Egypt. J. Chem. **Vol. 65,** No. SI:13B pp.1009-1021 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Genetic diversity estimates through yield performance and molecular markers in spring wheat genotypes.

Ramadan M.Esmail, Adel Ahmed El -Shafei , F. B. Fathallah, I.S.El-Demerdash.

Genetic and Cytology Department, Biotechnology Research Institute, National Research Centre, Dokki, Giza, Egypt

Abstract

This study was conducted to evaluate 32 spring wheat genotypes in a field experiment as well as at the molecular level using EST-SSR and SSR markers, to estimate genetic diversity. Results of field evaluation revealed a significant variation between the tested genotypes. The graphic analysis based on Euclidean distances, using the estimated values for each of the five traits separately, divided the 32 genotypes into two main groups, and each group was divided into small sub-clusters. The 32 wheat genotypes were divided into nine primary groups by a cluster analysis based on molecular data, with similarity coefficients ranging from 0.320 to 0.890, with an average of 0.687. The PIC values of the EST-SSR primers ranged from 0.111 to 0.665. But the PIC values of the SSR primers ranged from 0.17 to 0.737.

Based on the results obtained here, we have identified six new advanced breeding lines that can be recommended for use in future wheat breeding programs, four of them L7,L8,L31 and L38to improve grain yield productivity as well as , L20 to develop dwarf or semi-dwarf cultivars , and L 3 for breeding to short-season cultivars..

Key Words: Wheat, Yield traits, Genetic diversity, EST- SSR, Molecular marker.

Introduction

Wheat is one of the main staple grain crops that are critical to global food security. The majority of the world's population depends on wheat as food, and it occupies a leading place in the international grain trade.

The global population will be increased to 9.2 billion by 2050.To preserve wheat productivity, we will need to put in extra effort to boost grain production and yield stability through breeding programs. The challenges facing scientists interested in increasing wheat production locally and globally are; the lack of adequate agricultural land availability, climate change, biotic and abiotic stresses.

The availability of information about genetic variance helps breeders to identify genetically diverse parents to create a new set containing maximum variance and to introduce desired genes related to resistance to biotic and abiotic stress and the ability to adapt to a wider range of different materials into the available genetic material. (**Dangi et al., 2018).**

Many new alleles for higher grain yield, disease resistance, and abiotic stress tolerance have emerged as a result of increased genetic diversity in wheat. Previously, scientists realized the importance of genetic diversity, and there is still a large gap in the description of available genetic resources and their use in breeding programmers. Over time, conventional plant breeding techniques have successfully assimilated new alleles into elite germplasm, which has significant, effects on increasing production globally (**Patial and Pramanick, 2020).**

Several types of DNA molecular markers have been used to avoid the influence of environment such as Restriction Fragment Length Polymorphism (RFLP), Randomly Amplified Polymorphism DNA (RADP), Simple Sequence Repeat (SSR) and Single Nucleotide Polymorphism (SNP) markers have been widely used to measure genetic diversity studies in wheat and provided effective genotyping (**Landjeva et al,2007**).Presently, there are over 30 kinds of molecular markers accessible for surveying hereditary variety (**Mondini et al. 2009**).

As compares between DNA markers simple sequence repeats (SSRs) have many advantages because show high reproducibility, require a small amount of DNA, co-dominantly inherited, multi-allelic, highly polymorphic, abundant and evenly distributed in the genome, also the hyper-variable nature of SSRs produces very high allelic variations even amongst very closely related varieties. Numerous studies used EST-SSR and SSR markers in wheat ; **(Salem et al, 2008, Huang et al 2016,Zarei et al, 2016,Nihar et al 2017,Salehi et al 2018,Elshafei et al 2019a,b,van Frank et al, 2020 ,Yared et al, 2021 and Ayseet al,2021).**

Plant breeders are producing new cultivars with greater production potential, earlier maturity, better

*Corresponding author e-mail: lola_eldemardash@yahoo.com

Receive Date: 28 June 2022, Revise Date: 14 August 2022, Accept Date: 06 September 2022

DOI: 10.21608/EJCHEM.2022.140938.6396

adaptability, higher quality, and increased resistance to disease, insects, and environmental stress, to name a few of the features that benefit mankind, (**DePonti, 2009).**So, before performing hybridization in breeding programs the first step is the selection of parents from the available material possessing desired characters.

Therefore, this study was conducted with the aim of estimating genetic divergence by evaluate of 32 wheat genotypes for desirable traits under field conditions. Assessment of genetic diversity at molecular level between them using EST-SSR and SSR markers, to select the best diverse genotypes and exploiting in future wheat breeding programs.

Materials and Methods

1- Plant materials.

We used a set of 32elite wheat genotypes, consisting of 16historical released varieties commercially used in Egypt and 16 advanced lines from breeding programs, developed **by (Esmail, et al 2008),** Symbol, Name and Pedigree are presented in (Table 1).

2- Experimental design for field evaluation

The present investigation was carried out at the experimental farm in Shebin El-kom Menofiya, Egypt, during 2019/2020 winter growing season to study the performance of 32 genotypes for yield and some related traits. The field experiment was laid out in a randomized complete block design (RCBD) with three replicates. In the first half of November, each entry was planted in two rows 3 meter long and 30 cm apart, the seeding rate was 10 cm per plant. All the normal agronomic practices were followed as usual in the ordinary wheat field in the areas of study.

Table (1): Symbol, Name and Pedigree of the cultivars and advanced breeding lines used inthis study.

Symbol	Name	Pedigree
G1	Line-1	Advanced breeding line
G2	Line-2	Advanced breeding line
G3	Line- 3	Advanced breeding line
G4	Line- 4	Advanced breeding line
G5	Line- 5	Advanced breeding line
G6	Line- 6	Advanced breeding line
G7	Line- 7	Advanced breeding line
G8	Line -8	Advanced breeding line
G9	line 24	Advanced breeding line
G10	Sids-4	Maya "s"/Mon "S"/CM H74.A592/3/Giza 157*2
G11	C.306	Regent 1974/3*CHZ//2*C591/3819/C281
G12	Misr-1	Oasis/Skauz//4*BCN/3/2*PASTOR.CMSSOYO1881T- 050M-030Y–O3OM-030WGY-33M-0Y–0S
G13	Morocco	Susceptible Check
G14	Line 20	Advanced breeding line
G15	Pavon 76	VICAM-71//CIANO-67/SIETE-CERROS-66/3/KALYANSONA/BLUEBIRD
G16	Sids 14	SW8488*2/KUKUNA-CGSS01Y00081T-099M-099Y- 099M-099B–9Y–0B-0SD
G17	Line66	Advanced breeding line
G18	Shandwel.1	SITE/ MO/4/ NAC/ TH.AC//3* PVN/3/MIRLO/ BUC CMSS93B00567S-72Y-010M -010Y-010M-3Y-0M- 0HTY-0SH
G19	Sonora 64	YAKTANA-54//NORIN-10/BREVOR/3/2*YAQUI-54
G20	Beniswef-3	CORM"S"/RUFO"S" CD-48693-10Y-1M-1Y-0M
G21	Misr-2	Skauz/Bav92. CMSS96M0361S-1M-010SY-010M-010SY-8M-0Y-0S
G22	Sids 12	BUC//7C/ALD/5/MAYA74/ON//1160.147/3/BB/GLL/4/CHAT"S"/6/MAYA/VUL//CMH74A.630/4*SX SD70964SD-1SD-1SD-0SD
G23	C.B. Jo	Advanced breeding line,,
G24	Line 77	Advanced breeding line
G25	Line-37	Advanced breeding line
G26	Line- 31	Advanced breeding line
G27	Line -32	Advanced breeding line
G28	Line- 38	Advanced breeding line
G29	Giza168	MIL/BUC//SERI MIL/BUC//Seri CM93046-8M-0Y-0M-2Y-0B
G30	Gmiz.11	BOW"S"/ KVZ"S"// 7C/ SER182/3 / GIZA168/ SAKHA61 GM7892-2GM-1GM- 2GM-1GM-0GM
G31	White m	Advanced breeding line
G32	Giza-171	SAKHA93 /GEMMEIZA 9 S.6-1GZ- 4GZ- 1GZ- 2GZ-0S

Data collection

We evaluated five agronomic traits: days to heading, plant height (cm), number of spikes/plant, spike yield (g) and grain yield/plant (g). Data were collected on an individual plants basis for the above traits. The **statistical analysis** of data obtained is conducted according to (**Gomez and Gomez 1984**) using **SPSS** statistical program.

