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ESTIMATION OF GENETIC DIVERSITY AMONG SOME BREAD WHEAT (*Triticum aestivum* L.) GENOTYPES USING MOLECULAR MARKERS AND AGRONOMIC TRAITS

E. A. El-Absawy[(1)], A. A. Nawar[(2)], F. A. Feky[(3),] K. F. M. Salem[(4)] and Engy Edward[(4)]

[(1)] Department of Bioinformatics, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City University, Sadat City, P.O. Box, 79, Egypt

[(2)] Department of Crop Science, Faculty of Agriculture, Menoufiya University, Shebin El-Kom, Egypt

[(3)] Department of Biotechnology, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt

[(4)] Department of Plant Biotechnology, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City University, Sadat City, P.O. Box, 79, Egypt

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ABSTRACT: *Genetic diversity was investigated among some bread wheat varieties by nine simple sequence repeat markers and fifteen agronomic traits. The wheat simple sequence repeat markers (SSRs) used determined nine loci located on nine chromosomes and were capable of detecting 41 alleles with an average of 4.56 alleles per locus. The number of alleles per locus ranged from 3 to 7 and the allelic polymorphism information content (PIC) value ranged from 0.448 for the Xgwm333-7B to 0.857 for the Xgwm626-6B with an average of 0.665. The results revealed that the varieties differed for SSRs markers and agronomic traits. Significant correlation coefficient between gene diversity and the number of polymorphic bands was high, r = 0.852 (P <0.01). The genetic similarity based on agronomic traits ranged from 0.61 to 1.82 was higher than SSR markers which ranged from 0.136 to 0.991. Fifteen agronomic traits were used for morphological analysis. Cluster analysis was conducted based on SSRs and agronomic traits to group the wheat varieties and construct dendrogram. Three main groups distinguished by SSRs. However, the cluster analysis based on agronomic traits assigned the varieties into three different groups. SSR markers showed high level of polymorphism among the varieties examined. The present study indicates that SSR markers and agronomic traits could be successfully used in genetic characterization and diversity in wheat. Also, Information generated from this study can be used to select parents for hybrid development to maximize yield and its components.*

Key words: *Bread wheat (Triticum aestivum L.), SSR markers, Genetic diversity, Polymorphism information content (PIC), Agronomic traits.*

INTRODUCTION

Bread wheat (*Triticum aestivum* L.) is the first strategic important cereal crop in Egypt. The use of plant genetic resources and conservation are essential to the continued maintenance and improvement of agricultural production. Genetic diversity in wheats is essential for successful breeding and released of new cultivars. The study of phenotypic and genetic diversity to identify groups with similar genotypes is important for conserving, evaluating and utilizing plant genetic resources; studying the diversity of breeding germplasm; and for determining the uniqueness and distinctness of the phenotypic and genetic constitution of genotypes with the purpose of protecting a breeder's intellectual property rights (Franco *et al.,* 2001). Knowing the genetic diversity of wheat germplasm is necessary for identifying diverse parental combinations and creating segregating progeny with high genetic variability for selection. Criteria for assessment of genetic diversity can be different i.e., morphological characters (Maric *et al.,* 1998; Salem *et al.,* 2008; Sonmezoglu *et al.,* 2012), biochemical techniques or DNA markers. Molecular markers that reveal polymorphism at the

DNA level have been shown to be a very powerful tool for genotype characterization and estimation of genetic diversity (Plaschke *et al.,* 1995; Huang *et al.,* 2002; Salem 2009). The use of molecular markers for evaluation of genetic diversity is receiving much attention. Many wheat scientists have studied genetic diversity in wheat using different molecular markers such as RFLPs (Bohn *et al.,* 1999), RAPDs (Mukhtar *et al.,* 2002) and microsatellite (Huang *et al.,* 2002; Dreisigacker *et al.,* 2004 and Laido *et al.,* 2013).

Simple sequence repeats (SSRs) is one of the PCR based DNA marker (Roder *et al.,* 1998). SSRs can be used in studying genetic diversity, varietal identification, genetic map and marker assisted selection. It is simple to operate, less expensive, fast, does not involve radioactive labeling and does not require huge infrastructure to start with. The aims of this work were to: i) use SSR and agronomic traits to assess level and patterns of genetic variability among some bread wheat varietie**s,** ii) evaluate genetic relationships between some wheat varietie**s** and iii) compare results based on SSR markers and agronomic traits.

