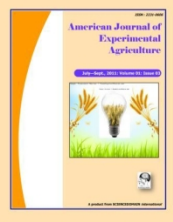

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Polymorphism in Some Egyptian Wheat Varieties Based on SSR-Markers

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Author's contribution

This whole work was carried out by author NRA.

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ABSTRACT

In this study 312 Microsatellite markers were used to analyze DNA polymorphism of three Egyptian wheat aiming to develop specific molecular markers useful in future Egyptian wheat breeding programs. DNA was extracted using the CTAB method and PCR products were separated in an ABI 3730 DNA analyzer. Data were scored using GeneMarker and 2.5% Agarose gel. A Total of 477 fragments were detected and among 312 simple sequences repeat markers 162 were proved to be polymorphic. The percentage of genetic polymorphism ranged from 33% to 100 % and fragment size from 112 to 535 bp. Results of these experiments consider the first step in the effective detection of polymorphism among some Egyptian wheat varieties in order to correct choose for parents in future.

Keywords: Wheat; MAS; SSR; PCR; polymorphism.

1. INTRODUCTION

Microsatellite or simple sequence repeats (SSRs) are highly mutable loci which may be present at many sites in a genome [1]. As the flanking sequence of these sites may be unique, primers can be designed to the flanking sequence [2]. SSRs provide highly informative markers and generally have high polymorphic information content [3]. DNA markers that are tightly linked to agronomically important genes (called gene 'tagging') may

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be used as molecular tools for marker-assisted selection (MAS) in plant breeding [4]. MAS involves using the presence/absence of a marker as a substitute for or to assist in phenotypic selection, in a way which may make it more efficient, effective, reliable and cost-effective compared to the more conventional plant breeding methodology. The use of DNA markers in plant breeding has opened a new realm in agriculture called 'molecular breeding'. DNA markers are widely accepted as potentially valuable tools for crop improvement in rice [5-6], wheat [7-8], maize [9-10], barley [11-12], tuber crops [13], pulses [14], oilseeds [15], horticultural crop species [16-18] and pasture species [19]. An understanding of the basic concepts and methodology of DNA marker development and MAS, including some of the terminology used by molecular biologists, will enable plant breeders and researchers working in other relevant disciplines to work together towards a common goal increasing the efficiency of global food production. Several reviews have been written about the construction of linkage maps, QTL analysis and the application of markers in marker-assisted selection [20-23]. Present research aimed to develop molecular markers associated with some different traits in Egyptian wheat using simple sequence repeat (SSR) markers and usefulness of these markers to detect possible specific markers to be utilized in the wheat future breeding programs in Egypt.

2. MATERIALS AND METHODS

Three Egyptian bread wheat varieties (2n=42, AABBDD) i.e. Egypt 1 (E.1), Gemmeiza 9 (G.9) and Sakha 93 (S.93) were used in the current experiment. Leaf tissues from 15 plants per line (single seed single plant) were sampled at the two-leaf stage in 1.1-mL deep-well plates and freeze-dried for 2 days (Thermo Fisher, Waltham, MA, USA) for DNA isolation. Each well of the plates contained a 3.2-mm stainless steel bead and dried tissue, and the plates were shaken in a Mixer Mill (Retsch GmbH, Germany) at 25 times s-1 for 3 min. Genomic DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method [24]. The quantity and quality of DNA were evaluated by spectrophotometery gel documentation and 0.8% Agarose gel respectively. 312 SSR markers were used. A 13-µl Polymerase chain reactions (PCR) mixture contained 1.0 µl of 10× NH_4 PCR buffer (Bioline, Taunton, MA, USA), 2.50 mM $MgCl_2$, 200 µM each dNTP mix, 40 nM M13 fluorescent-dye-labeled primer (ACGACGTTGTAAAACGAC), 50 nM tailed forward primer (adding the M13 tail sequence to 50-end of forward primer), 90 nM reverse primer, 1.0 U Taq DNA polymerase, and about 25 ng of template DNA. A touchdown PCR program was used for PCR amplification. Briefly, the reaction was incubated at 95°C for 5 min, and then continued for 5 cycles of 1 min at 96°C at 68°C with a decrease of 2°C in each subsequent cycle, and 1 min at 72°C. For another five cycles, the annealing temperature started at 58°C for 2 min, with a decrease of 2°C for each subsequent cycle. Reactions then went through an additional 40 cycles of 1 min at 96°C for 2 min at 58°C, and 1 min at 72°C with a final extension at 72°C for 5 min. PCR products were separated on an ABI Prism 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA) and Agarose gel 2.5%. Data collected from an ABI DNA Analyzer (Applied Biosystems) were processed by GeneMarker version 1.6 (Soft Genetics LLC, State College, PA, USA) and manually checked twice for accuracy. Past program (PAleontological Statistics, version 2.17) to calculate the similarity among varieties.

