and *Aegilops juvenalis* (Thell.) Eig and determination of genetic similarity with ISSRs. Genet. Mol. Res., 11 (3): 2147-55.
- El-Henawy, A.A.A., H.A.E. Hashem and N.Kh.M. El-Safty (2018). Molecular systematic and productivity evaluation of some ecotypes of Mentha longfolia under different ecological conditions. IOSR Journal of Agriculture and Veterinary Science, 11 (1): 57-67.
- Ibrahim, S.D., S.S. Adawy, M.A.M. Atia, M.A. Alsamma and M.M. Mokhtar (2016). Genetic diversity, variety identification and gene detection in some Egyptian grape varieties by SSR and SCoT markers. Plant Omics, 9 (5): 311-318.
- Idris, A.E., N.B. Hamza, S.O. Yagoub, A.I.A. Ibrahim and H.K.A. El-Amin (2012). Maize (*Zea mays* L.) genotypes diversity study by utilization of inter-simple sequence repeat (ISSR) markers. Australian Journal of Basic and Applied Sciences, 6 (10): 42-47.
- John Staughton (BASc, BFA) (2019). Available online: https://www.organicfacts.net/health-benefits/cereal/Triticale.html last updated - October 04, 2019

- Jonnala, R.S., S. Irmak, F. MacRitchie and S.R. Bean (2010). Phenolics in the bran of waxy wheat and Triticale lines. Journal of Cereal Science, 52: 509-515.
- Joshi, C.P., H. Zhou, X. Huang and V.L. Chiang (1997). Context sequences of translation initiation codon in plants. Plant Mol. Biol., 35: 993-1001.
- Kallamadi, P.R., V.P.R. Ganga Rao Nadigatlab and S. Mulpurib (2015). Molecular diversity in castor (*Ricinus communis* L.). Industrial Crops and Products, 66: 271–281.
- Kuleung, C., P.S. Baenziger and I. Dweikat (2004). Transferability of SSR markers among wheat, rye and Triticale. Theoretical and Applied Genetics, 108: 1147-1150.
- Kuleung, C., P.S. Baenziger, S.D. Kachman and I. Dweikat (2006). Evaluating the genetic diversity of Triticale with wheat and rye SSR markers. Crop Sci., 46: 1692–1700.
- Khaled, A.G.A., M.H. Motawea and A.A. Said (2015). Identification of ISSR and RAPD markers linked to yield traits in bread wheat under normal and drought conditions. J. Genet. Eng. Biotechnol., 13 (2): 243-252.
- Leonova, I.N., O.B. Dobrovolskaya, L.N. Kaminskaya, I.G. Adonina, L.V. Koren, L.V. Khotyljova and E.A. Salina (2005). Molecular analysis of the Triticale lines with different vrn genes systems using microsatellite markers and hybridization *in situ*. Russian Journal of Genetics, 41: 1014-1020.
- Mahjbi, A., G. Baraket, A. Oueslati and A. Salhi-Hannachi (2015). Start Codon targeted (SCoT) markers provide new insights into the genetic diversity analysis and characterization of Tunisian Citrus species. Biochemical Systematics and Ecology, 61: 390–398.
- Ma, X., X.Q. Zhang, Y.H. Zhou, S.Q. Bai and W. Liu (2008). Assessing genetic diversity of *Elymus sibiricus* (Poaceae: Triticeae) populations from Qinghai-Tibet Plateau by ISSR markers. Biochem. Syst. Ecol., 36: 514–522.
- Moll, R.H., J.H. Lonnquist, J. Wlez Fortuno and E.C. Johnson (1965). The relationship of heterosis and genetic divergence in maize. Genetics, 52: 139–144.
- Mohamed, A.S.H., M. Ibrahim, S. Teleb and M. Tantawy (2017). SEM and SCoT markers unveil new taxonomic and genetic insights about some Northern African *Triticum aestivum* L. cultivars. Vegetos., 30 (1): 34-44.
- Pondini, F., F. Carvalbo, J. Neto, De. Car Valho and Dao-Amaral (1997). Evaluation of genetic variability for heading by induced mutations and artificial crosses. Pesquisa Agropecuaria Gaucha, 3 (1): 55-61.

