

Original Research Article

SSR-DNA Polymorphism in some Egyptian Wheat

ABSTRACT

WG-SSR: Whole genome simple sequence repeat was considered as an efficient method for screening polymorphic of three Egyptian bread wheat genotypes. Fundamentally, the markers cover the whole wheat genome. 312 SSR markers were selected from previous reports and USDA, Hard Winter Wheat Genetics Research Unit, Kansas State University, Manhattan KS, USA to using in the current study. After DNA extraction PCR products were separated in an ABI 3730 DNA analyzer (Applied Biosystems, Foster City, CA) and data were scored using GeneMarker (version 1.6; SoftGenetics LLC. State College, PA). Results of these experiments consider the first step in the effective detection of polymorphism among some Egyptian wheat in order to correct choose for parents in future. This study aimed to develop molecular markers associated with some different traits in 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.

Key words: Wheat, Egypt, SSR, Polymorphism

INTRODUCTION

Microsatellite or simple sequence repeats (SSRs) are highly mutable loci which may be present at many sites in a genome (**Morgante *et al.*, 1998**). As the flanking sequence of these sites may be unique, primers can be designed to the flanking sequence (**Jones *et al.*, 1997**). SSRs provide highly informative markers and generally have high polymorphic information content (**Gupta *et al.*, 1996**). DNA markers that are tightly linked to agronomically important genes (called gene ‘tagging’) may be used as molecular tools for marker-assisted selection (MAS) in plant breeding (**Ribaut & Hoisington, 1998**). 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 (**Mackill *et al.*, 1999**; **McCouch&Doerge, 1995**), wheat (**Eagles *et al.*, 2001**; **Koebner and Summers, 2003**), maize (**Stuber *et al.*, 1999**; **Tuberosa *et al.*, 2003**), barley (**Thomas, 2003**; **Williams, 2003**), tuber crops (**Barone, 2004**), pulses (**Kelly *et al.*, 2003**), oilseeds (**Snowdon & Friedt, 2004**), horticultural crop species (**Baird *et al.*, 1996, 1997**; **Mehlenbacher, 1995**) and pasture species (**Jahufer *et al.*, 2002**). 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. Different reviews have been written about the construction of linkage maps, QTL analysis and the application of markers in marker-assisted selection (**Haley & Andersson, 1997**; **Jones *et al.*, 1997**; **Paterson, 1996ab**; **Staub *et al.*, 1996**).

The present research will be a useful reference and initial step for conventional plant breeders, physiologists, pathologists and other plant scientists in Egypt whom work in wheat breeding for decrease the cost and time on select the markers in the future studies.

MATERIAL AND METHODS

Three Egyptian bread wheat genotypes (2n = 42, AABBDD) i.e. Egypt 1 (E.1), Gemmeiza 9 (G.9) and Sids 1 (S.1) 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 cetyl trimethyl ammonium bromide (CTAB) method (**Saghai-Marooof *et al.* 1984**). The quantity and quality of DNA were evaluated by spectrophotometry gel documentation and 0.8% Agarose gel respectively. 312 SSR markers were selected from previous reports and USDA, Hard Winter Wheat Genetics Research Unit, Kansas State University, Manhattan KS, USA. A 13-μl Polymerase chain reactions (PCR) mixture contained 1.0 μl of 10× NH₄ PCR buffer (Bioline, Taunton, MA, USA), 2.50 mM MgCl₂, 200 IM of each dNTP, 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 five cycles of 1 min at 96_C, 3 min. 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, 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). 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.

RESULTS

Specific peaks were scored among all cultivars. Polymorphic in this study is significant among wheat cultivars. The observed of polymorphism could be attributed to selection of genotypes with diverse characteristics, as well as to specific markers used in the future assay. The percentage ranged from 33% up to 100 % (Table 1), these genotypes will be useful for developing mapping populations. The polymorphism observed in the present study represents inherent variability among genotypes at the DNA level. A total of 162 out of 312 markers were polymorphic detected in Table (1) among which 72 markers were specific to one cultivar (44.4%) and 90 markers (55.5%). 477 peaks of 486 were detected and 9 peaks not amplified. The fragment size ranged from 112 to 535 bp. In conclusion, the present study indicated the presence of specific markers and the chromosomal location of each marker in some cultivated Egyptian wheat using SSR markers. This opens up a possibility for developing a molecular genetic map that will lead to the application of marker-assisted selection tools to genetic enhancement of cultivated Egyptian wheat in the future. Based on 162 different markers SSR markers data in Figure (1) grouped the investigated cultivars into two main clusters. The first cluster included Gemmeiza 9 and Sakha 93, the second cluster contained Egypt 1. Data in Table (2) showed the monomorphic markers used in the current research (150 Markers).

