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DETERMINATION OF GENETIC DISTANCE AMONG GENOTYPES OF BREAD WHEAT *TRITICUM AESTIVUM* L., USING ISSR MARKERS

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Abstract

This research was conducted in biotechnology laboratories of grain test and validation office-Ministry of Agriculture in 2017. Fourteen wheat genotype that represented pure local and imported classes, were cultivated to evaluate genetic diversity and determine genetic affinity among them by using 12 primer inter Simple Sequence Repeats (ISSR), 11 of showed an activity to give a polymorphism among the studied genotypes which give 118 alleles, these primers ratio of this polymorphism was 89. The primer UBC827 gave higher bands of 17, while the primer A33 gave lower bands of 5. The study showed that genetic similarity of the genetic affinity ranged between 0.174 – 0.843 in which the higher genetic affinity (0,843) was for V1 and V2 varieties followed by (0.680) for V1 and V3 varieties, The lower genetic affinity (0.174) was for V10 and V11 varieties.

Key words: Bread Wheat, polymorphism, ISSR.

Introduction

Bread wheat (*Triticum aestivum* L.) is the most important cereal crop in the world that evolved from wild grasses, probably somewhere in the Near East (Briggle and Curtis, 1987). .

Genetic diversity patterns can provide in sights into evolutionary and demographic history of a taxon. Moreover, exploitation, evaluation, and strategies for the conservation *in situ* and *ex site* of genetic diversity present in natural populations or local cultivars are essential to guarantee sustainable development (Smolik *et al.,* 2011). Dreisigacker *et al.,* (2005) reported that the genetic variation of bread wheat cultivars conserved in seed banks is usually unknown. Thus, in many countries, strategies have been devoted to characterize and cultivates, providing considerable opportuction of core collections (Hou *et al.,* 2005).

Genetic diversity among individuals or populations can be determined using morphological and molecular markers. Phenotypic characters have limitations since they are influenced by environmental factors and the developmental stage of the plant (Archak *et al.*, 2003). Different molecular marker systems have been used to assess genetic diversity in wheat, including random amplified polymorphic DNA (RAPD) (Abdollahi Mondoulakani *et al.,* 2010), amplified fragment length polymorphism (AFLP) (Khalighi *et al.,* 2008), simple sequence-tagged-sites (STS) (Talbert *et al.,* 1994), and retrotransposon-based markers (Carvalho *et al.,* 2010). Inter simple sequence repeat (ISSR) markers is a PCR –based technique, amplify regions between adjacent and inversely oriented microsatellites using di-, tri-, tetra-, and penta-nucleotide SSR primers, with the advantage that knowledge of the DNA sequence of the target region is not required. ISSR amplification depends on the variation, motif, and frequency of SSRs that change among species (Zietkiewicz *et al.,* 1994). ISSR is an ideal method for fingerprinting and a useful alternative to single-locus or hybridization-based methods. It is a powerful technique to determine intra- and inter-specific genetic diversity (Bornet and Branchard, 2001; Myskow *et al.,* 2011; Pradeep Reddy *et al.,* 2002; Smolik *et al.,* 2011; Sofalian *et al.,* 2009). The ISSR markers are increasingly applied in the plant sciences and have detected a sufficient degree of polymorphism in faba bean (Terzopoulos and Bebeli, 2008), safflower (Golkar *et al.,* 2011), rice (Blair *et al.,* 1999), and barley (Hou *et al.,* 2005). ISSR markers have a better reproducibility than RAPDs, They are easy to detect and can be produced at lower costs that AFLPs.

They are simpler to use than the SSR technique and they are less restrictive than restriction fragment length polymorphism (RFLP), and may offer considerable variation among species (Archak *et al.*, 2003).

This research aimed to characterizing the genetic of some cultivated wheat varieties, and determining the genetic affinity among them using ISSR technique.

Materials and Methods

Plant material consisted of fourteen of *Triticum aestivum* L. collected from different locations. The research was conducted in the biotechnology laboratories of the Grain Test and Validation Office-Ministry of Agriculture. Grains of the fourteen wheat genotypes were planted, to sustain their grains and increase their genetic originality, in the Center of Research fields. Genotypes grains, resulting from the previous season, were planted into field and the samples were taken at the appearance of actual leaves to be used in DNA isolation (table1).