MATERIALS AND METHODS

Plant materials and cultivation

Seven bread wheat varietie**s** namely; Giza 157, Giza 160, Giza 163, Giza 164, Giza 165, Gemmiza 1 and Gemmiza 9 were grown at the Experimental Research Farm, Faculty of Agriculture, Menoufiya University, Egypt, during the two wheat successive growing seasons, 2010/2011 and 2011/2012 (Table 1). Wheat varietie**s** were grown in a randomized complete block design (RCBD) with three replicates with each plot consisting of 1.5 m long rows with 20 cm apart and 5 cm between hills. Normal agronomic practices were followed as recommended in the wheat production area.

Evaluation of Agronomic traits

Ten guarded plants from each wheat varietie**s** were evaluated for a range of agronomic traits. Data were determined on ten random plants per each plot for fifteen agronomic traits of randomly selected plants, as follows: ear date, flowering date, plant height, number of tillers per plant, grain yield per plant, 1000-grain weight, spike weight, no of spikes per plant, no of grains per spike, hectoliter weight, spike length, no of spikelets per spike, spike density, grain weight per spike and spike fertility.

DNA Isolation

Total genomic DNA was extracted from leaf tissue per each genotype. Young leaves from four weeks old plants were cut as tissue samples for DNA extraction. DNA was isolated from these genotypes as described by Plaschke *et al.* (1995).

Table 1: Names of the seven wheat genotypes, their pedigree and year of release

Genotypes	Pedigree	Year of release
GIZA 157	Giza 155//Pit 62/LR 64/3/Tzpp/Knott	1977
Giza 160	Chenab70/Giza 155	1982
Giza 163	*T. aestivum*/Bon//Cno/7C CM33009-F-15 M-4Y-2 M-1 M-1 M-1Y-0 M	1987
Giza 164	Kvz/Buha ‘‘s’’//Kal/Bb CM33027-F-15 M-500y-0 M	1987
Giza 165	0Mcno/Mfd//Mon ‘‘S’’ CM43339-C-1Y-1M-2Y-1M-2Y-0B	1987
Gemmiza 1	Maya 74/On//1160.147/3/Bb/Gall/4/Chat‘‘s’’ CM58924-1GM-OGM	1991
Gemmiza 9	Ald‘‘s’’/Huac‘‘\s’’//CMH74A.630/5x CGM.4583-5GM-1GM-0GM	2000

SSR markers analysis

SSR analysis was conducted using the nine polymorphic SSR markers (Table 2). PCR reactions were carried out in a volume of 26 µl. PCR mixtures contained 2.0 µl wheat genomic DNA, 2.5 µl 10x PCR buffer, 1.5 µl 25 mM $MgCl_2$, 0.5 µl dNTP, 1.0 µl SSR primer, 0.1 µl *Taq* DNA polymerase and 18.4 µl diH2O. Amplification for all SSR markers were carried out according to Roder et al., 1998, the following program conditions, initial denaturation at 94°C for 3 min, then denaturation for 45 cycles at 94°C for 1 min, annealing for 45 cycles at 50, 55 or 60°C for 3 min, extension for 45 cycle at 72° C for 1.3 min, followed by final extension at 72°C for 5 min.

Marker Polymorphism

Amplification products of PCR were analyzed by electrophoresis on 1.2% agarose gels in 1x TBE buffer, stained by ethidium bromide, visualized and photographed under ultraviolet illumination UV light.

Data Collection and Diversity Analysis

SSR markers were scored for the presence (1) or absence (0) of amplified bands for each sample. Genetic similarity coefficients were calculated using the Numerical Taxonomy Multivariate Analysis System (NTSYSpc) Version 2.1 software package. The resulting similarity coefficients were used to perform the cluster analysis by the unweighted pair group method of arithmetic mean (UPGMA). All calculations were performed using the NTSYS-pc version 2.1 software package Biostatistics Inc., USA, (Rohlf, 2000). SSR polymorphism rates were determined using polymorphism information content (PIC) value, which were calculated according to the formula: $\mathrm{PIC} = 1 - \sum_{i=1}^{k} P_i^2$, where k is the total number of alleles detected for a locus of a marker and P*i* is the frequency of the *i*th allele in the set of seven genotypes investigated (Anderson *et al.,* 1993). Data for agronomic traits were standardized as described by Roldan-Ruiz *et al.* (2001) and then used to estimate the Euclidean distances.