91 Table (1) genetic polymorphic based on 162 SSR-markers among three Egyptian wheat
92 cultivars
93

Primer	Chr.	G.9	S.93	E.1	Poly	Primer	Chr	G.9	S.93	E.1	Poly
BAR0001	5	140	136	136	33	CFA2262	3	148	127	127	33
BAR0008	1	164	274	270	100	CFD0008	5	164	160	164	33
BAR0017	1	303	170	294	100	CFD0014	7	137	141	141	33
BAR0055	2	148	139	148	33	CFD0018	5	120	0	117	100
BAR0074	5	108	112	112	33	CFD0039	4	178	170	178	33
BAR0079	6	178	185	178	33	CFD0040	5	217	196	198	100
BAR0091	4	194	180	188	100	CFD0047	6	212	214	104	100
BAR0111	7	193	193	119	33	CFD0051	2	0	169	165	100
BAR0126	7	141	133	139	100	CFD0060	5	241	212	247	100
BAR0131	1	233	233	254	33	CFD0066	7	136	172	186	100
BAR0133	3	132	138	132	33	CFD0069	7	215	213	181	100
BAR0134	6	217	209	178	100	CFD0076	6	169	174	169	33
BAR0137	1	251	274	274	33	CFD0084	4	198	188	176	100
BAR0139	3	148	146	152	100	CFD0095	6	172	169	152	100
BAR0141	5	150	134	134	33	CFD0106	4	108	172	299	100
BAR0143	5	186	186	195	33	CFD0219	6	303	305	280	100
BAR0154	7	236	236	246	33	CFD0257	4	300	300	422	33
BAR0160	2	123	129	129	33	CFD0223	3	149	154	0	100
BAR0163	4	131	197	200	100	GDM0046	7	157	157	127	33
BAR0170	4	0	144	169	100	GDM0125	4	252	229	229	33
BAR0172	7	170	186	411	100	GDM0127	6	206	206	307	33
BAR0175	6	244	244	230	100	GDM0132	6	363	368	368	33
BAR0177	5	194	181	194	33	GDM0138	5	171	176	176	33
BAR0178	6	137	138	115	100	GWM0005	3	160	142	136	100
BAR0183	6	188	169	240	100	GWM0006	4	160	157	157	33
BAR0186	5	177	157	177	33	GWM0052	3	141	131	147	100
BAR0196	6	138	180	180	33	GWM0102	2	196	0	151	100
BAR0198	6	138	141	141	33	GWM0107	6	179	179	195	33
BAR0217	4	205	173	173	33	GWM0108	3	155	152	124	100
BAR0235	7	139	121	130	100	GWM0111	7	153	158	158	33
BAR0284	3	173	193	173	33	GWM0113	4	204	289	286	100
BAR0286	5	164	220	172	100	GWM0120	2	170	161	161	33
BAR0294	3	166	162	166	33	GWM0130	7	148	135	156	100
BAR0303	5	201	200	193	100	GWM0133	6	121	116	0	100
BAR0314	3	156	156	151	33	GWM0149	4	265	260	320	100
BAR0316	5	140	148	148	33	GWM0161	3	188	0	194	100
BAR0321	3	262	195	191	100	GWM0186	5	259	244	244	33
BAR0354	6	148	167	124	100	GWM0190	5	216	184	0	100
BAR1118	4	153	150	150	33	GWM0194	4	132	138	132	33
CFA2049	7	171	171	154	33	GWM0219	6	205	197	141	100
CFA2121	5	194	174	181	100	GWM0249	2	184	199	199	33

Continue Table (1) genetic polymorphic based on 162 SSR-markers among three Egyptian wheat cultivars

