The Usage of Specific Markers for Some Major Traits in Egyptian Wheat (*Triticum aestivum* L.) and Their Wild Relatives

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ABSTRACT: Wheat (*Triticum aestivum* L.) is one of the world's major cereal crops. The present work was carried out at the Faculty of Agriculture, (Saba Basha), Alexandria University, Egypt during the seasons of 2014 up to 2016 to study the implementation of specific markers on some major traits in Egyptian wheat. Five Egyptian bread wheat genotypes (2n=42, AABBDD), Egypt 1, Gemmeiza 9 & 11, Sakha 93, Sids 1 and one wild wheat *Aegilops ventricosa* Tausch (2n=28, DvDvNvNv) were used in the current experiment. Specific peaks for target genes were scored across all genotypes in addition to other unique peaks for susceptible genotypes of all the traits. Polymorphic level was (90%) across all wheat accessions, especially between wild and domesticated wheat. High level of polymorphism could be attributed to selection of genotypes with diverse characteristics. A total of 110 alleles were detected, among which 21 alleles (19%) were polymorphic and 89 alleles were specific for target genes (81%). For SSR, it is very common that each primer set amplifies multiple fragments and they are either different alleles in one locus or different loci. The result reveals significant differences in allelic diversity among wheat cultivars studied. The fragment sizes ranged from 103 to 440bp. This opens up a possibility to apply marker-assisted selection (MAS) in developing new Egyptian wheat cultivars.
Key words: Wheat, SSR, specific markers**,** genetic polymorphism

INTRODUCTION

Wheat (*Triticum aestivum*) is one of the world's major cereal crops. As the unique molecular make up of its grain allows its use as a primary structural ingredient of breads, pastas, tortillas, and other products worldwide. To achieve the food production levels needed to supply worldwide demands, plant breeders have focused on the development of agricultural varieties possessing two characters: high yield potential and high end-usequality. In order to meet the demands of the future populations, there is a need to develop new methods not only for increasing wheat yield, but also for increasing the utility and reliability of the resultant grain (Collard *et al.,* 2005).

Wheat is a major crop for human consumption. Its importance hinges upon unique rheological properties of wheat flour which allow for the production baked goods. In recent years, wheat production has been increasing rapidly enough to keep pace with population growth, and is predicted to continue increasing at an average yearly rate of 1.9%, rising from 609 million tons in 1997 to a projected 641 million tons**.**

Genetic markers represent genetic differences between individual organisms. Generally, they do not represent the target genes them selves but act as 'signs'or 'flags'. Genetic markers that are located in close proximity to genes (i.e. tightly linked) may be referred to as gene 'tags'. Such markers themselves do not affect the phenotype of the trait of interest because they are located only near or 'linked' to genes controlling the trait.

All genetic markers occupy specific genomic positions within chromosomes (like genes) called 'loci' (singular 'locus') (Hammer *et al.,* 2000).

Simple seqance repeat (SSR) markers are abundant, highly polymorphic, evenly distributed throughout the genome and require only small amounts of genomic DNA for analysis (Nicot *et al.,* 2004). Further, SSR markers were shown to be successfully able across different wheat species, making them a powerful tool for population genetics and mapping studies in wild and cultivated wheat (Fahima *et al.,* 2002). Recent studies on SSR markers published a high level of polymorphism among diploid wheat species (Hammer *et al.,* 2000), tetraploid wild wheat accessions (Fahima *et al.,* 2002) and hexaploid wheat varieties (Plaschke *et al.,* 1995). Nader (2014) used 312 microsatellite markers to analyze DNA polymorphism of three Egyptian wheat aiming to develop specific molecular markers useful in future Egyptian wheat breeding programs.

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. The present reasarch aims to usage of some specific markers for major traits such as drought, aluminum tolerance, quality of gluten (Low and high molecular weight), fungal disease resistance (stem, stripe, leaf rust, pre harvest sprouting resistance and Fusarium head blight resistance in some Egyptian wheat (*Triticum aestivum* L.) and their wild relatives, and detect the genetic distance and similarity between the Egyptian wheat studied varieties based on SSR markers.