Molecular analysis

Molecular analysis was carried out at the Biotechnology Lab, Department of Genetics and Cytology, National Research Center (NRC), Dokki, Giza, Egypt. DNA was extracted from wheat genotypes using the Wizard Genomic DNA purification Kit (Promega Corporation Biotechnology, Madison, WI, USA). Then, the extracted DNA was treated with RNase and stored in a refrigerator at −20˚C.Before conducting the EST-SSR and SSR analysis, DNA was diluted to 25 ng/µL. Nineteen EST-SSR primers **(Peng and Lapitan 2005**) and nine SSR primers **(Somers et al. 2004)** were used (Table 4). The PCR mixture comprised 50 ng of genomic DNA, 1X PCR buffer, 1.5 mMMgCl2, 0.1 mM each dNTP, 0.5 µM each of forward and reverse primers, and 1 UTaq polymerase in a volume of 0.015 cm^3.The PCR program for the EST-SSR and SSR analyses involved a primary denaturation at 94˚C for 3 min, followed by 35 cycles of denaturation at 94˚C for 1 min, annealing at 50, and 61C˚ (dependent on EST-SSR and SSR primers) for 1 min, and extension at 72˚C for2min, followed by a final extension at 72˚Cfor 10 min. The amplified PCR products were applied to 3% (m/v) agarose gel containing 0.1 µgcm^3ethidium in TBE buffer. After electrophoresis, a photograph of the gel was captured using a UV trans-illuminator. The EST-SSR and SSR data were scored on the basis of presence (1) or absence (0) of a given marker, after excluding un-reproducible bands.

Molecular marker data and genetic variation

A similarity matrix was estimated according to (**Nei and Li,1979**) using molecular marker data as follows:

SM = 2N ij/(N i + N j) where, Nij is the number of alleles present in both the ith and jth genotypes, Ni is the number of bands present in the ith genotype, and Nj is the number of alleles present in the jth genotype. The similarity matrix was then subjected to the rate un-weighted pair group method with arithmetic average(UPGMA) grouping algorithm. Principal coordinate analysis (PCoA) was used as an alternative to hierarchical clustering in that the similarity matrix was used to obtain the coordinates. These coordinates were then used to create scatterplots that represent the relationships among genotypes. Both UPGMA and PCoA were conducted using PAST version 1.62 **(Hammer et al. 2001).** Furthermore, to ensure the reliability of the generated dendrogram, 1000 simulations were performed using PAUP* version 4.0.b5 **(Swofford2001).** Polymorphic information content (PIC) was calculated as follows **(Smith et al. 1997)**:PIC = $1 - \sum^{n} pi^2$

Where, pi is the frequency of the ith allele across genotypes. To identify the informative markers and study the correlation between genetic diversity and average grain yield for each genotype in the stressed environment. The discrimination power was calculated by dividing the number of polymorphic alleles amplified for each primer by the total number of polymorphic alleles obtained (**Khierallahet al.2011**).

Results

The analysis of variance for all studied characteristics is presented in Table (2). Highly significant genotype differences were recorded for all traits indicating the presence of considerable variability between the tested genotypes.

Table (3) shows that genotypes significantly differed for all traits studied. Average heading date was 77.45 day and ranges between genotypes were 45.3 days. The early heading date was taken from Sids-4 (57 days, to heading) whereas Sonora variety had late flowering time (102.33 days).

Table (2): Analysis of variance of the traits measured on the thirty-two spring wheat genotypes.

S.O.V.	D.F	Days to heading	Plant height (cm)	Spikes no /plant	Spike yield (g)	Grain yield /plant (g)
Reps.	2	0.48	0.525	0.374	0.0334	2.94
Genotypes	32	292.93**	250.87**	8.02**	2.29**	163.28**
Error	64	1.31	2.69	1.28	0.076	26.92

*and ** significant at 0.05 and 0.01 levels of probability, respectively.

Table (3): Mean performance of the traits measured on the thirty two spring wheat genotypes.

Genotypes Name		Days to heading	Plant height (cm)	Spikes no /plant	Spike yield(g)	Grain yield /plant (g)
1.	Line-1	90.00	114.0	5.0	4.37	21.77
2.	Line-2	90.66	108.33	6.67	3.90	29.78
3.	Line- 3	63.33	96.33	3.33	5.67	18.99
4.	Line- 4	68.00	104.66	3.66	5.74	20.86
5.	Line- 5	72.66	103.66	5.67	5.23	29.69
6.	Line- 6	73.33	110.33	5.01	4.16	20.95
7.	Line- 7	71.66	106.0	6.0	6.38	38.29

8.	Line -8	69.00	108.66	8.0	4.63	36.97
9.	Line- 24	66.66	107.33	5.66	4.63	26.49
10.	Sids4	57.00	113.33	3.33	4.51	15.03
11.	C.306	80.00	119.66	6.0	4.12	24.84
12.	Misr 1	87.00	108.33	7.66	3.94	30.25
13.	Morocco	77.66	119.0	5.0	4.18	20.99
14.	Line 20	77.66	74.0	7.33	4.67	33.89
15.	Pavon 76	70.66	108.0	5.0	4.42	22.25
16.	Sids 14	94.33	116.33	6.0	4.53	27.78
17.	Line66	71.33	107.66	5.33	3.77	20.18
18.	Shandwel.1	77.00	117.66	8.66	4.20	36.44
19.	Sonora 64	102.33	119.66	6.0	3.44	20.52
20.	Bensiwef-3	90.00	103.66	9.0	4.15	35.25
21.	Misrt-2	89.00	97.0	6.33	4.21	26.73
22.	Sids 12	81.00	121.33	7.33	4.47	32.97
23.	C.B. Jo	68.00	107.33	5.0	3.65	18.39
24.	Line 77	72.66	110.66	5.33	4.64	24.79
25.	Line-37	80.00	122.33	6.33	3.79	23.84
26.	Line- 31	76.33	110.0	4.66	7.32	35.32
27.	Line -32	77.33	114.66	4.33	6.25	26.91
28.	Line- 38	75.00	103.0	6.0	5.96	35.94
29.	Giza168	76.66	106.66	9.66	3.80	37.16
30.	Gmiz.11	70.33	107.00	7.0	4.99	35.11
31.	White m	70.66	107.33	7.0	4.63	32.41
32.	Giza-171	76.66	113.33	6.33	5.01	31.18
Mean		77.45	109.31	6.16	4.54	28.52
LSD .05		1.86	2.68	1.84	0.45	8.47
LSD .01		2.48	3.56	2.45	0.59	11.27

Prior to performing cross-breeding program, the plant breeder must have sufficient information about flowering time, to overcome differences in flowering dates between available plant genetic materials by planting them at intervals. Cluster analysis based on Euclidean distances of the first measure trait days to heading, grouped the 32 genotypes into two major groups (Fig.1) in general agreement with the mean performance obtained (Table 3). However, genotypes in each main group were sub-divided into two sub–clusters. The first sub-cluster consisted of 6 genotypes, while the second ones included the highest number of days to heading (102.33days), variety Sononra 64 only. The second main group was again subdivided into two sub-clusters. The first sub-cluster in group 2 included 23 genotypes and branched into two sub—sub – clusters, its contains the best early twelve genotypes eight advanced lines; no. 24,8,3,7,5,6,66 and 77 and four varieties C.B.Jo, pavon 76 , White M and Gimmiza 11.On the other hand, the second sub-cluster in group 2 included two early genotypes, Sids 4 and Line 3 which had the lowest number of days to heading(57.0 days and 63.33 days) , respectively.