Table 2: Description of nine wheat microsatellites markers, their chromosomal location, motif and annealing temp.

No	SSRs	Chromosome	Motif	Annealing temp. (°C)
1	*Xgwm164*	1A	(CT)16	55
2	*Xgwm268*	1B	(GA)17TA(GA)27	55
3	*Xgwm312*	2A	(GA)37	60
4	*Xgwm374*	2B	(GT)17	60
5	*Xgwm608*	4D	(GA)16	60
6	*Xgwm071*	3D	(GT)20	60
7	*Xgwm601*	4A	(CT)17	60
8	*Xgwm626*	6B	(CT)5(GT)13	50
9	*Xgwm333*	7B	(GA)19	55

RESULTS AND DISCUSSION

SSRs Polymorphism

In total, nine SSR markers for nine loci were tested for their ability to generate SSR banding patterns from DNA corresponding and evaluate the genetic diversity of seven bread wheat varieties. All SSR markers used in this study yielded polymorphic fragments, yielding a polymorphism rate of 100% among the seven varieties. A total of 41 alleles were detected. The number of alleles per locus ranged from 3 to 7 with an average number of 4.56 alleles per locus (Table 3). A wide range allelic variants was observed for each locus (Table 3). The maximum number of alleles was observed at *Xgwm626* and their size ranged from 96 to 108 bp. A similar pattern of allelic variation was also detected at other loci. Different number of alleles has been detected in wheat using microsatellite markers. Huang *et al.* (2002) reported an average allele number of 18.1 in 998 gene bank accessions of hexaploid wheat originated from 68 countries of five continents. Khlestkina *et al.* (2004) found an average allele number of 6.6 in 54 Siberian old and modern common spring wheat varieties. Roussel *et al.,* 2005 reported an average allele number of 16.4 in 480 wheat varieties originating from 15 European geographical areas and released from 1840 to 2000. Salem *et al.* (2008) detected an average of 3.2 alleles in seven wheat varieties. In the present study, the average number of alleles was 4.56 in seven wheat varieties. The value was lower than most previous studies, but it was comparable with Stachel's results, which detected 4.8 alleles per locus in wheat varieties (Stachel *et al.,* 2000) and was high than the average of 3.2 alleles per locus in seven wheat varieties detected by Salem *et al.* (2008). In the present study, the average number of alleles was different for individual genomes: 11 for A genome, 24 for B genome and 6 for D genome. This might suggest that D genome is the most conserved. This may be due to the evolution of wheat genomes, as D genome was incorporated into hexaploid wheat much later than A and B genomes, so it may be less diverse. On the other hand, the number of SSR alleles located on B genome may reflect its greater variability sustained during evolution (Feldman 2001). Those results are consistent with data achieved by Roder *et al.* (1998) and Huang *et al.* (2002) for SSR markers.

Gene diversity

The allelic polymorphism information content (PIC) values ranged from 0.448 for the *Xgwm333* to 0.857 for the *Xgwm626* with an average of 0.665 (Table 3). Nevertheless, these results confirm the conclusion of Plaschke *et al*., 1995 that the small number of markers is sufficient to distinguish closely related wheat varieties and carry out phylogenetic studies, hence could select genotypes for highest genetic diversity. Wheat SSR markers showed an average PIC value of 0.665, which confirms that wheat SSR markers are highly informative (Botstein *et al.,* 1980). Gene diversity obtained in the present investigation was comparable with previous results on genetic diversity of wheat using SSR analysis.

The present results revealed that, the value of gene diversity increased with the increasing number of alleles at a given locus (Fig. 1). The correlation coefficient between gene diversity and the number of polymorphic bands was high, *r = 0.852 (P <0.01)*. The linear relationship between them is shown in Figure 1. Therefore the number of alleles can be used for the evaluation of genetic diversity. Our results agree with those of Huang *et al.* (2002) and Salem *et al.* (2010) who reported that the PIC value was correlated with the number of alleles, and did not agree with those of Prasad *et al.* (2000).