MATERIALS AND METHODS

The present research was carried out at the Faculty of Agriculture (Saba Basha), Alexandria University, Egypt during the seasons of 2014 up to 2016 to study the implementation of specific markers on some major traits in Egyptian wheat (*Triticum aestivum* L.)

Plant materials:

Five Egyptian bread wheat genotypes, Egypt 1, Gemmeiza 9 & 11, Sakha 93, Sids 1 and one wild wheat *Aegilops ventricosa* Tausch were used in the current experiment. Grain samples were obtained from Field Crops Research Institute, Agriculture Research Center, Giza.

Molecular analysis:

DNA Extraction:

Twenty known wheat DNA from USDA Genotyping Lab, Manhattan KS, USA were used as controls for different genes. Leaf tissue was collected from 14 d old seedlings into 96-well plate (1mL), dried for two d in a freeze drier (Thermo Fisher, Waltham, MA), and ground by shaking the plate containing a 3.2 mm metal bead in each well for 3 min at 25 times per second using a Mixer Mill (Retsch GmbH, Haan, Germany).

Genomic DNA was extracted using the CTAB (cetyltrimethylammonium bromide) method (Saghai-Maroof *et al*., 1984). The quantity and quality of DNA were evaluated by running it in 0.8% agarose gel. Twenty SSR markers associated with important wheat genes were selected based on the previous reports (Tables 1 and 2).

Polymerase cycle reaction (PCR) amplifications were performed in a Tetrad Peltier DNA Engine (Bio-Rad Laboratories, Hercules, CA). A 13µl PCR mixture contained 1.0 µl of 10×NH_4 buffer (Bioline Inc. Taunton, MA), 2.50mM $MgCl_2$, 200µM each dNTP, 50nM forward-tailed primer, 90 nM reverse primer, 40 nM M13 fluorescent-dye-labeled primer, 1.0U of Taq DNA polymerase and 40ng of template DNA. Briefly, the reaction was incubated at 95℃ for 5 min, and then continued for 5 cycles of 1 min at 96℃ at 68℃ with a decrease of 2℃ in each subsequent cycle, and 1 min at 72℃. For another five cycles, the annealing temperature started at 58℃ for 2 min, with a decrease of 2℃ for each subsequent cycle.

Reactions then went through an additional 40 cycles of 1 min at 96℃ for 2 min at 58℃, and 1 min at 72℃ with a final extension at 72℃ for 5 min. PCR products were separated in an ABI 3730 DNA analyzer (Applied Bio systems, Foster City, CA) and data were scored using Gene Marker (version 1.6; Soft Genetics LLC. State College, PA).

Table (1). SSR markers associated with important traits selected for the current study.

Markers	Trait Category	References
WMC0331/Al 4DL	Aluminum tolerance	Theor Appl Genet (2005) 112: 51–57
BAR0344/Al 3BL	Aluminum tolerance	Theor Appl Gene 2002, 104:286–293
TSM0120/1RS Rye	Drought tolerance	Theor Appl Gene 2008,117:915–926
Glu-A3ac/Glu-A3	Gluten strength (LMW)	T. Appl Genet (2004) 108:1409–1419
Glu-A3d/Glu-A3	Gluten strength (LMW)	T. Appl Genet (2004) 108:1409–1419
UMN19/Glu-A1	Gluten strength (HMW)	Theor Appl Genet (2008) 118:177–183
UMN25/Glu-D1	Gluten strength (HMW)	Theor Appl Genet (2008) 118:177–183
UMN26/Glu-D1	Gluten strength (HMW)	Theor Appl Genet (2008) 118:177–183
UHW89/Yr36	protein content (HGPC)	Dr. St. Amand (KSU, KS, USA)
Sr35- 64A22-1/Sr35	Stem rust resistance	Dr. St. Amand (KSU, KS, USA)
Sr36-STM773-2/Sr36	Stem rust resistance	Nucl. Appl. Res., 2002, Vol. 30, No. 23
Sr28-wPt-7004/Sr28	Stem rust resistance	Theor Appl Gene (2012) 125:877–885
csSr2-CAP/Sr2	Stem rust resistance	Theor Appl Genet (2011) 122:735–744
GWM0413/Yr15	Stripe rust resistance	Genetics 1998,149:2007–2023
GWM0273/Yr15	Stripe rust resistance	Genetics 1998,149:2007–2023
GWM0614/Lr17	leaf rust resistance	Genetics 1998,149:2007–2023
VEN./Lr37, Sr38, Yr17	Leaf rust resistance	Crop Science, 2003, 43:1839-1847
BAR0055/Sr32	Preh. Spro. resistance	T. A. Genet (2009) 119:1223–1235
Lr21-214/Lr21	Leaf rust resistance	www.k-state.edu/wgrc/Protocols
UMN10/Fhb1	F. head blight (FHB)	C. Res. Comm. 2008.B 36:195-201