Primer	Chr	G.9	S.93	E.1	Poly	Primer	Chr	G.9	S.93	E.1	Poly
CFA2147	1	308	267	308	33	GWM0251	4	175	175	225	33
CFA2153	1	175	209	216	100	GWM0272	5	138	142	138	33
CFA2219	1	266	264	260	100	GWM0275	2	136	136	130	33
GWM0293	5	206	201	206	33	WMC0154	2	138	164	173	100
GWM0295	7	211	268	0	100	WMC0161	5	351	0	180	100
GWM0311	6	221	163	148	100	WMC0177	2	210	196	204	100
GWM0325	6	155	161	215	100	WMC0201	6	255	269	262	100
GWM0333	7	169	171	189	100	WMC0247	5	164	178	186	100
GWM0334	6	140	133	140	33	WMC0285	4	300	286	258	100
GWM0356	2	201	197	197	33	WMC0291	3	115	145	115	33
GWM0358	5	187	203	187	33	WMC0326	3	152	211	209	100
GWM0369	3	120	117	173	100	WMC0327	5	113	131	131	33
GWM0371	5	187	192	185	100	WMC0331	4	220	220	224	100
GWM0372	2	305	352	381	100	WMC0332	2	188	188	149	100
GWM0375	4	243	243	234	33	WMC0361	2	238	244	238	100
GWM0400	7	163	165	193	100	WMC0396	7	173	166	173	33
GWM0413	1	107	127	107	33	WMC0413	4	161	174	159	100
GWM0427	6	139	215	369	100	WMC0416	1	187	238	230	100
GWM0428	7	159	159	149	33	WMC0441	2	171	176	179	100
GWM0429	2	216	228	228	33	WMC0463	7	201	102	133	100
GWM0445	2	204	204	535	33	WMC0468	4	237	231	231	33
GWM0456	3	251	124	251	33	WMC0473	4	146	154	154	33
GWM0469	6	187	191	164	100	WMC0479	7	193	231	163	100
GWM0493	3	218	218	230	33	WMC0488	7	136	141	123	100
GWM0494	6	198	215	360	100	WMC0517	7	215	206	206	33
GWM0495	4	263	263	232	33	WMC0524	5	127	131	123	100
GWM0533	3	154	133	0	100	WMC0525	7	215	238	381	100
GWM0537	7	228	225	139	100	WMC0619	1	0	204	229	100
GWM0539	2	149	155	155	33	WMC0702	7	185	200	215	100
GWM0540	5	169	164	166	33	WMC0705	5	261	259	261	33
GWM0544	5	155	167	169	100	WMC0756	6	202	200	200	33
GWM0558	2	131	143	133	100	WMC0757	4	182	0	213	100
GWM0583	5	166	160	282	100	WMC0786	6	138	176	138	33
GWM0604	5	143	151	143	33	WMC0790	7	146	146	200	33
GWM0617	6	140	221	150	100	WMC0830	1	302	305	239	100
GWM0626	6	0	154	140	100	WMC0116	7	232	374	128	100
GWM0636	2	125	404	127	100	WMC0121	7	326	328	173	100
WMC0014	7	193	343	367	100	WMC0125	4	197	195	191	100
WMC0031	1	160	160	148	33	WMC0134	1	169	169	194	33
WMC0052	4	198	207	195	100	WMC0083	7	182	177	179	100

* Chr: Chromosome, G.9: Gemmeiza 9, S. 93: Sakha 93, S.1.: Sids 1

Table (2) List of Monomorphic SSR Markers of three Egyptian wheat cultivars

BAR0003	BAR0169	CFA2256	GWM0018	GWM0291	UMN0010	WMC0283
BAR0013	BAR0181	CFD0009	GWM0046	GWM0296	WMC0024	WMC0296
BAR0015	BAR0184	CFD0012	GWM0060	GWM0299	WMC0041	WMC0311
BAR0028	BAR0197	CFD0023	GWM0088	GWM0301	WMC0044	WMC0313
BAR0042	BAR0198	CFD0031	GWM0102	GWM0304	WMC0047	WMC0323
BAR0056	BAR0222	CFD0045	GWM0124	GWM0341	WMC0059	WMC0357
BAR0057	BAR0227	CFD0049	GWM0136	GWM0357	WMC0073	WMC0364
BAR0072	BAR0271	CFD0057	GWM0147	GWM0375	WMC0079	WMC0366
BAR0073	BAR0279	CFD0063	GWM0148	GWM0408	WMC0144	WMC0388
BAR0074	BAR0288	CFD0102	GWM0154	GWM0458	WMC0167	WMC0397
BAR0075	BAR0319	CFD0116	GWM0156	GWM0499	WMC0169	WMC0420
BAR0077	BAR0356	CFD0132	GWM0157	GWM0513	WMC0181	WMC0426
BAR0080	BAR1005	CFD0189	GWM0181	GWM0518	WMC0216	WMC0428
BAR0085	BAR1032	GDM0040	GWM0182	GWM0526	WMC0219	WMC0459
BAR0089	BAR1119	GDM0072	GWM0210	GWM0566	WMC0222	WMC0474
BAR0094	BAR1162	GDM0088	GWM0247	GWM0570	WMC0233	WMC0494
BAR0095	BAR1165	GDM0101	GWM0261	GWM0595	WMC0235	WMC0549
BAR0107	BAR1182	GDM0129	GWM0276	GWM0624	WMC0256	WMC0622
BAR0117	BAR1183	GDM0146	GWM0284	SCM0009	WMC0274	WMC0671
BAR0127	CFA2058	GWM0003	GWM0285	SWM0010	WMC0278	WMC0687
BAR0130	CFA2110	BAR0135	CFA2134	BAR0148	CFA2135	BAR0158
BAR0164	CFA2155	CFA2170				