Table 1: Wheat cultivars used its pedigree

No.	Cultivar Name	Pedigree
Vl	Sham 6	Input
V2	Alaze	Kilrad to irradiation of Hybrid class success with Mixipack cultivar using Gama rays
V3	Aliraq	A mulation was selected in the seventh mutant generation of Mixipack wheat grown with Gama rays
V4	IPA 95	Inpyt
V5	Altahdy	Inbreed selected from the hybridization of Sabir Beek cultivar and Mixipack
V6	Hashmeya	A-901 Y- 902 D OY\ LD* 6\F B6628-F3 0069 BR 12* 3\3 BR 14
V7	Rabeaa	Inbreed selected from irradiation of the third generation of Sabir Beek wheat with the wheat Glo Kirad 10 (60 dosage) using Indian Kobat Gama rays HD831
V8	Alfateh	Input
V9	Abo Graib 3	Hybridization between (Maxico 24 × Linsa R66) × Ajeba cultivar
V10	Tamoze 3	Inbreed selected from irradiation of wheat of isolated second generation of wheat hybridatal seeds Sabir Beek with Maxioack fast Nitrons will dosage 400 in 1983
V11	Alnaaema	Inbreed selected from irradiation isolated second generation planting cultivar 131 kokurate 71
V12	Aladnanya	Inbreed selected from hybridization Abo Graib and Maxipack cultivar
V13	Alnoor	21 YOM / COC CAR853 \ BOW CM86106\3\ VEE CY2M-OY-
V14	Alrashed	Inbreed selected from irradiation of clear breed of Mixipack cultivar using Gama rays with a dosage Glo Kilrad

Extraction Genome DNA

DNA was extraction from the young studied genotypes leaves and DNA quantity was about 50 – 150 Mg per 1.5 g leaves for each wheat genotype with a purity ranging between 1.7 – 2 which measured by Nanodrop device. DNA samples dilutions were fitted to obtain 50 ng.ML^{-1} concentration that appropriate for PCR. There are many methods to isolate nucleic acid from the plants due to the fact that plant diversity contains different quantities of plant compounds such as proteins, polysaccharides and nucleic acids. ISSR Markers that depend on PCR and 12 primers, were used as shown in (table 2). The method of (Weigand *et al.,* 1993), was adopted to isolate DNA from wheat which had an efficiency to isolate the DNA (Doyle and Doyle 1990). Relatively, DNA isolation from the plant is more difficult than other organisms due to the presence of the thick wall surrounding the cell membranes. Further more some plants contain large amounts of phenol materials and polysaccharides which are considered as pollutants that sometimes deposit with the DNA giving a high viscosity liquid. Moreover they are considered as inhibitor for PCR. To get rid of these materials, a dilution of the extracted DNA was conducted to reduce the ratio of saccharide inhibitors (Malik *et al.,* 2012)

Table 2: Nucleotide Sequence of primer used in ISSR

Temperature	Nucleotide Sequence 3 - 5	Starter
33.8	GAGAGAGAGAGAGAGATT	UBC478
35.6	GAGAGAGAGAGAGAGA	UBC844
42.6	TGTGTGTGTGTGTGTG	UBC860
29.7	AGAGAGAGAGAGAGAG T	UBC819
57.8	AGCAGCAGCAGCAGCAGC	UBC866
45.5	TCTCTCTGTGTGTGTG	UBC468
30.8	ACACACAGAGAGAGAGAC	UBC872
33.3	GAGACCGG	N35
40	GCGCGTGTGTGTGTGT	N37
41.9	CTCTCTCTCTCTCTCT	A36
34.7	CTCTCTCTCTCTCTGT	B39
28.6	AGAGAGAGAGAGAGAGCTT	A33

ISSR Application

In this study, 12 primers were used after importing from Bioneer Co. Table 2 show the Nucleotide Sequence and the coalescence temperature of the starters used in

the study.