Table (3): SSR markers, position, number of alleles, size range of alleles (bp) and (PIC)

Locus	Position	Number of alleles	Size range of alleles (bp)		PIC
			Min alleles	Max alleles	
Xgwm164	1A	3	129	147	0.449
Xgwm268	1B	7	85	243	0.844
Xgwm312	2A	5	212	241	0.755
Xgwm374	2B	7	91	343	0.837
Xgwm608	4D	3	188	192	0.612
Xgwm071	3D	3	212	238	0.612
Xgwm601	4A	3	147	177	0.571
Xgwm626	6B	7	96	108	0.857
Xgwm333	7B	3	122	144	0.448
Total		41			
Mean		4.56			0.665

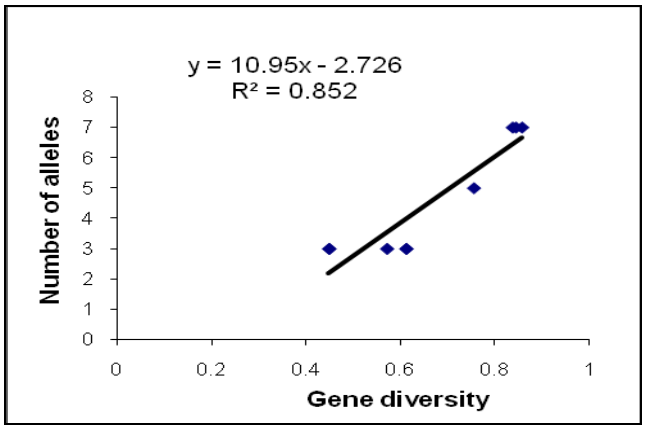

Figure 1: Relationship between gene diversity and the number of alleles detected using nine SSR markers.

Genetic relationship and diversity among different wheat varieties

To assess the genetic diversity of wheat varieties, marker data were converted into binary matrix, which in turn allowed to calculate the genetic similarity index. A dendrogram derived from UPGMA cluster analysis based on the GS coefficient matrix for the seven varieties was constructed. Basically, all varieties could be distinguished. The genetic similarity coefficient for all varieties ranged from 0.136 to 0.991 (Table 4).

Table 4: Genetic similarity estimates for seven wheat genotypes based on nine SSR markers.

	Giza 163	Gemmiza 1	Giza 157	Giza 160	Giza 164	Giza 165	Gemmiza 9
Giza 163	1						
Gemmiza 1	0.599	1					
Giza 157	0.542	0.877	1				
Giza 160	0.510	0.966	0.468	1			
Giza 164	0.136	0.653	0.683	0.547	1		
Giza 165	0.622	0.991	0.566	0.797	0.692	1	
Gemmiza 9	0.572	0.740	0.884	0.668	0.642	0.915	1

A dendrogram was created with the use of these data (Fig. 2) for each pairwise similarity estimation. The consensus tree showed that the seven wheat varieties were divided into three major groups. Group I consisted of five bread wheat varieties 'Giza 163', 'Giza 164' , 'Giza 157', 'Giza 160' and 'Giza 165' and was divided into three subgroup. Subgroup IA consisted of two varieties 'Giza 163' and 'Giza 164'. The subgroup IB included the two varieties 'Giza 157' and Giza 160'. However, The subgroup IC included only one variety Giza 165'. However, Group II consisted of one variety 'Gemmiza 9'. Also, group III consisted of only one variety 'Gemmiza 1'. In general, the similarity indices showed that the two most closely related varieties were Giza 163 and Giza 164 (Fig 2). On the other hand, the two Egyptian wheat varieties Gemmiza 1 and Giza 165 were the most genetically diversified from other varieties sources and could be important sources for new cultivar development if they differ in useful agronomic traits. It should be noted here that varieties grouping here by cluster analysis depended on the polymorphic SSR markers. Cultivars grouped together by the SSR markers could have noticeable phenotypic differences in morphology, growth habits and agronomic traits.