Results concerning plant height mean performance revealed that Line 20 had the shortest plant height (74.cm) while, the tallest taken from Line 37 (122.33cm). As shown in (Fig.2) the 32 wheat genotypes classified into two major groups, line 20 was alone in a first group as the semi-dwarf genotype, as well as line. 3 and Misr 2 fallen in lower diagrams also exhibited shortest plant height. Sub group 2 contains four genotypes; sub- group 3 included 14 genotypes and divided into four sub clusters. Sub -group 4 containing 6 wheat genotypes and fifth group included five genotypes had the tallest plant height i.e, C306,Sonora,Morocco, Sids 12 and line 37.The genotypes in the same group are more similar than those in different groups, this is evident in Table 3.

Spike numbers per plant is a major contribution to grain yield. Results in Table .3 showed that 13 genotypes exceeded in their performance than the overall mean (6.16) of all genotypes under study, the four advanced lines no, 2,8,20, and 37 demonstrated superior performance. Clustering of 32 wheat genotypes produced two main groups Fig 3, the first one demonstrated the three superior varieties under field conditions Giza 168, Shandwel 1 and Beniswef 3, while the second group was divided into three sub clusters composed all other genotypes including the best four advanced breeding lines.

Spike yield (g) between genotypes ranged from 7.32g (Line 31) to 3.44g (Sonora 64). High spike yield (7.32g) were taken from high fertility genotype Line 31and followed by Lines 7 and 32 (6.38g and 6.25g, respectively) .These three genotypes may be used as a best germplasm to increase grain number per spike.

Cluster diagram using spike yield data (Fig.4) classified genotypes into two main clusters, each of them again sub-divided into two sub-clusters. The first main cluster included 6 genotypes, line 31(had high grain number per spike) lies in the first sub-cluster only. However, the two other sub-clusters branched from the second main cluster included 26 genotypes.

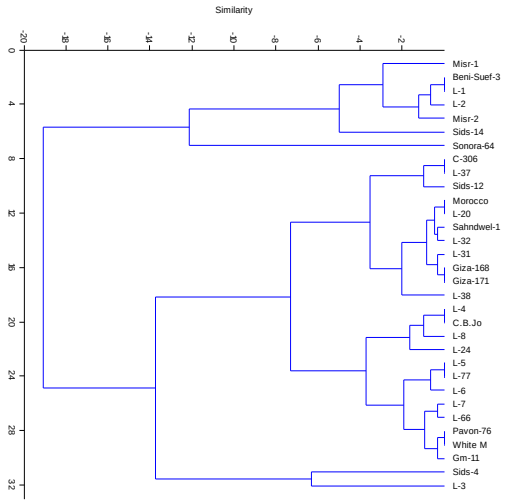

Fig (1): Cluster diagram for 32 wheat genotypes classified by Days to heading data.

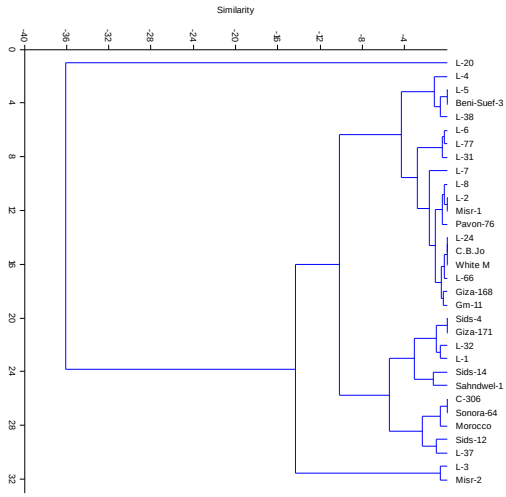

Fig (2): Cluster diagram for 32 wheat genotypes classified by plant height data.

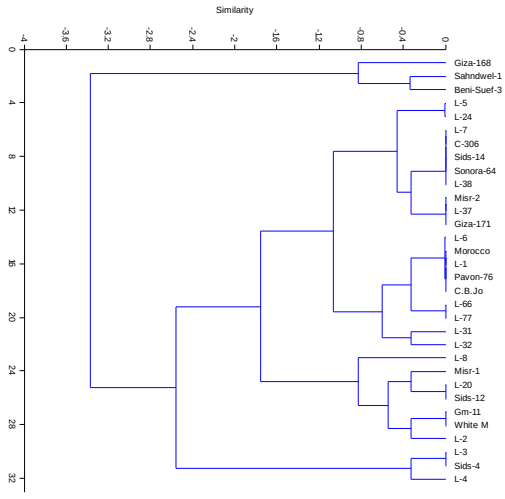

Fig (3): Cluster diagram for 32 wheat genotypes classified by spikes no per plant data.

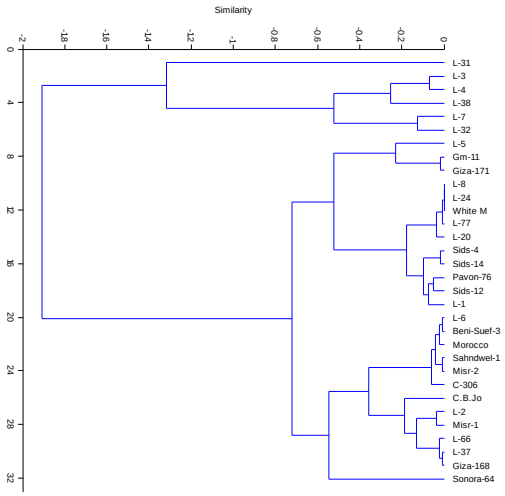

Fig (4): Cluster diagram for 32 wheat genotypes classified by spike yield data.

Grain yield is a complex trait and resulting from contribution to other yield components. Results in Table (3) revealed that variations between genotypes in grain yield per plant ranged from (38.29 g to 15.03 g). The highest grain yield /plant in 16 advanced breeding lines were produced by genotype L7 which produced 38.29g followed by L8 L38, L31 and L20 which recorded 35.94g, 35.32g and 33.89g, respectively. The lowest genotype among all evaluated varieties was Sdis-4 which gave 15.03g. Four advanced breeding lines gave maximum grain yield per plant L7, L8, L31 and L38, while the next four, L1, L3, L6 and L66 showed the lowest values.

Cluster analysis of grain yield per plant divided the 32 wheat genotypes into five sub-clusters originated from two major clusters (Fig. 5) in general agreement with their mean performance obtained. The first sub-cluster composed seven genotypes; four advanced lines L24, L32, L77 and L37 and three varieties Misr 2, Sids 14 and C306.The second cluster included nine genotypes that had intermediate grain yield values. On the other hand, Sids 4 fell alone in the third sub-group. Moreover, four outstanding lines in grain yield performance i.e., L7, L8, L31, and L 38 falling in the fourth sub –cluster. Likewise, seven genotypes with intermediate grain yield values make up the fifth sub-group.

.

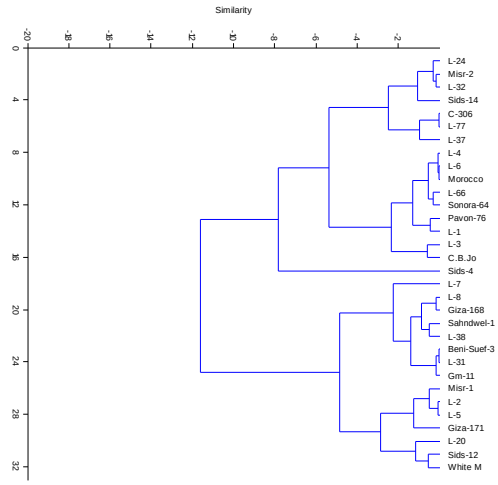

Fig (5): Cluster diagram for 32 wheat genotypes classified by Grain yield / plant data.