PCR was conducted according to (Williams *et al.,* 1990), with some modifications and the final reaction volume was 25 µl using 2 × Master mix obtained from Bioneer Co. The reaction consisted from 2ul of distilled water, and DNA with concentration of 40 ng.µl^{-1} . This reaction occurred into thermo-rotation system according to the following conditions:

1- Separation: at 94^{0}C for 5 minutes, the two series of DNA to be separated.
2- 40 rotations, each includes the following stages:
   2-1 Separation occurred at 94^{0}C for 30 sec.
   2-2 Coalescence: according to the temperature of each starter, from table 2, for 1 minute.
   2-3 Elongation at 72^{0}C for 1 minute.
3- Reaction completion at 72^{0}C for 1 minute.

The samples were kept under 4^{0}C, then deported on Agarose gel.

The Electric Deportation, Coloring and Imaging

The deportation on Agarose gel (2%) was done in the buffer solution TBE 1x.

TBE 1x = (10x TBE buffer = 108g Tris borate + 55g Boric acid + 9.2 EDTA. Ph 0.8)

5µl of ethidium bromide stain (10 mg.ml^{-1}) where DNA samples were loaded on agarose gel by adding 5 µl of the special loading liquid (Bromophenol blue 1 × loading buffer). 1kbp DNA, from Geneaid Co., was injected to determine the volume and molecular weight of the resulted bands, then deporting by passing through electric field of 100 v to separate DNA bands resulting from the amplification, then imaging the gel by the image analyzer (Eagle Eye II Stratagene). .

Statistical Analysis

The results of the amplification process were gathered in a table depending on the presence or absence of DNA bands in the studied samples, the number 1 indicates the presence of clear DNA band only, Where number 0 indicates absence of the band. Individually, the tables were organized for each starter and the dendrogram was drawn by applying Unweighted Pair Group Method with Arithmetic Averaging (UPGMA) using the Statistical Past.

Result & Discussion

Polymorphism

Of the 12 used primer, 11 primers had an ability to detect the genetic differences among different pure varieties. Table 3 showed that used primers gave 117 bands, 90 of them had a polymorphism with ratio of 76.92%. That indicated the genetic distance among the pure wheat varieties used in the study. The table show that the primer UBC827 gave greater band number (17 band) compared to the other used primers, while the primer A33 showed the least bands number (5 bands) through sample deportation on poly acryl amide gel. The variation in the number of bands, resulting from each pair of the used primer, depends on the correspondence extent of the primer link with plant genome as well as the components of each primer of nitrogenous bases. The sequence difference of the used primers bases led to the difference of their link sites with the plant genome and this led to difference in bands number resulting from the used primers (Vos *et al.,* 1995*)*. In spite of the difference in the bands number, the primers succeeded to give polymorphism among the resulted bands which reached 100% in the primers UBC844, while the primer A35 had no more than 42.8% of polymorphism. This variation in the percentages attributed to the difference in the sequence of bases in the plant genome, which affected the primers link sites. This difference was caused by genetic rearrangement, link, passing and other. Different bands appeared clearly on the gel or do not appear in certain sites on the gel (Rohlf, 2000).

Table 3: Number of bands and polymorphism of the used primer

Polymorphism %	Polymorphism	Bands No.	Starters
85.7	6	7	UBC478
100	9	9	UBC844
91	11	10	UBC860
69.2	9	13	UBC819
50	7	14	UBC866
88.2	15	17	UBC872
85.7	6	7	N35
77.8	7	9	N37
42.8	6	14	A36
83.3	10	12	B39
80	4	5	A33
	90	117	Total
76.92			Average

Determination of Genetic Affinity among Studied Genotypes

Genetic distance among studied genotypes was calculated according to the equation of (Powell *et al.,* 1996). The result showed the similarity and dissimilarity extent with the pure ancestries. The higher similarity percentage, which corresponded to less genetic distance (0.843) was between V1 and V2 followed by V1 and V3 which formed a small group. On the other hand, less