Agronomic traits analyses

Genetic distances for agronomic traits

Distance estimates based on 15 agronomic traits ranged from 0.61 to 1.82 (Table 5). The lowest distance was between Gemmiza 9 and Giza 164 indicting the close relationship within each of this pair of bread wheat varieties. However, the highest genetic distance was between Giza 157 and Gemmiza 1 indicating the wide relationship between these wheat varieties (Table 5).

Relationship among different wheat varieties based on agronomic traits

Cluster analysis based on the agronomic traits assigned the varieties into three major groups (Fig. 3). The first group includes Giza 163 and Gemmiza 1. While, the second group was divided into two sub-groups; the first sub-group IIA included Giza 164 and Gemmiza 9. However, the second sub-group IIB included Giza 165. However, Group III consisted of two varieties 'Giza 157' and 'Giza 160'. In general, The similarity indices showed that the two most closely related varieties were Giza 164 and Gemmiza 9 (Fig. 2). On the other hand, the two varieties Gemmiza 1 and Giza 157 were the most agronomically diversified from other genotypes sources and could be important sources for new cultivar development.

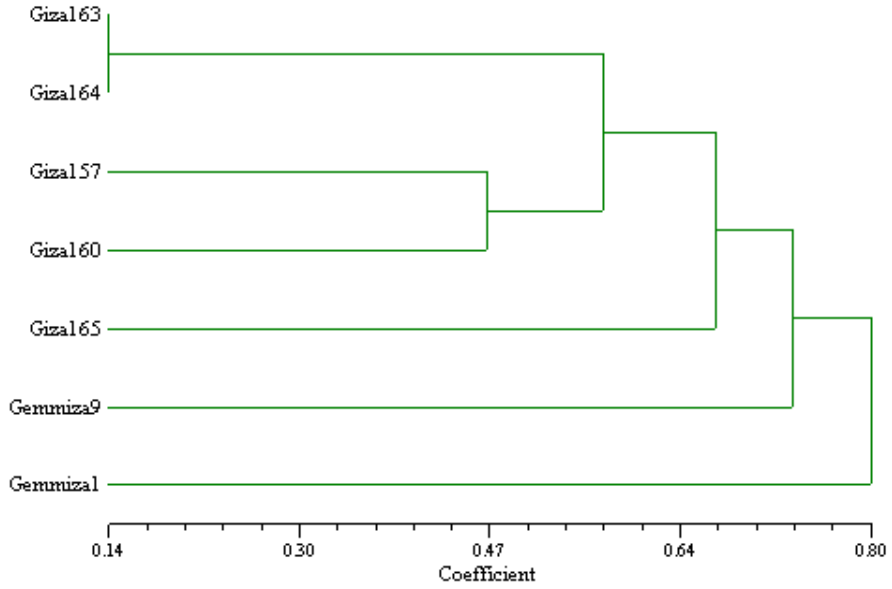

Fig. 2: Dendrogram generated based on UPGMA clustering using SSR analysis among seven bread wheat genotypes.

Table 5: Genetic distance estimates for seven bread wheat varieties based on agronomic traits.

	Giza 163	Gemmiza 1	GIZA 157	Giza 160	Giza 164	Giza 165	Gemmiza 9
Giza 163	1						
Gemmiza 1	0.84	1					
GIZA 157	1.37	1.82	1				
Giza 160	1.44	1.74	1.18	1			
Giza 164	1.27	1.44	1.46	1.26	1		
Giza 165	1.43	1.58	1.80	1.71	0.92	1	
Gemmiza 9	1.31	1.66	1.61	1.34	0.61	1.17	1