Microsatellite Marker combined analysis.

Only 51 of the 28 EST-SSR and SSR primer pairs tested exhibited polymorphic bands throughout the 32 wheat genotypes, including 16 released varieties and 16 advanced breeding lines. The similarity coefficients derived from the EST-SSR and SSR combination information of the 62 scored alleles were used to perform a cluster analysis. Cluster analysis was used to split the 32 wheat genotypes into nine primary groups, with similarity coefficients ranging from 0.320 to 0.890, with a mean of 0.687. The lines L-7 and L-4, as well as L-38 and L-6, had the highest genetic similarity (0.89), whereas White M and L-1 had the lowest (0.32). (See tables 4 and 5 for more information.) The first group has one genotype, (G171). The second group contained one wheat genotype (L-24). The third cluster comprising fife sub-groups. The first subgroup upheld by a bootstrap value of 1% had genotype Misr-1that was advanced to Spikes no/plant. The second sub-group supported by a bootstrap value of 16% included the three genotypes (Sids-14, L-37 and Misr-2). The third sub-group supported by a bootstrap value of 24% included the two genotypes (L-77 and L-31 that were the highest plant). The fourth subgroup contained three genotypes (L-20, L-2 and L-66 that were low in grain yield/p). The two genotypes (L-6 and L-38) that were identical in days to heading formed the fifth sub-group, which had an 80 percent bootstrap value. The fourth cluster comprising seven sub-groups. The first sub-group supported by a bootstrap value of 28% included two genotypes (L-4 and L-7).The second, third, fourth, fifth, sixth, and seventh sub-groups each contained one genotype, the second subgroup genotype Morocco that was test their resistance to rust disease, the third sub-group genotype L-5, the fourth sub-group genotype Pavon-76, the fifth sub-group genotype L-8, the sixth subgroup genotype Sonora-64(that was the lowest in Spikes no/plant and the highest in Days to heading). The seventh sub-group genotypeL-1(that was the lowest in Days to heading).The fifth group supported by a bootstrap value of 9% included three varieties (Sids-4, Sahndwel-1 and Giza-168). The six cluster containing two sub-groups. The first sub-group enclosed line (L-32). Three wheat genotypes (L-3, C.B.jo and Sids-12) were included in the second sub-group, which had a bootstrap value of 16 % that were a lowest grain yield /plant. The seventh group supported by a bootstrap value of 18% consist of two varieties (C.306 and Gm-11) that were equal in Plant height. The eighth group contained one wheat genotype(Beni-Suef-3). The ninth group contained one wheat genotype (White M). Results of the study indicated that based on agronomic parameters and molecular markers are promising lines (L-7, L-8, L-38 and L-31), that gave a high grain yield (38.29, 36.97, 35.94 and 35.32g/plant) that will be evaluated for resistance to rusts in another study.

Molecular markers (EST-SSR and SSR) and Pedigree-based genetic diversity estimates showed a similar hierarchical type of genetic diversity among the thirty-two genotypes evaluated. However, genetic diversity estimate was not specific for identifying degree of relatedness among individuals.

Genetic information produced by EST-SSR and SSR markers

The PIC of 19EST-SSR primers was calculated to determine their discriminating power (DP). Using 19 EST-SSR primers, 39 alleles were amplified from the 32 wheat genotypes. The average number of amplified bands (alleles) for each primer was 2.05, ranging from one for primer 'Xcwem1' to four for primer 'Xcwem17' (Tables 4, 5). Amplified alleles have lengths ranging from 68 to 320 bp. The PIC values for each of the nineteen EST-SSR loci were used to calculate the levels of polymorphism among the 32 genotypes. All of the EST-SSR loci tested had a wide range of PIC values. Four primers had 0 PIC values because they only recognized one allele. The PIC values for the next fifteen primers ranged from 0.059 (Xcwem32) to 0.665 (Xcwem14) (Tables 4, 5). The number of amplified was favourably linked (r = 0.377) with the PIC values. alleles per primer. The polymorphism detected by EST-SSR primers was similar to that detected by gSSR markers, according to the current study. These might efficiently distinguish between various genotypes.

Table 4. Details of 19 genomic EST-SSR and nine SSR makers including their annealing temperature (TA), location on the wheat genome, amplicon size, number of alleles, number of polymorphic alleles detected, polymorphism information content (PIC), and discrimination power (Dp) for 32 wheat genotypes.

Source of primers	Primers	Chromosome location	TAC°	Amplicon size range (bp)	Number of alleles	Polymorphic allele	PIC value	Dp.
EST-SSR	Xcwem1	1D	50	80	1	0	0.0	0.0
	Xcwem2	3B	50	80-85	2	1	0.111	0.062
	Xcwem3	1A	55	100-90	2	2	0.392	0.062
	Xcwem4	3B	50	85-90	2	2	0.114	0.062
	Xcwem5	1D	55	90-126	2	2	0.499	0.062
	Xcwem6	1B	55	98-170	2	2	0.485	0.062
	Xcwem7	6B	55	90-95	2	2	0.445	0.062
	Xcwem8	2A	55	85-95	2	2	0.496	0.062
	Xcwem9	3A, 1B	55	78-115	2	2	0.500	0.062
	Xcwem10	1D	50	68	1	0	0.0	0.0
	Xcwem11	4A	60	90-120	2	2	0.504	0.062
	Xcwem12	1D	60	95-320	3	2	0.436	0.062
	Xcwem13	5B	55	95	1	1	0.0	0.031
	Xcwem14	2D	55	85-130	3	2	0.665	0.062
	Xcwem15	2B	60	85	1	0	0.0	0.0
	Xcwem16	2B	55	130-200	3	3	0.395	0.094
	Xcwem17	6B, 5D	55	58-300	4	3	0.655	0.094
	Xcwem19	3BB	50	85-90	2	2	0.483	0.062
	Xcwem32	1D	55	80-85	2	2	0.059	0.062
SSR	Barc130	5D	52	100-290	3	3	0.531	0.158
	Barc147	3B	52	110-140	2	2	0.170	0,105
	Barc167	2B	50	80-290	3	3	0.546	0.105
	Barc173	5D	50	10-260	3	3	0.700	0.158
	Barc180	6B	52	150-200	2	1	0.342	0,053
	Barc200	2B	52	100	1	1	0.0	0.053
	Wmc44	1B	61	90-290	4	3	0.737	0.158
	Wmc169	3A	61	225-270	3	2	0.621	0,105
	Wmc175	2D	61	90-280	2	1	0.474	0.053

Table5. The levels of genetic diversity in 32 wheat genotypes by 28EST- SSR and SSR combined analysis.

Source of primers	No. of primers	No. of loci	No. of alleles	Allele/ Loci		Similarity coefficient		PIC value		DP	
				Range	Main	Range	Main	Range	Main	Range	Main
EST-SSR	19	32	39	1 to 4	1.22	0.21- 0.88	0.680	0.111-0.665	0.328	0.031-0.094	0.054
SSR	9	19	23	1 to 4	1.21	.41-1.0	0.690	0.17-0.737	0.458	0.053-0.158	0.114
Combination	28	51	62	1 to 4	1.215	0.32-0.89	0.687	--	--	--	--

By measuring the PIC of their loci, nine SSR markers were employed to investigate their discrimination power (DP). Nine SSR markers were used to amplify a total of 23 bands (alleles) among the 32 wheat genotypes. The average number of amplified alleles (bands) per primer ranged from one for the barc200 primer to four for the wmc44 primer, with 2.555 alleles on average (Tables 4, 5). The amplified alleles were between 80 and 290 bp in length. The average number of amplified alleles (bands) per primer ranged from one for the barc200 primer to four for the wmc44 primer, with 2.555 alleles on average (Tables 4, 5). The amplified alleles were between 80 and 290 bp in length. Calculating the PIC values for each of the nine SSR primers was used to evaluate the level of polymorphism among the 32 genotypes. For each SSR locus studied, the PIC values differed substantially. The PIC value of one SSR primer was zero, indicating that it detected only one allele. The remaining eight primers had PIC values ranging from 0.170 (barc147) to 0.737 (wmc44) (Tables 4, 5). The number of amplified alleles per marker was strongly linked (r = 0.406) with the PIC values. According to Botstein et al. (1980), PIC values can be categorized into three categories: The marker is regarded substantially informative if its PIC value is more than 0.5. The marker is somewhat informative if the PIC value falls between 0.25 and 0.5. The marker is slightly informative if the PIC value is less than 0.25. The EST-SSR and SSR markers' average PIC value is fairly informative.