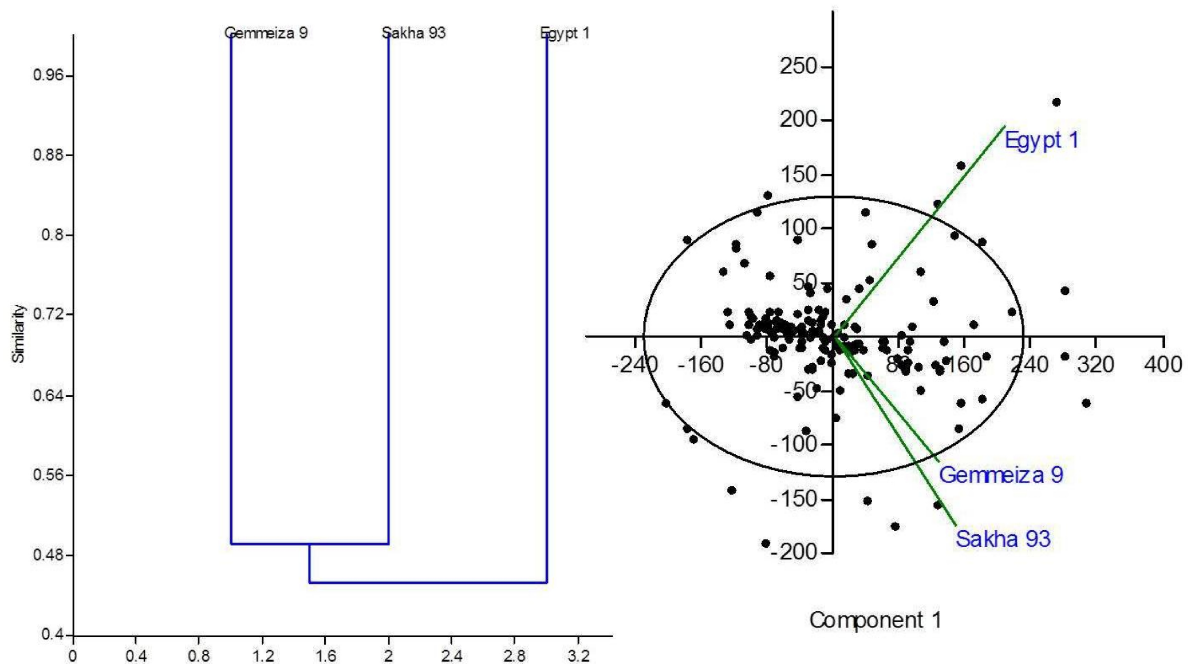


Figure (1) UPGWA dendrogram based on 162 SSR markers showing the genetic diversity among three Egyptian wheat cultivars.