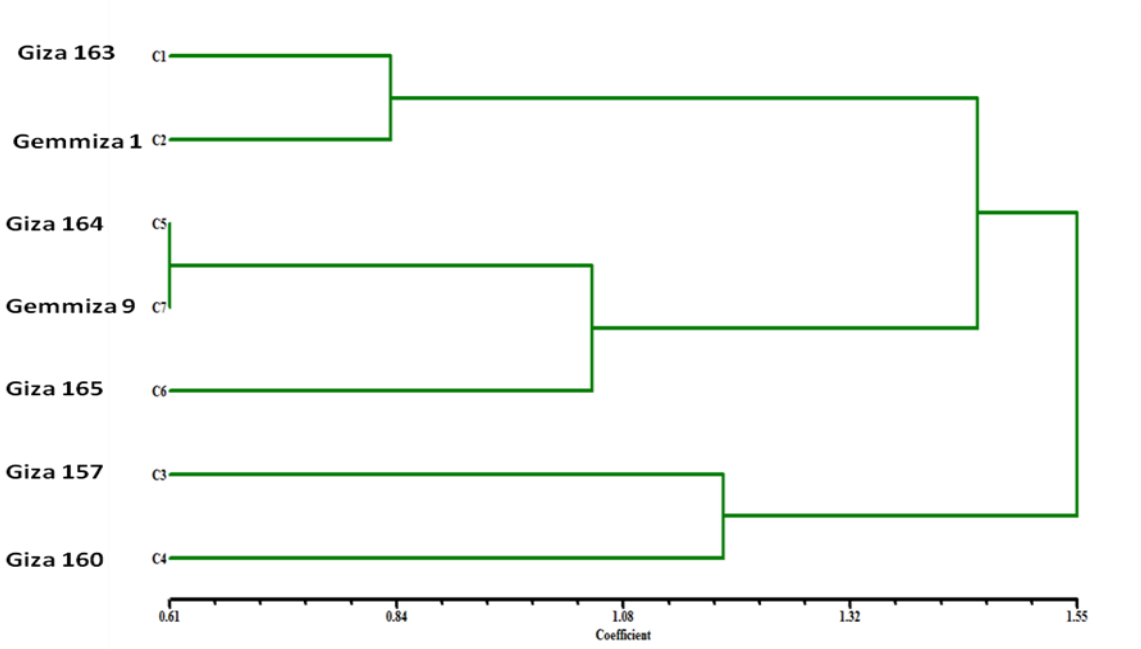

Fig. 3: Dendrogram generated based on UPGMA clustering method and Euclidean similarity coefficient among seven wheat varieties.

Comparison between molecular marker and agronomic traits

Agronomic traits analysis of wheat varieties was coupled with molecular analyses (SSR markers) to investigate the genetic relationships among seven varieties. The varieties showed diverse agronomic traits and distinct SSR markers patterns (Fig. 2 & Fig. 3). It means that the dendrogram clusters disagree with agronomic traits distance. Also, the range of genetic distance based on agronomic traits was on average higher than SSR markers, which may reflect the influence of the environment on the performances of the materials. Therefore, the DNA markers and agronomic traits will not necessarily gain closely matching results (Vollmann *et al.,* 2005, Martnez *et al.,* 2005; Sonmezoglu *et al.,* 2012). Three reasons for the low correlation between DNA markers and agronomic traits: (a) DNA markers cover a larger proportion of the genome, including coding and noncoding regions, than the agronomic traits (Semagn, 2002 and Salem *et al*., 2008), (b) DNA markers are less subjected to artificial selection compared with agronomic traits and (c) the screened SSR markers did not necessarily amplify the DNA regions linked to the gene regions expressing the agronomic traits used in this study Sonmezoglu *et al.* (2012). Martnez *et al.* (2005) believed that the correspondence between different methods might be improved by analyzing more agronomic traits and DNA markers.

The knowledge about the genetic relationships of varieties provides useful information to address breeding program and germplasm resource management (Roldan-Ruiz *et al.,* 2001). In summary, our data showed significant variation in agronomic traits and SSR polymorphisms among bread wheat varieties. This study using SSR markers and agronomic traits revealed considerable amount of genetic diversity among seven wheat varieties. The SSR data can be used in selecting diverse parents in breeding program and in maintaining genetic variation in the germplasm, is crucial in utilizing the genetic potential of these varieties for improvement of traits needed for adaptation to various stress conditions. Also, this study shows that

analyzing higher numbers of varieties may not add much practical value to a general plant improvement program, unless a specific crossing program is aimed towards the improvement of specific traits. It is therefore suggested that a focused breeding scheme should be adopted while analyzing genome diversity estimates for parent selection to gain maximum value and practical impact on a breeding program.