The PIC of nineteen EST-SSR primers was calculated to investigate their DP. All of the EST-SSR loci analysed had a wide range of DP values. Three primers detected only one allele and had 0 DP values. The following sixteen primers had DP values ranging from 0.031 to 0.094, with an average of 0.054. (Tables 4, 5). The two primes with the greatest DP (0.094) were Xcwem16 and Xcwem17. One of the primers, Xcwem13, had the lowest DP (0.031). (Table 4, 5).

The PIC of nine SSR primers was calculated to examine their DP. All of the SSR loci analysed had a wide range of DP values. The average of the DP nine SSR primers was 0.114, with a range of 0.053 to 0.158. (Tables 4, 5). The highest DP (0.158) was observed in three markers, barc130, barc173 and wmc44. The lowest DP (0.053) was observed in three markers, barc180, and barc200 and barc175 (Table 4, 5).

PCoA stands for principal coordinate analysis (Figure 7). The first and second coordinates in PCoA explained 17.64 and 15.20 percent of genotype variation, respectively. Genotypes were divided into four groups. PCOA presents results that are somewhat similar to those of cluster analysis, where Giza-171, Beni-Suef-3 and White M were grouped into three different cluster and the remainder of the genotypes were close to each other.

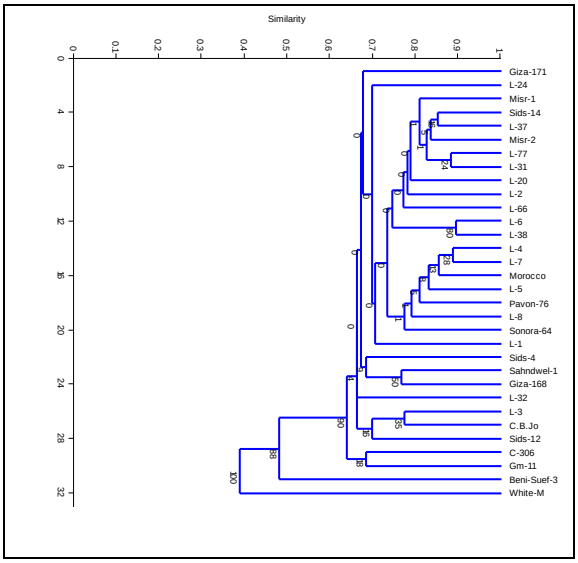

Figure 5: UPGMA dendrogram for 32 wheat genotypes containing 28 polymorphic EST-SSR and SSR combinations.

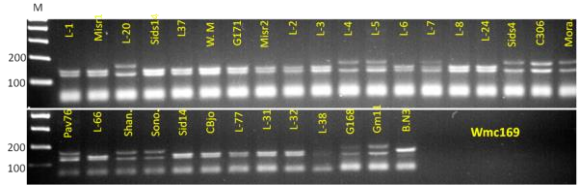

Figure 6: Amplified products of SSR marker using primer Wmc169 for analyzing32 wheat genotypes

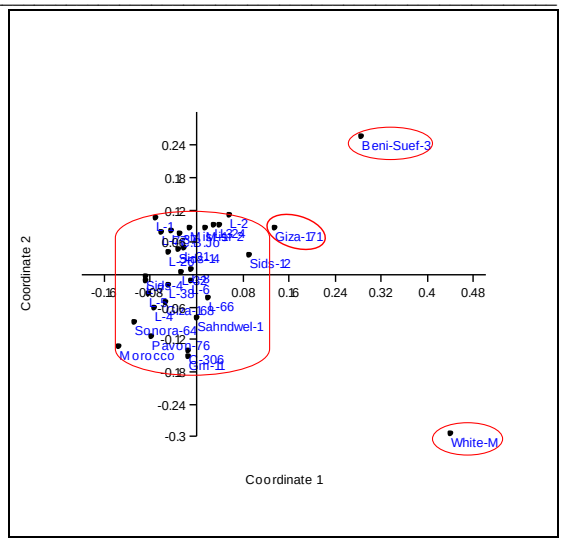

Figure 7: PCoA of the thirty-two wheat genotypes using 28 polymorphic EST-SSR and SSR combinations.

Discussion

Field evaluation experiment: Yield is a complex trait that is influenced by yield components as well as the action of multiple genes and their interactions with one another as well as environmental conditions., therefore yield and its components could be considered and exploit in breeding programs. The screening and field evaluation of available genetic materials allow the selection of outstanding ones and subsequently improved varieties. So, genetic variance among 32 spring wheat genotypes for yield and related traits were assessed under field conditions. As explained by the results we obtained here. The difference in all traits studied might be due to differences in genetically make-up of the parental materials.

The presence of wide genetic variability in genotypes (Table 2) creates opportunity to select superior genotypes **(Weikai and Hunt, 2001**). Wheat must establish develop biomass, and flower at times that correspond to optimal seasonal conditions in order to achieve maximum seed size and number (potential grain yield). **(Trethowan 2014).** Cultivars with short stature are required to restrain lodging in wheat. Suitable plant height is associated with a reduced range of lodging, an increased grain number per spike, and an improved harvest index and also increased grain yield and quality, **(Hedden 2003 and Griffiths, et al. 2012).** This suggests that early maturity improvement in wheat could be successful by selecting parents from these groups and exploit it in breeding programs. This suggested that improvement in earliness in wheat could be succeeded by selecting parents from the diverse cluster. As an example, based on cluster mean data, it is predicted that a cross between genotypes of

cluster 1 and cluster 2 might results in transgressive segregates for heading date , and short maturity traits.

Shorter stem lengths in wheat contribute to higher yields by improving lodging resistance. **(Chairi et al. 2019).**
Plant height (PH) is an important feature of yield components related to plant morphology and other yield-related traits, such as spike length, number of spikelets per spike, spike compactness (SC), and thousand kernel weight (TKW), thus affecting the yield potential. **(** Gao **et al., 2015; Kowalsk et al., 2016; Guan et al., 2018).**

Line 20 showed the shortest plant height, indicate that it contains dwarfing genes, which can be exploited in breeding programs as a parent for hybridization to produce a new dwarf and semi-dwarf cultivars. However , (**Rinkiet al., 2019)** reported that lodging, due to rain and high-speed winds causes significant economic and yield losses in cereals. Hence, lodging is emerging as a major obstacle to achieving the required yield targets. Because of the complex nature of the loading phenomenon, it remains unclear which traits are best correlated for genotype screening in breeding programs. Genotypes with more closely related traits such as plant height and stem wall thickness should be introduced/selected into breeding programs to inoculate lodging tolerance in new high yielding cultivars to enhance productivity and farmer incomes as well.

The results indicate that lines 7,8,31 and 38 performed better. These lines can be exploited as a new genetic material to enhance grain yield production in wheat. These results are in accordance with the findings reported by (**Esmail 2002;Esmail and Kattab 2002 ;Esmail et al, 2016 ;Amanuel et al(2018) ; Abdelkhalik ,2019and Ibrahim 2019)** .Therefore, there is great potential among the advanced lines that have been studied under field conditions to be used as breeding material to produce new improved wheat cultivars. The introduction of new beneficial alleles from exotic genotypes into commercial cultivars could increase genetic diversity., **(Abaza et al, 2020 and Mansour, et al 2021|).**
The graphic analysis, using the field evaluation results for each of the five traits separately, divided the 32 genotypes into two main groups, and each group was divided into small sub-groups. Cluster analysis was approved as a suitable method for data classifying and suggested by **(Mohammadi and Prasanna, 2003).** On the other hand,(**Ibrahim et al. 2011),** mentioned that agronomic parameters were useful in clustering barley varieties using traditional cluster analysis.