- Randhawa, S.H., M. Asif, C. Pozniak, J.M. Clarke, R.J. Graf, et al. (2013). Application of molecular markers to wheat breeding in Canada. Plant Breed., 132: 458–471.
- Saad-Allah, K.M. and M.S. Youssef (2018). Phytochemical and genetic characterization of five quinoa (*Chenopodium quinoa* Willd.) genotypes introduced to Egypt. Physiol. Mol. Biol. Plants, 24 (4): 617-629.
- Sadek, M.S.E. and S.D. Ibrahim (2018). Genetic relationships among maize inbred lines as revealed by start codon targeted (SCoT) analysis. Journal of Innovations in Pharmaceutical and Biological Sciences, 5 (1): 103-107.
- Sawant, S.V., P.K. Singh, S.K. Gupta, R. Madnala and R. Tuli (1999). Conserved nucleotide sequences in highly expressed genes in plants. J. Genet., 78: 123-131.
- Shahlaei, A., S. Torabi and M. Khosroshahli (2014). Efficiacy of SCoT and ISSR marekers in assessment of tomato (*Lycopersicum esculentum* Mill.) genetic diversity. International Journal of Biosciences, 5 (2): 14-22.
- Sneath, P.H.A. and R.R. Sokal (1973). In: 'Numerical Taxonomy'. Freeman, San Francisco, California.
- Sokal, R. and C. Michener (1958). A statistical method for evaluating systematic relationships. University of Kansas Science Bulletin, 38: 1409-1438.
- Trebichalský, A., Ž. Balážová, Z. Gálová, M. Chňapek and M. Tomka (2013). Detection of genetic diversity of Triticale by microsatellite markers. Journal of Microbiology, Biotechnology and Food Sciences, 2: 1898– 1906.
- Tams, S.H., E. Bauer, G. Oettler and A.É. Melchinger (2004). Genetic diversity in European winter Triticale determined with SSR markers and coancestry coefficient. Theoretical and Applied Genetics, 108: 1385-1391.
- Vaillancourt, A., K.K. Nkongolo, P. Michael and M. Mehes (2007). Identification, characterisation, and chromosome locations of rye and wheat specific ISSR and SCAR markers useful for breeding purposes. Euphytica, 159: 297–306.
- Vivodík, M., Ž. Balážová, Z. Gálová and L. Petrovičová (2017). Genetic diversity analysis of maize (*Zea mays* L.) using scot markers. J Microbiol. Biotech. Food Sci., 6 (5): 1170-1173.
- Wang, X.M. (2010). Optimization of DNA isolation, ISSR-PCR system and primers screening of genuine species of rhubarb, an important herbal medicine in China. J. Med. Plants Res., 4 (10): 904-908.
- Žiarovská, J., K. Ražná and M. Labajová (2013). Using of inter microsatellite polymorphism to evaluate gamma-irradiated *Amaranth mutants*. Emir. J. Food Agric., 25 (9): 673-681.

التقييم الوراثي والإنتاجي لبعض أصناف التريتكال تحت ظروف شمال سيناء

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التريتكال من محاصيل الحبوب الجديدة في المملكة النباتية وهو عبارة عن أول هجين بين القمح وحشيشة الراي يجمع بين الصفات التكنولوجية والإنتاجية للقمح وصفات الراي وقدرته على تحمل الإجهاد البيئي والحيوي ويتميز بارتفاع البروتين والأحماض الأمينية الأساسية وخاصبة حامض الليسين عن القمح. يتناول البحث دراسة التقييم الوراثي والإنتاجي لخمسة تراكيب وراثية لنبات التريتكال وارده من المركز الدولي للزراعة الملحية بدبي، جمهورية الإمارات العربية المتحدة وهم إيكبًا ٢ وتريتكال ٨ و٦ و٧ و١٦ لزَّر اعتها بالأراضي الهامشية بجمهورية مصر العربية، وتقييمها من خلال مشروعات مع مركز بحوث الصحراء. وتمت الدراسة تحت ظروف محافظة شمال سيناء في منطقة بالوظة ذات المناخ الجاف والتربة الرملية. وتم عمل التقييم الوراثي باستخدام واسمات وراثية تهدف مناطق متكررة على مستوى الجينوم وأخرى تهدف لبداية المناطق المشفرة للجينات. وتمت دراسة التوصيف الوراثي والعلاقات الوراثية من التشابه والاختلاف الجيني والبصمة الوراثية لتراكيب التريتكال الوراثية تحت ظروف الدراسة. وكان ناتج القطع الجينية المبلمرة ١٦٧ قطعة من بينهم ٥٨ مختلفة و١٠٩ متشابهة ونسبة الاختلاف الكلية ٣٤.٧٪ باستخدام ١٢ برايمر للواسمات المستخدمة. وتبين أن تريتكال ١١ وإيكبا ٢ الأكثر تميزًا واختلافًا مما يفيد في انتخابهم في در اسات لاحقة. حيث تغيد الواسمات الور اثية في تحسين المحاصيل عن طريق انتخاب التراكيب المتباعدة لتهجينها للحصول على هجن أفضل إنتاجية وجودة مما يوفر الوقت والجهد عن عشرات السنين باستخدام طرق التربية التقليدية. ونجحت الواسمات في فصل التراكيب في شجرة القرابة وتميز ها وكانت تراكيب تريتكال ٧ و٦ و٨ الأعلى تشابهًا وتراكيب تريتكال١١ وإيكبا ٢ الأكثر تباعدًا عنهم. وتم تقييم الإنتاجية من خلال تقدير صفات المحصول ومكوناته و هي ارتفاع النبات والسنابل و عدد السنابل والسنيبلات/سنبلة وتقدير كل من محصول القش والحبوب والمحصول البيولوجي، وكانت إنتاجية المحصول مرتفعة وتراكيب تريتكا ١١ ثم إيكبا ٢ الأكثر تميزًا وفي المجمل يوجد اختلاف لكن لا يوجد فروق معنوية بين صفات المحصول المقدرة. وتؤكد نتائج الدراسة بعضها بعضًا حيث توجد أيضًا نسبة تشابه وراثي مرتفعة نسبيًا مما يدل على احتمالية أن الأباء لهذه التراكيب قريبين لبعضهم بنسب مرتفعة مما أدى إلى عدم وجود فروق معنوية في تقديرات المحصول، حيث تشير الدراسات إلى أهمية التباعد في التهجينات في المحاصيل و أن ذلك ينعكس على جودة المحصول وكميته