3. RESULTS AND DISCUSSION

Three major wheat varieties from Egypt were used in the current research to analyze the genetic polymorphism. Specific band was scored among all varieties. Polymorphic in this

study is significant among wheat varieties. The observed of polymorphism could be attributed to selection of genotypes with diverse characteristics, as well as to specific markers that may be used in the future assay. The percentage of genetic polymorphism ranged from 33% to 100% (Table 1) and assay in Figure (1), these varieties will be useful for developing mapping populations depending on percentage of polymorphism. The polymorphism observed in the present study represents inherent variability among varieties at the DNA level. A total of 162 out of 312 markers were polymorphic detected in Table 1 and Figure 1. 72 markers (44.4%) for 33% polymorphism and 90 markers (55.5%) for 100% polymorphism. A total of 477 fragments were detected. The fragment size ranged from 112 to 535 bp. Based on 162 SSR-markers data in Figure 2 grouped the investigated varieties into two main clusters. The first cluster included Gemmeiza 9 and Sakha 93, the second cluster contained Egypt 1. Similarity percentage among the three varieties was ~ 45% and between Gemmeiza 9 and Sakha 93 was ~ 50%.

Table 1. Genetic polymorphic based on 162 SSR-markers among three Egyptian wheat varieties

Primer	Chr.	G.9	S.93	E.1	Poly.	Primer	Chr	G.9	S.93	E.1	Poly.
BAR0008	1	164	274	270	100	GWM0533	3	154	133	0	100
BAR0017	1	303	170	294	100	CFA2262	3	148	127	127	33
BAR0131	1	233	233	254	33	CFD0223	3	149	154	0	100
BAR0137	1	251	274	274	33	GWM0005	3	160	142	136	100
CFA2147	1	308	267	308	33	GWM0052	3	141	131	147	100
CFA2153	1	175	209	216	100	GWM0108	3	155	152	124	100
CFA2219	1	266	264	260	100	GWM0161	3	188	0	194	100
GWM0413	1	107	127	107	33	WMC0291	3	115	145	115	33
WMC0031	1	160	160	148	33	WMC0326	3	152	211	209	100
WMC0416	1	187	238	230	100	BAR0091	4	194	180	188	100
WMC0619	1	0	204	229	100	BAR0163	4	131	197	200	100
WMC0830	1	302	305	239	100	BAR0170	4	0	144	169	100
WMC0134	1	169	169	194	33	BAR0217	4	205	173	173	33
BAR0055	2	148	139	148	33	BAR1118	4	153	150	150	33
BAR0160	2	123	129	129	33	GWM0375	4	243	243	234	33
GWM0356	2	201	197	197	33	GWM0495	4	263	263	232	33
GWM0372	2	305	352	381	100	WMC0052	4	198	207	195	100
GWM0429	2	216	228	228	33	CFD0039	4	178	170	178	33
GWM0445	2	204	204	535	33	CFD0084	4	198	188	176	100
GWM0539	2	149	155	155	33	CFD0106	4	108	172	299	100
GWM0558	2	131	143	133	100	CFD0257	4	300	300	422	33
GWM0636	2	125	404	127	100	GDM0125	4	252	229	229	33
CFD0051	2	0	169	165	100	GWM0006	4	160	157	157	33
GWM0120	2	170	161	161	33	GWM0113	4	204	289	286	100
GWM0275	2	136	136	130	33	GWM0149	4	265	260	320	100
WMC0154	2	138	164	173	100	GWM0194	4	132	138	132	33
WMC0177	2	210	196	204	100	GWM0251	4	175	175	225	33
WMC0332	2	188	188	149	100	WMC0285	4	300	286	258	100
WMC0361	2	238	244	238	100	WMC0331	4	220	220	224	100
WMC0441	2	171	176	179	100	WMC0413	4	161	174	159	100
GWM0249	2	184	199	199	33	WMC0468	4	237	231	231	33
GWM0102	2	196	0	151	100	WMC0473	4	146	154	154	33
BAR0133	3	132	138	132	33	WMC0757	4	182	0	213	100
BAR0139	3	148	146	152	100	WMC0125	4	197	195	191	100
BAR0284	3	173	193	173	33	BAR0001	5	140	136	136	33
BAR0294	3	166	162	166	33	BAR0074	5	108	112	112	33
BAR0314	3	156	156	151	33	BAR0141	5	150	134	134	33
BAR0321	3	262	195	191	100	BAR0143	5	186	186	195	33
GWM0369	3	120	117	173	100	BAR0177	5	194	181	194	33

Table 1 Continued …..