Similarity index and cluster analysis

(Tomkowiak et al., 2020) the parental components for heterosis crosses can be chosen based on genetic similarity evaluated using molecular SSR markers and the Jaccard, Kluczyski, Nei, and Rogers coefficients, according to our findings. In the analysis of genetic diversity among 68 advanced wheat lines by using fifty-two EST-SSR and ninety-nine SSR markers, the average number of alleles detected was higher SSRs (5.4) than EST-SSR (3.3) **(Dreisigacke 2004**). Within 54 old and modern common spring wheat varieties, there is a lot of genetic variability. 151 alleles were discovered using 22 SSR markers, with an average of 6.6 alleles per locus, ranging from 3 to 11 alleles per locus, and genetic similarity values between types ranging from 0.19 to 0.96. **(Khlestkin et al. 2004)**. In the analysis of genetic diversity among 12 Saudi wheat genotypes by RAPD and ISSR primers, the average genetic similarity among the twelve wheat genotypes were 0.50, with values ranging from 0.34 to 0.68(**Barakat et al 2010**). Nineteen SRAP and nine TRAP markers were checked to locate the genetic diversity of 6 durum wheat genotypes, as a result the coefficient of similarity was ranging from 0.71 to 0.93 for SRAP and 0.46 to 0.84 for TRAP markers and the cluster based on SRAP markers differed from that based on TRAP markers **(Al-Doss et al 2010).** while using Thirty microsatellite markers in twenty-one wheat genotypes, the genetic diversity for all loci ranged from 0.74 to 0.90 with a mean of 0.83**(Salehi et al 2018).**

Cluster analysis for old and modern 54 common spring wheat genotypes. The genotypes were clustered in two nearly equal groups including of predominantly old (liberated before 1960) and modern (liberated in 1960–90s) genotypes **(Khlestkina et al 2004).** All 253 *Triticumtauschii* genotypes (T. *tauschii*), 119 wheat landrace cultivars (LCs), and 123 current wheat cultivars are represented in this phylogenetic tree (MWCs). T. *tauschii* accessions were well distinguished from LCs and MWCs by genotypes, with only one T. *taschii* accession (Reif et al. 2005). Cluster analysis using 242 SSR markers with 43 wheat genotypes were further divided into three groups, two groups for winter wheat (18 and 10 genotypes) one group spring wheat (15 genotypes) **(Chao et al 2007).**The combined cluster based on RAPD and ISSR markers agreed superior with the groups of the wheat cultivars based on pedigree analysis than the cluster created by ISSR or RAPD data alone **(Barakat et al 2010).**

Cluster analysis using SRAP and TRAP data, separated into two main clusters **(Al-Doss et al 2010).** Estimated the genetic diversity using agronomic performance and molecular markers to find an association between molecular markers and agronomic traits. The combined analysis produced similar cluster to that produced using TRAP marker

alone **(Barakat et al 2013).** The use of forty EST-SSR and SSR markers to assess genetic diversity in sorghum. Cluster analysis revealed that the majority of genotypes within geographic origins were mostly based on race. The EST-SSR markers utilized in this study were shown to have stronger discriminating power than the genomic SSRs (Ramu et al., 2013).

The cluster separated the genotypes of the three species into three distinctive groups **(Salehi et al 2018).** Only 24 SSR makers generated polymorphisms among the 11 wheat advanced lines. The eleven wheat genotypes were categorised into two main clusters using SSR data, with similarity coefficients ranging from 0.086 to 0.88. (ELshafei et al 2019). Using wheat genotypes, a comparison of molecular marker (AFLP) and pedigree-based genetic diversity estimation approaches was made. The forty-three genotypes studied demonstrated a comparable hierarchical kind of genetic diversity using AFLP and Pedigree-based genetic diversity estimates. Though the genetic diversity estimate was not well suited to determining degree of relatedness among individuals, it could be useful for evaluating overall patterns of genetic variation among regional germplasm (Barrett et al 1998).

PCoA based on modified Rogers' distances did not separate the genotypes according to their targeted mega environments (MEs) (Dreisigacke 2004). Principal coordinates analysis for 20 early and 20 recent wheat cultivars with 2010 SSR alleles. The main coordinate plot revealed not only two completely separate groups of 20 early and recent cultivars, but also two completely separate groups of 20 early and recent cultivars (Fu and Somers 2009).The PCoA was used to imagine the genetic relationships among 21 wheat genotypes. The first three components accounted for 25.71% of the total variation, embodiment that the used marker possessed a suitable dispersion of markers in the genome. Since the first components do not explain much of the total variation **(Salehi et al 2018).**Principal coordinate analysis (PCoA) and cluster analysis, Synthetic hexaploid wheat (SHWs) were again divided into two subgroups (SpringSHW and WinterSHW) similar to that of Bayesian clustering **(Bhatta et al 2018).**The PCoA plot for the first Three coordinates, explaining 84.659(%) of variance among eleven advanced lines. lines were separated into two main groups presenting salt tolerant lines and salt sensitive lines **(ELshafei et al 2019b)**.

The PIC value reported in this investigation was very close to the previously estimated mean PIC value of 0.37. (Somers et al. 2003; Chao et al.,2009; Edwards et al.,2009; Allen et al.,2011). The polymorphism information content (PIC) an average of 0.28 among 20 US elite wheat cultivars using 359 SNP markers **(Chao et al.,2009).** The 162 DArT polymorphism information content (PIC) values ranged from 0.035 to 0.50, with an average of 0.27. (Edwards et al.,2009). The validated markers' PIC values ranged from 0.08 to 0.375, with an average value of 0.300. (Allen et al.,2011). Polymorphic information content (PIC) ranged from 0.70 to 0.89 with an average of 0.82 **(Salehi et al 2018).** Genetic diversity of 230 Nebraska winter wheat genotypes using single nucleotide polymorphism (SNPs). The polymorphism information content (PIC) across chromosomes ranged from 0.09 to 0.37 with an average of 0.23 **(Eltaher et al 2018).** The PIC values measured from 8 SRAP combination primers were ranged from 0.445 to 0.896, with a mean 0.764 per primer. The PIC values were positively correlated (r = 0.896) **(Elshafei et al 2019a).**

Using thirty CAAT box–derived polymorphism (CBDP) and fifteen start codon targeted (SCoT) markers. PIC values the ranged from 0.031 to 0.39, with an average value of 0.34 within the set of ninety one *Triticumaestivum*, *Aegilopscylindrica*, and *Aegilopscrassa* species **(Ghobadi et al 2021).** All of the SSR loci studied had a wide range of PIC values. Sixteen SSR markers detected only one allele and had zero PIC values. The remaining 7 markers had PIC values ranging from 0.18 to 0.576. The number of amplified alleles per primer was strongly linked (r = 0.95) with the PIC values (ELshafei et al., 2019b).

Each marker's measured DP ranged from 0.033 to 0.067, with an average of 0.042. (ELshafei et al 2019b). Using six (AFLP) combination primers, Khierallah et al. (2011) found a DP range of 0.31 to 0.06 among eleven date palms. The DP of the primer ranged from 0.0435 to 0.195, with 0.125 being the average (Elshafei et al 2019a).