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تقدير التباعد الوراثي بين بعض أصناف قمح الخبز باستخدام المعلمات الجزيئية والصفات المحصوليه

السيد العبساوي[1] ، عبد الحميد نوار[2] ، فوزي الفقي[3] ، خالد فتحي سالم[4] ، انجي ادوارد[4]

[1] قسم المعلوماتيه الحيويه، معهد الهندسه الوراثيه والتكنولوجيا الحيويه، جامعه مدينه السادات, مصر

[2] قسم المحاصيل, كليه الزراعه، جامعه المنوفيه، مصر

[3] قسم البيوتكنولوجيا، كليه الزراعه، جامعه الازهر، مصر

[4] قسم البيوتكنولوجيا النباتيه، معهد الهندسه الوراثيه والتكنولوجيا الحيويه، جامعه مدينه السادات، مصر

الملخص العربى

يعد استخدام المعلم الجزئ الميكروستاليت مهم لتقدير التنوع الوراثي في القمح وكذلك الصفات المحصوليه، ويهدف إجراء هذا البحث إلى:

ا) استخدام المعلم الجزيئي الميكروستاليت والصفات المحصوليه لتقييم التنوع الوراثي بين بعض أصناف قمح الخبز، ب) تقييم العلاقات الوراثية بين بعض أصناف القمح, ج) مقارنة النتائج على أساس استخدام كلا من المعلمات

الجزيئيه والصفات المحصوليه في تقدير التنوع الوراثي. وقد استخدم لتنفيذ هذا البحث تسعه معلمات جزيئيه ميكروستاليت لدراسة التنوع الوراثى لسبعه أصناف من قمح الخبز والتابعة للنوع (*Triticum aestivum L.*) وهم جيزة 157 ، جيزة 160 ، جيزة 163، جيزة 164، جيزة 165، جميزة 1، جميزة 9. وفيما يلي ملخص لأهم النتائج :

1- كان إجمالي عدد الأليلات 41 أليل وراثي بمتوسط 4.56 أليل لكل موقع وراثى وتراوح عدد الأليلات بين 3 الى 7 أليل.

2- كانت قيم PIC والتي تعبر عن التنوع الوراثى للتسعه معلمات جزيئيه مابين 0.448 للمعلم الجزيئي *Xgwm333* علي الكروموسوم سبعه B ، 0.857 للمعلم الجزيئي *Xgwm626* علي الكروموسوم سته B , وبمتوسط قدرة 0.665.

3- كان معامل الارتباط عالي المعنويه "*r = 0.852 , (P <0.01)*" بين التنوع الوراثي وعدد الأليلات.

4- تراوحت قيم التشابه الوراثي على أساس الصفات المحصوليه من 0.61 الي 1.82 , وكانت أعلى من علامات قيم التشابه الوراثي على أساس المعلمات الجزيئيه التي تراوحت من 0.136 الي 0.991.

5- اشتملت نتائج الدندوجرام على أساس المعلمات الجزيئيه و على أساس الصفات المحصوليه علي ثلاثه مجموعات رئيسيه ولكن غير متطابقين مما يدل علي وجود اختلافات بين الأصناف مورفولوجيا و جزيئيا.

6- أوضحت هذه الدراسة أنة يمكن الحصول على أعلى اختلافات وراثية بين أصناف القمح المستخدمه باستخدام أقل عدد من المعلم الجزئي الميكروستاليت وكذلك عمل البصمة الو راثية لهذه الأصناف.

7- تشير الدراسة إلى أن المعلمات الجزيئيه والصفات المحصوليه يمكن أن تستخدم بنجاح في تقدير التنوع الوراثي في القمح. أيضا المعلومات الناتجه من هذه الدراسة يمكن أن تستخدم لتحديد الآباء الداخله في التهجينات بين الأصناف لتحقيق أقصى زيادة فى المحصول ومكوناته فى برامج تربية القمح.

8- يمكن استخدام بيانات SSR فى اختيار الآباء المختلفة فى برامج التربية و فى الحفاظ على التنوع الوراثى فى الاصول الوراثية، و هذا يعتبر أمر حاسم فى الاستفادة من الإمكانات الوراثية لهذة الاصناف لتحسين الصفات اللازمة للأقلمة مع ظروف الإجهادات البيئية المحتلفة.