GWM0456	3	251	124	251	33	BAR0186	5	177	157	177	33
GWM0493	3	218	218	230	33	BAR0286	5	164	220	172	100
Primer	Chr	G.9	S.93	E.1	Poly.	Primer	Chr	G.9	S.93	E.1	Poly.
BAR0303	5	201	200	193	100	CFD0076	6	169	174	169	33
BAR0316	5	140	148	148	33	CFD0095	6	172	169	152	100
CFA2121	5	194	174	181	100	CFD0219	6	303	305	280	100
GWM0293	5	206	201	206	33	GDM0127	6	206	206	307	33
GWM0358	5	187	203	187	33	GWM0107	6	179	179	195	33
GWM0371	5	187	192	185	100	GWM0133	6	121	116	0	100
GWM0540	5	169	164	166	33	GWM0219	6	205	197	141	100
GWM0544	5	155	167	169	100	WMC0201	6	255	269	262	100
GWM0583	5	166	160	282	100	WMC0756	6	202	200	200	33
GWM0604	5	143	151	143	33	WMC0786	6	138	176	138	33
CFD0008	5	164	160	164	33	GDM0132	6	363	368	368	33
CFD0018	5	120	0	117	100	BAR0111	7	193	193	119	33
CFD0040	5	217	196	198	100	BAR0126	7	141	133	139	100
CFD0060	5	241	212	247	100	BAR0154	7	236	236	246	33
GDM0138	5	171	176	176	33	BAR0172	7	170	186	411	100
GWM0186	5	259	244	244	33	BAR0235	7	139	121	130	100
GWM0190	5	216	184	0	100	CFA2049	7	171	171	154	33
GWM0272	5	138	142	138	33	GWM0295	7	211	268	0	100
WMC0161	5	351	0	180	100	GWM0333	7	169	171	189	100
WMC0247	5	164	178	186	100	GWM0400	7	163	165	193	100
WMC0327	5	113	131	131	33	GWM0428	7	159	159	149	33
WMC0524	5	127	131	123	100	GWM0537	7	228	225	139	100
WMC0705	5	261	259	261	33	WMC0014	7	193	343	367	100
BAR0079	6	178	185	178	33	CFD0014	7	137	141	141	33
BAR0134	6	217	209	178	100	CFD0066	7	136	172	186	100
BAR0175	6	244	244	230	100	CFD0069	7	215	213	181	100
BAR0178	6	137	138	115	100	GDM0046	7	157	157	127	33
BAR0183	6	188	169	240	100	GWM0111	7	153	158	158	33
BAR0196	6	138	180	180	33	GWM0130	7	148	135	156	100
BAR0198	6	138	141	141	33	WMC0396	7	173	166	173	33
BAR0354	6	148	167	124	100	WMC0463	7	201	102	133	100
GWM0311	6	221	163	148	100	WMC0479	7	193	231	163	100
GWM0325	6	155	161	215	100	WMC0488	7	136	141	123	100
GWM0334	6	140	133	140	33	WMC0517	7	215	206	206	33
GWM0427	6	139	215	369	100	WMC0525	7	215	238	381	100
GWM0469	6	187	191	164	100	WMC0790	7	146	146	200	33
GWM0494	6	198	215	360	100	WMC0116	7	232	374	128	100
GWM0617	6	140	221	150	100	WMC0121	7	326	328	173	100
GWM0626	6	0	154	140	100	WMC0083	7	182	177	179	100
CFD0047	6	212	214	104	100	WMC0702	7	185	200	215	100

** Chr.: Chromosome, G.9: Gemmeiza 9, S. 93: Sakha 93, E.1.: Egypt 1, Poly. Polymorphism*

Microsatellite or SSR (simple sequence repeat) markers have been more widely used in major crops [25-27] because of their ease of analysis [28-29]. Microsatellite markers are becoming the markers of choice due to the level of polymorphism, as well as higher reliability [30-31]. In wheat, abundant wheat genomic SSR markers are now available and mapped [4], making them a useful resource for further studies. In this study, polymerase chain reaction (PCR)-based system (SSR) have been used for studding the genetic polymorphism between three wheat varieties. The highest levels of polymorphism for SSRs system reported in previous studies [32-39]. This high level of polymorphism, associated with SSR markers, is to be expected because of the unique mechanism responsible for generating SSR allelic diversity by replication slippage. It should be noted that multiple allelism is very common in SSR markers and they are able to produce different alleles in one locus [40]. The authoress