Conclusions

Results of the study count on agronomic parameters and molecular markers of promising lines (L-7, L-8, L-38 and L-31), that gave a high grain yield/plant. Furthermore, when it came to identifying wheat genotypes, the EST-SSR and SSR markers were extremely helpful. SSR markers can be used to distinguish wheat lines as well as measure genetic variation within the genetic materials being investigated. In addition, studying the level of polymorphism among wheat genotypes is greatly aided by genetic diversity and PIC values. Consequently, wheat breeders can carry use of this information when selecting parents to start new breeding programs, with the goal of maximizing genetic variability or focusing on specific traits associated with molecular markers. Based on the results obtained here, we have identified four new high yielding advanced breeding lines that can be recommended for use in future wheat breeding programs.

References

Abaza, G.M.S.M.; Awaad, H.A.; Attia, Z.M.; Abdel-lateif, K.S.; Gomaa, M.A.; Abaza, S.M.S.M.; Mansour, E. Inducing potential mutants in bread wheat using different doses of certain physical and chemical mutagens. *Plant Breed. Biotech.* **2020**, *8*, 252–264.

Abdelkhalik,S.A.M. (2019) Assessment of some genetic parameters for yield and its components in four bread wheat crosses using six parameter model.Egypt. J. Plant Breed, 23 (2019), pp. 719-736.

Al-Doss, A.A., Saleh, M., Moustafa, K.A., Elshafei, A.A. and Barakat, M.N., 2010. Grain yield stability and molecular characterization of durum wheat genotypes under heat stress conditions. African Journal of Agricultural Research, 5(22), pp.3065-3074.

Allen, A. M., Barker, G. L. A., Berry, S. T., Coghill, J. A., Gwilliam, R.,Kirby, S., et al. (2011). Transcript-specific, single-nucleotide polymorphism discovery and linkage analysis in hexaploid bread wheat (Triticuma estivumL.). Plant Biotechnol. J. 9, 1086–1099.doi: 10.1111/j.1467-7652.2011.00628.x

Amanuel, M. D. Gebre, T. Debele (2018)**.**Performance of bread wheat genotypes under different environment in lowland irrigated areas of Afar Region, Ethiopia.African J. Agric. Res., 13 (2018), pp. 927-933.

Ayse Y., Mortaza H., Kursad O., Muhammad A. N. & Danny H.(2021) Molecular characterisation of the oldest domesticated Turkish einkorn wheat landraces with simple sequence repeat (SSR) markers, Biotechnology & Biotechnological Equipment, 35:1, 1291-1300.

Barakat, M., Al-Doss, A.A., Moustafa, K. and Ibrahim, E., 2010. Morphological and molecular characterization of Saudi wheat genotypes under drought stress. J Food, Agric Environ. Journal of Food, Agriculture & Environment, 8(1), pp.220-228.

Barakat, M.N., Al-Doss, A.A., Elshafei, A.A., Ghazy, A.I. and Moustafa, K.A., 2013. Assessment of genetic diversity among wheat doubled haploid plants using TRAP markers and morpho-agronomic traits. Australian Journal of Crop Science, 7(1), pp.104-111.

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.

Bhatta, M., Morgounov, A., Belamkar, V., Poland, J. and Baenziger, P.S., 2018. Unlocking the novel genetic diversity and population structure of synthetic hexaploid wheat. BMC genomics, 19(1), pp.1-12.

Chairi F, Sanchez-Bragado R, Serret MD, Aparicio N, Nieto-Taladriz MT, Luis AJ. Agronomic and physiological traits related to the genetic advance of semi-dwarf durum wheat: the case of Spain. Plant Sci. 2019;295:110210.

Chao, S., Zhang, W., Akhunov, E., Sherman, J., Ma, Y., Luo, M. C., et al. (2009).Analysis of gene-derived SNP marker polymorphism in US wheat (Triticumaestivum L.) cultivars. Mol. Breed. 23, 23–33.doi: 10.1007/s11032-008-9210- 6.

Chao, S., Zhang, W., Dubcovsky, J. and Sorrells, M., 2007. Evaluation of genetic diversity and genome-wide linkage disequilibrium among US wheat (Triticumaestivum L.) germplasm representing different market classes. Crop Science, 47(3), pp.1018-1030.

Dangi, R., A. Kumar and A. Khar.(2018). Genetic variability, heritability, and diversity analysis in short day tropical onion (Allium cepa). Indian J. Agric. Sci., 88: 948-957.

De Ponti,O (2009) The Role of Plant Breeding in Meeting The Multiple Challenges of a Fast – Changing World ,2nd World Seed Conference,Rome, Italy. DOI: 10.1080/13102818.2021.1970023.

Dreisigacker, S., 2004. SSR and pedigree analyses of genetic diversity among CIMMYT wheat lines targeted to different megaenvironments. Crop science, 44(2), pp.381-388.

Edwards, K. J., Reid, A. L., Coghill, J. A., Berry, S. T., and Barker, G. L.(2009). Multiplex single nucleotide polymorphism (SNP)-based genotypinginallohexaploid wheat using padlock probes. Plant Biotechnol. J. 7, 375–390. doi: 10.1111/j.1467-7652.2009.00413.x

Elshafei, A., Barakat, M., Milad, S., Khattab, S. and Al-mutlaq, M., 2019c. Regeneration of rice somaclons tolerant to high level of abscisic acid and their characterization via RAPD markers. Bulletin of the National Research Centre, 43(1), pp.1-10.

ELshafei, A.A., Afiah, S.A., Amer, M.A. and Magda, A.M., 2019bValidation of molecular markers linked with salinity tolerance in wheat (TriticumaestivumL.) grown on saline soil. Bioscience Research16(2): 963-978.

Elshafei, A.A., Afiah, S.A.E.A., Al-Doss, A.A. and Ibrahim, E.I., 2019d. Morphological variability and genetic diversity of wheat genotypes grown on saline soil and identification of new promising molecular markers associated with salinity tolerance. Journal of Plant Interactions, 14(1), pp.564-571.

Elshafei, A.A.M., Amer, M.A.E., Elenany, M.A.M. and Helal, A.G.A.E., 2019a. Evaluation of the genetic variability of faba bean (Viciafaba L.) genotypes using agronomic traits and

molecular markers. Bulletin of the National Research Centre, 43(1), pp.1-10.

Eltaher, S., Sallam, A., Belamkar, V., Emara, H.A., Nower, A.A., Salem, K.F., Poland, J. and Baenziger, P.S., 2018. Genetic diversity and population structure of F3: 6 Nebraska winter wheat genotypes using genotyping-by-sequencing. Frontiers in genetics, 9, p.76.

Esmail R. M., A.A. Abdel Sattar, M. A. Abou-Ellail, Heba A. Mahfouze,Hoda M.A. Waziri, Sherin A. Mahfouze (2017) :Identification of Isozyme and Molecular Markers Linked To Leaf Rust Resistance in New Egyptian Wheat lines. App. Sci. Report.19 (1), 2017: 20-26.

Esmail, R.M Sara E.I. Eldessouky, sherin A. mahfouz and I.S. EL-Demardash (2016). Evaluation of new bread wheat lines (Triticumaestivum L) under normal and water stress conditions. International Journal of ChemTech Research,9(5),: 89-99. 99.

Esmail, R.M. (2002) Estimation of Genetic parameters in the F1 and F2 Generations of Diallel Crosses in Bread Wheat (Triticumaestivum L) . Bull. NRC,Egypt, 27 (1) 85-106 .

Esmail, R.M.and S.A.M. Kattab (2002) . Genetic Behavior of Yield and its Components in Three Bread Wheat Crosses . Minufiya J. Agric. Res., 27(2) 215 – 224 .

Esmail, R.M; M.E.S.Ottai, and E.A.H. Mostafa, (2008) Germplasm enhancement for water stress tolerance and storage insect resistance in bread wheat (Triticumaestivum L).World J. of Agricultural Sciences, 4(2):230-240.

Fu, Y.B. and Somers, D.J., 2009. Genome-wide reduction of genetic diversity in wheat breeding. Crop Science, 49(1), pp.161-168.