Table 4: Values of the genetic similarity among 14 wheat genotypes

V1	V2	V3	V4	V5	V6	V7	V 8	V8	V 9	V 10	V 11	V 12	V 14	
													1.00	**V 1**
												1.00	**0.843**	**V 2**
											1.00	0.666	**0.680**	**V 3**
										1.00	0.654	0.541	0.618	**V 4**
									1.00	0.460	0.659	0.558	0.505	**V 5**
								1.00	0.423	0.547	0.500	0.567	0.585	**V 6**
							1.00	0.395	0.417	0.554	0.551	0.505	0.477	**V 7**
						1.00	0.382	0.545	0.623	0.524	0.620	0.561	0.555	**V 8**
					1.00	0.554	0.444	0.640	0.525	0.466	0.551	0.657	0.675	**V 9**
				1.00	0.587	0.518	0.354	0.483	0.564	0.386	0.588	0.567	0.586	**V10**
			1.00	**0.174**	0.416	0.541	0.637	0.562	0.468	0.624	0.557	0.486	0.482	**V11**
		1.00	0.424	0.368	0.358	0.371	0.337	0.313	0.365	0.387	0.421	0.309	**0.305**	**V12**
	1.00	0.468	0.528	0.309	0.348	0.421	0.391	0.373	0.416	0.433	0.368	0.423	0.365	**V13**
1.00	0.530	0.377	0.495	0.375	0.365	0.484	0.451	0.344	0.478	0.392	0.504	0.477	0.446	**V14**

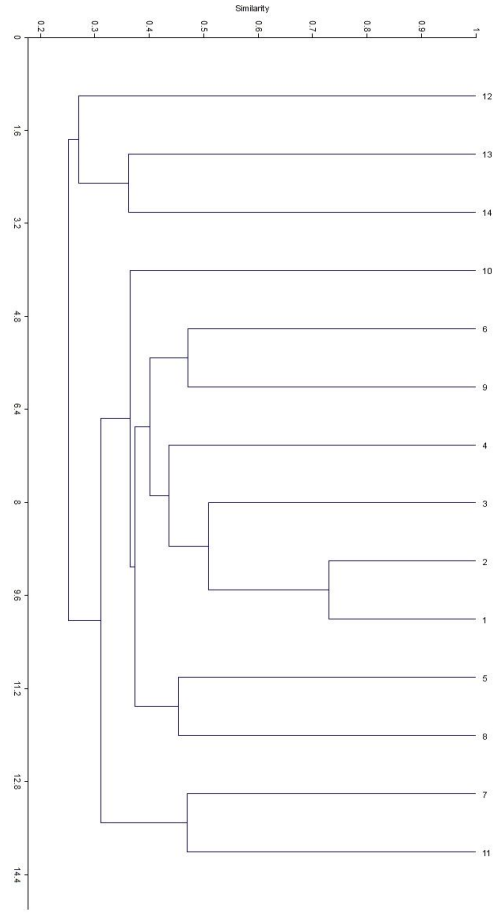

Fig 1 : Dendrogram of the Studied Genotypes

similarity percentage that corresponded to great genetic distance (0.174), was between V10 and V11 followed by V1 and V12 genotypes. The results, are shown in table 4 below.

Cluster Analysis

Cluster analysis was used to divide the studied genetic structures into groups reflecting affinity among them according to their originality. The dandrogram that depended on values of the genetic distance using UPGMA method (fig. 1), which were created according to the results of ISSR indicators, showed that the 14 genotypes were distributed into two main groups, First and Second. The First cluster included 3 genetic structures (V12, V13 and V14), and it can be observed in the group First that V13 and V14 genotypes ratio was 38%. The second cluster was divided into two main branches: the (A) branch which included V7 and V11 while the (B) branch included V1, V2, V4, V5, V6, V8, V9, and V10, and between V1 and V2 similarity the ratio was 73%. It can be concluded that molecular study showed the studied wheat genotypes contained high genetic variety and these results can be used as an essential material in the programs of breeding by hybridizing to improve the quantity and quality properties and produce wheat in divided hybrids. The results are found to be similar to (Saada and Lawand, 2015; Dashchi *et al.,* 2016).

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