Table (2). Primers name and sequences of the SSR loci reaction.

Primers	Sequence
WMC0331	F: ACGACGTTGTAAAACGACCCTGTTGCATACTTGACCTTTTT R: GGAGTTCAATCTTTCATCACCAT
BAR0344	F:ACGACGTTGTAAAACGACGCGCGTCGACATGTATTTCTTGAT R: GCGTTTCATCTGGTATCTGGTGTAT
TSM0120	F: ACGACGTTGTAAAACGACCCGCCGTCCTCCTCCT R: AGACGGCAGGCATGGAT
Glu-A3ac	F: ACGACGTTGTAAAACGACCACAATTTTCACAGCAACAGCAG R: TTGGTGGCTGTTGTGAAGACGA
Glu-A3d	F: ACGACGTTGTAAAACGACACCAGTTATTCATCCATCTGCTC R: GTGGTTTCGTACAACGGCTCG
UMN19	F: ACGACGTTGTAAAACGACCGAGACAATATGAGCAGCAAG R: CTGCCATGGAGAAGTTGGA
UMN25	F: ACGACGTTGTAAAACGACGGGACAATACGAGCAGCAAA R: CTTGTTCCGGTTGTTGCCA
UMN26	F: ACGACGTTGTAAAACGACCGCAAGACAATATGAGCAAACT R: TTGCCTTTGTCCTGTGTGC
UHW89	F: ACGACGTTGTAAAACGACTCTCCAAGAGGGGAGAGACA R: TTCCTCTACCCATGAATCTAGCA
Sr35-Cyrille-64A22-1	F: ACGACGTTGTAAAACGACATTCGTTGCGTGTTGGCTGATG R: GCTCGGGATGCATGGTATTGGTA
Sr36-STM773-2	F: ACGACGTTGTAAAACGACATGGTTTGTTGTGTTGTGTGTAGG R: AAACGCCCCAACCACCTCTCTC
Sr28-wPt-7004	F: ACGACGTTGTAAAACGACCTCCCACCAAAACAGCCTAC R: AGATGCGAATGGGCAGTTAG
csSr2-CAP	F:ACGACGTTGTAAAACGACAGATAACTCTTATGATCTTACATTTTTCTG R: CAAGGGTTGCTAGGATTGGAAAAC
GWM0273	F: ACGACGTTGTAAAACGACATTGGACGGACAGATGCTTT R: AGCAGTGAGGAAGGGGATC
GWM0413	F: ACGACGTTGTAAAACGACTGCTTGTCTAGATTGCTTGGG R: GATCGTCTCGTCCTTGGCA
GWM0614	F: ACGACGTTGTAAAACGACGATCACATGCATGCGTCATG R: TTTTACCGTTCCGGCCTT
VENTRIUP-LN2	F: ACGACGTTGTAAAACGACAGGGGCTACTGACCAAGGCT R: TGCAGCTACAGCAGTATGTACACAAAA
Lr21-214	F: ACGACGTTGTAAAACGACTGAGGTCAACAAAGAAAACCTG R: ATCCAATGCAGTGGCATTCT
BAR0055	F:ACGACGTTGTAAAACGACGCGGTCAACACACTCCACTCCTCTCTC R: CGCTGCTCCCATTGCTCGCCGTTA
UMN10	F: ACGACGTTGTAAAACGACCGTGGTTCCACGTCTTCTTA R: TGAAGTTCATGCCACGCATA