Gao, F. M., Wen, W., Liu, J. D., Rasheed, A., Yin, G. H., Xia, X. C., et al. (2015). Genome-wide linkage mapping of QTL for yield components, plant height and yield-related physiological traits in the chinese wheat cross zhou 8425B/chinese spring. Front. Plant Sci. 6:1099. doi: 10.3389/fpls.2015.01099.

Ghobadi, G., Etminan, A., Mehrabi, A.M. and Shooshtari, L., 2021. Molecular diversity analysis in hexaploid wheat (Triticumaestivum L.) and two Aegilops species (Aegilopscrassa and Aegilopscylindrica) using CBDP and SCoT markers. Journal of Genetic Engineering and Biotechnology, 19(1), pp.1-11.

Gomez, K.A. and A.A. Gomez (1984).Statistical Procedures for Agricultural Research.John Wiley and Sons. New York. 680.

Griffiths, S. et al. (2012) Meta-QTL analysis of the genetic control of crop height in elite European winter wheat germplasm. Mol. Breed. 29, 159–171.

Guan, P. F., Lu, L. H., Jia, L. J., Kabir, M. R., Zhang, J. B., Lan, T. Y., et al. (2018). Global QTL analysis identifies genomic regions on chromosomes 4A and 4B harboring stable loci for yield-related traits across different environments in wheat (Triticumaestivum L.). Front. Plant Sci. 9:529. doi: 10.3389/fpls.2018.00529.

Hammer D, Harper A, Ryan P. (2001). PAST:Palaeontological Statistics software packagefor education and data analysis.Palaeontol.Electron., 4-9.

Hedden, P. (2003) The genes of the Green Revolution. Trends Genet. 19, 5–9.

Huang, L., Raats, D., Sela, H., Klymiuk, V., Lidzbarsky, G., Feng, L., Krugman, T. and Fahima, T., 2016. Evolution and adaptation of wild emmer wheat populations to biotic and abiotic stresses. Annual review of phytopathology, 54, pp.279-301.

Ibrahim(2019) Genetic variability, Correlation and Path analysis for Yield and yield components in F6 generation of Wheat (TriticumaestivumEm. Thell.) IOSR J. Agric. Vet. Sci., 12 (2019), pp. 17-23.

Khierallah, H.S.M., Bader, S.M., Baum, M., Hamwieh, A., 2011. Genetic Diversity of Iraqi Date Palms Revealed By Microsatellite Polymorphism. Journal of the American Society for Horticultural Science 136, 282–287.. doi:10.21273/jashs.136.4.282

Khlestkina, E.K., Röder, M.S., Efremova, T.T., Börner, A. and Shumny, V.K., 2004. The genetic diversity of old and modern Siberian varieties of common spring wheat as determined by microsatellite markers. Plant breeding, 123(2), pp.122-127.

Kowalsk, A. M., Gooding, M., Ferrante, A., Slafer, G. A., Orford, S., Gasperini, D., et al. (2016). Agronomic assessment of the wheat semi-dwarfing gene Rht8 in contrasting nitrogen treatments and water regimes. Field Crops Res. 191, 150–160. doi: 10.1016/j.fcr.2016.02.026

Landjeva S, Korzun V, Börner A (2007) Molecular markers: actual and potential contributions to wheat genome characterization and breeding. Euphytica 156: 271–296.

Mansour, E.; Desoky, E.M.; Ali, M.M.A.; Abdul-Hamid, M.I.; Ullah, H.; Attia, A.; Datta, A. Identifying drought-tolerant genotypes of faba bean and their agro-physiological responses to different water regimes in an arid Mediterranean environment. *Agric. Water Manag.* **2021**, *247*, 106754.

Mondini L, Noorani A, Pagnotta MA (2009) Assessing plant genetic diversity by molecular tools. Diversity 1:19–35.

Nei M, Li WH. (1979). Mathematical model forstudying genetic variation in terms ofrestriction endonucleases. Proceedings ofthe National Academy of Sciences 76(10):5269–5273.

Nihar, S., Ramesha, M.S., Sundaram, R.M., Neeraja, C.N. and Kemparaju, K.B., 2017. Genetic Diversity Studies using SSR and EST-SSR Markers in Maintainer Lines of Rice Hybrids.Journal of Rice Research 10 (1) pp. 44-48.

Patial, M. and Pramanick, K. K. 2020. Genetic diversity in Wheat for Biotic Stress Resistance. VigyanVarta 1(7): 63-67.

Peng JH, Lapitan NL. 2005. Characterization of EST-derived microsatellitesinthe wheat genome and development of eSSR markers. FunctIntegr Genomics. 5(2):80–96.

Ramu, P., Billot, C., Rami, J.F., Senthilvel, S., Upadhyaya, H.D., Reddy, L.A. and Hash, C.T., 2013. Assessment of genetic diversity in the sorghum reference set using EST-SSR markers. Theoretical and Applied Genetics, 126(8), pp.2051-2064.

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.

Rinki K.,Vinod T.,Sindhu S.,G.P. Singh (2019) Exploring the traits for lodging tolerance in wheat genotypes: a review.Physiology and Molecular Biology of Plants 25(1). DOI: 10.1007/s12298-018-0629-x

Salehi, M., Arzani, A., Talebi, M. and Rokhzadi, A., 2018. Genetic diversity of wheat wild relatives using SSR markers. Genetika, 50(1), pp.131-141.

Salem, K. F. M. , A. M. El-Zanaty and R. M. Esmail(2008) Assessing wheat (Triticumaestivum L.) Genetic Diversity Using Morphological Characters and Microsatallite Markers. World J. of Agricultural Sciences, 4(5) 538-544.

SAS Institute Inc. (2002) SAS Software v.9.1.3 sp4, Cary, NC: SAS Institute Inc.

Smith JSC, ECL, Chin H, Shu OS, Smith SJ, WalML Senior SE, Mitchell S, Kresovich, ZiegleJ. (1997). An evaluation of the utility of SSRloci as molecular markers in maize (Zeamays L.): Comparisons with data fromRFLPS and pedigree. Theoretical andApplied Genetics 95(1–2): 163–173.

Somers, DJ, Isaac, P and Edwards, K (2004) A high-density wheat microsatellite consensus map for bread wheat (Triticumaestivum L.). Theoretical and Applied Genetics 109: 1105–1114.

Swofford,DL.(2001).PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) Version 4.0 beta (2001)

Tomkowiak, A., Bocianowski, Jan, Kwiatek, M. and Kowalczewski, P. Ł.(2020). "Dependence of the heterosis effect on genetic distance, determined using various molecular markers" Open Life Sciences, vol. 15, no. 1, 2020, pp. 1-11. https://doi.org/10.1515/biol-2020-0001

Trethowan R.M. (2014) Defining a genetic ideotype for crop improvement. Methods MolBiol 1145:1–20.

van Frank, G.; Rivière, P.; Pin, S.; Baltassat, R.; Berthellot, J.-F.; Caizergues, F.; Dalmasso, C.; Gascuel, J.-S.; Hyacinthe, A.; Mercier, F.; Montaz, H.; Ronot, B.; Goldringer, I.(2020). Genetic Diversity and Stability of Performance of Wheat Population Varieties Developed by Participatory,Breeding. *Sustainability* , *12*,384. ttps://doi.org/10.3390/su12010384.

Weikai,Y.;Hunt, L.A. (2001) Interpretation of genotype x environment interaction for winter wheat yield in Ontario. Crop Sci. Vol. 41, p. 19-25.

Yared B., H. Shimelis, M. Laing & I. Mathew (2021) Genetic diversity and population structure of bread wheat genotypes determined via phenotypic and SSR marker analyses under drought-stress conditions, Journal of Crop Improvement, 35:3, 303-325, DOI: 10.1080/15427528.2020.1818342

ZareiAbbasabad, E, Mohammadi, SA, Moghaddam, M, and Jalal Kamali, MR (2016). Analysis of genetic diversity, population structure and linkage disequilibrium in Iranian wheat landraces using SSR markers. Plant Genetic Resources. 1, 1-8.