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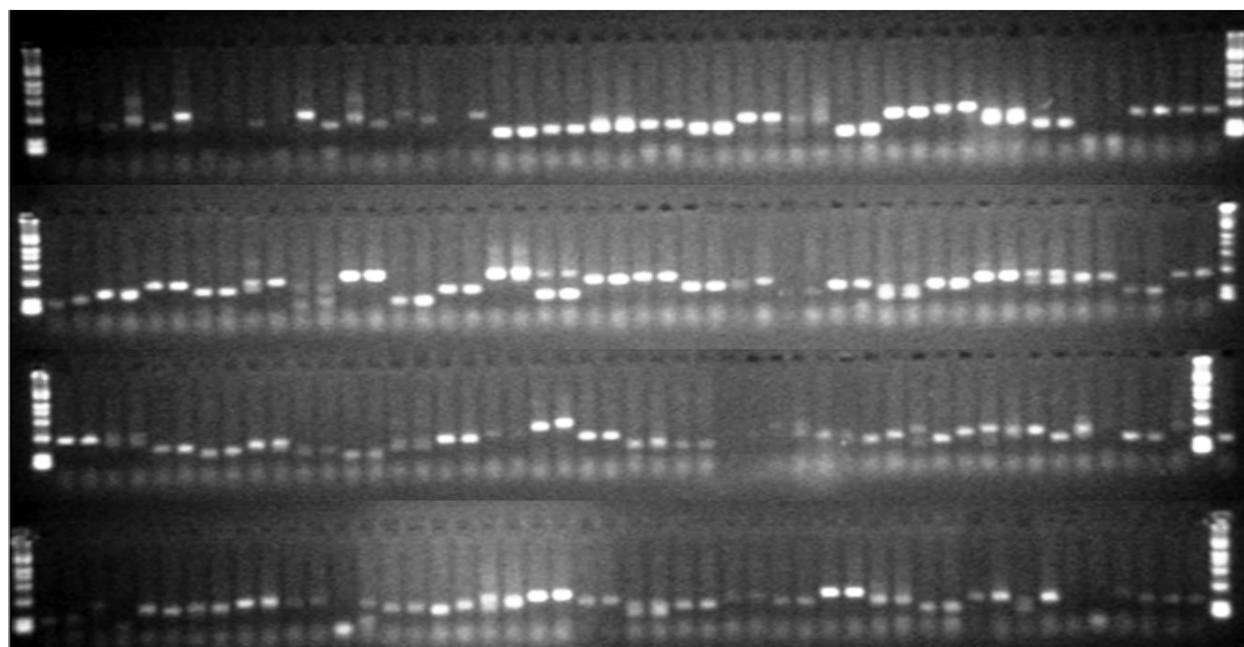


Figure (2) An example of SSRs banding pattern showing polymorphism obtained using different markers of wheat genotypes

DISCUSSION

Microsatellite or SSR (simple sequence repeat) markers have been more widely used in major crops (Röder *et al.* 1998; Ramsay *et al.* 2000; Smith *et al.* 1997; Temnykh *et al.* 2001) because of their ease of analysis (Powell *et al.* 1996; Donini *et al.* 1998). Microsatellite markers are becoming the markers of choice due to the level of polymorphism, as well as higher reliability (Plaschke *et al.* 1995 and Fu *et al.* 2005). In wheat, abundant wheat genomic SSR markers are now available and mapped (Röder *et al.* 1998), making them a useful resource for further studies. In this study, polymerase chain reaction (PCR)-based system (SSR) have been used for studying the genetic polymorphism between three wheat cultivars. 312 SSR markers were used, 162 of them were polymorphic and 150 were monomorphic. The level of polymorphism was ranged from 33 to 100%. The highest levels of polymorphism for SSRs system reported in previous studies (Belaj *et al.*, 2003; Russel *et al.*, 1997; Bohn *et al.*, 1999; Gomes *et al.*, 1998; Maguire *et al.*, 2002; Palombi and Damiano, 2002; Rajora and Rahman, 2003; Ferreria, *et al.*, 2004). 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 (Naghavi *et al.*, 2007). The fragment size ranged from 112 to 535 bp. While Salem *et al.* (2008) obtained an allelic size range between 77 to 266 bp on using 15 microsatellite markers on some wheat genotypes, also, Moghaieb *et al.* (2011) reported an allelic size range between 82 to 1620 bp on using SSR markers associated with salt tolerance in Egyptian wheats. There are different reports for obtaining different alleles of using SSR markers to study genetic polymorphism among different wheat cultivars and lines. Reddy *et al.* (2002) confirmed that inter simple sequence repeats PCR (ISSR-PCR) is a simple, cost-efficient, robust, multi locus marker method which extremely useful in determining genetic

variability. Marker assisted selection (MAS) has been becoming the method of choice in facilitating tagging of the desirable traits in many crops (Abdel-Tawab *et al.*, 2003). The genotype specific SSR markers were determined, some important markers can be considered as a useful marker for screening biotic and Abiotic stress traits in the Egyptian wheat genotypes. Based on 162 different markers SSR markers grouped the investigated cultivars into two main clusters. Different allelic variations in the same species and even monoallelic differences in subspecies cussed the different clusters (Naghavi *et al.* 2007). More also, SSR were characterized in different eukaryotic organisms, being the most variable component of the genome with high rate of molecular evolution. The distribution and sequence of SSR markers may therefore provide insight into phylogenetic relationships among varieties and species (Zeb *et al.*, 2004).

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