reported that fragment size ranged from 112 to 535 bp. While [41] obtained an allelic size range between 77 to 266 bp using 15 microsatellite markers on some wheat genotypes. In addition. [42] reported an allelic size range between 82 to 1620 bp using SSR markers associated with salt tolerance in Egyptian wheat varieties. There are different reports for obtaining different alleles of using SSR markers to study genetic polymorphism among different wheat varieties and lines. Marker assisted selection (MAS) has been becoming the method of choice in facilitating tagging of the desirable traits in many crops [43-44]. The results in line with [44] reported different allelic variations in the same species and even monoallelic differences in subspecies cussed the different clusters. The distribution and sequence of SSR markers may therefore provide insight into phylogenetic relationships among varieties and species [45].

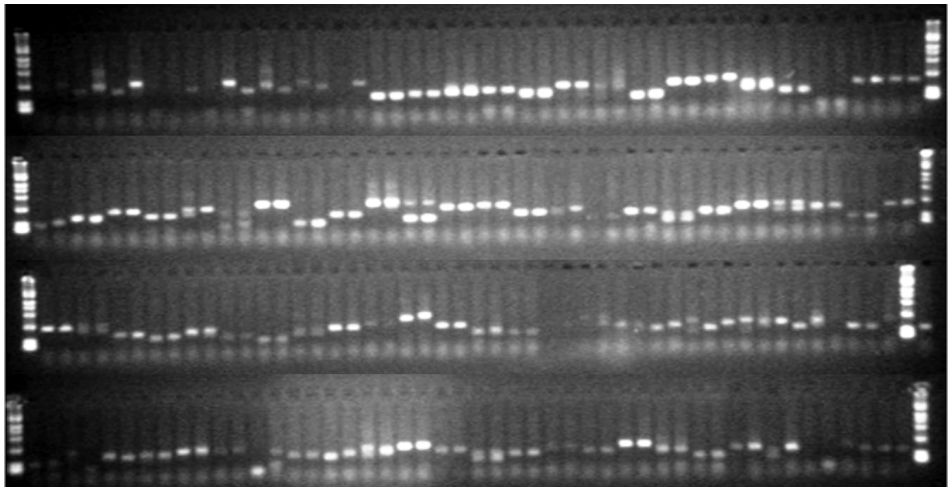

Fig. 1. An example of SSRs banding pattern showing genetic polymorphism based on different markers of wheat varieties using 2.5% Agarose gel

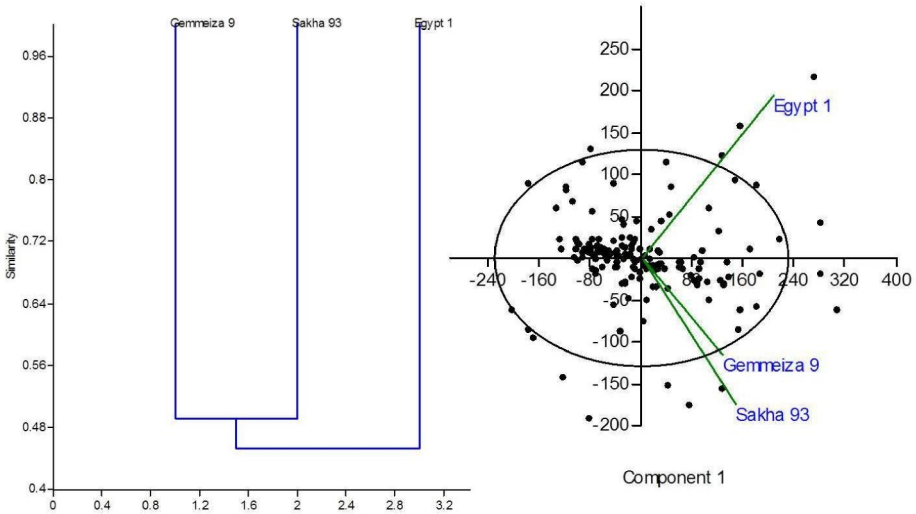

Fig. 2. Cluster analysis of similarity among three Egyptian whet varieties using 162 SSR markers

4. CONCLUSION

The study indicated the presence of specific markers in wheat varieties using 312 SSR markers opens up a possibility to apply marker-assisted selection (MAS) in some Egyptian wheat varieties. Current research may be a useful reference and first step for conventional plant breeders, physiologists, pathologists and other plant scientists in Egypt to decrease the cost and time for detect DNA polymorphism among these varieties. These results indicated that some selected markers were able to screen the Egyptian wheat genotypes for some major traits.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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