(source: USDA Genotyping Lab, Manhattan KS, USA)

Data analysis:

Specific peaks for target genes were scored across all genotypes in addition to other unique peaks for susceptible genotypes of all the traits. Fragments scored as present/absent. Fragment scoring and lane matching performed automatically on digital images of the gels, using geneMarker programe.

RESULTS AND DISCUSSION

Specific peaks for target genes were scored across all genotypes in addition to other unique peaks for susceptible genotypes of all the traits. Polymorphic level in this study is high (90%) across all wheat accessions, especially between wild and domesticated wheat used in this study (Table 3). The high level of polymorphism could be attributed to selection of genotypes with diverse characteristics. These genotypes will be useful for developing mapping populations between wild and domesticated wheat. The polymorphism observed in this study represents inherent variability among genotypes at the DNA level. Microsatellite markers are becoming the markers of choice due to the level of polymorphism, as well as higher reliability (Plaschke *et al.,* 1996 and Fu *et al*., 2005). In wheat, abundant wheat genomic SSR markers are now available and mapped, making them a useful resource for further studies.

A total of 110 alleles were detected (Table 3) among which 21 alleles (19%) were polymorphic and 89 alleles were specific for target genes (81%). For SSR, it is very common that each primer set amplifies multiple fragments and they are either different alleles in one locus or different loci. The result reveals significant differences in allelic diversity among wheat accessions studied. The fragment sizes ranged from 103 to 440bp. The study indicated the presence of specific markers in cultivated wheat using SSR markers. This opens up a possibility to apply marker-assisted selection (MAS) in developing new Egyptian wheat cultivars. The results showed one specific allele per locus, except some markers showed both resistant and susceptible allele (Table 3) while (Fahima *et al*., 1998) reported an average of 10 alleles per locus on some wild wheat accessions; also, Zeb *et al.* (2009) reported an average of 5.2 alleles per cultivar.

In addition, Salem *et al*. (2008) reported an average of 3.2 alleles from seven wheat cultivars. These allelic variations in different studies are mostly attributed to the kind of wheat genotypes for the mentioned studies (Abdel Tawab *et al*. 2003). The introduction of some traits into plants can be very difficult and expensive. Some important markers can be considered as a useful marker for screening some biotic and a biotic stress trait in the Egyptian wheat genotypes.

In many studies they are mostly attributed to the kind of wheat genotypes. Considerable amount of natural out crossing that occurs in wild wheat accessions and also the landraces which are selected from local germplasm have a wide range of diversity and thus will result in higher alleles (Salem *et al.* 2008). However, cultivars which are product of repeated inbreeding would have lower alleles than both of wild genotypes or landraces. The size of the detected alleles produced from using the SSR primer sets ranged from 59 to 635 bp (Table 3) which reflects not a large difference in the number of repeats between different alleles.

While, Salem *et al.* (2008) obtained an allelic size range between 77 to 266 bp on using 15 microsatellite markers on some wheat genotypes. In addition, Moghaieb *et al*. (2011) reported an allelic size range between 82 to

1620bp on using SSR markers associated with salt tolerance in Egyptian wheats. It should be noted that SSR markers can not only show different allelic variations in the same species but they are also able to assess even monoallelic differences in subspecies specifically (Naghavi *et al*. 2007).

The microsatellite variation is thought to be due to slippage of the DNA polymerase during replication of unequal crossing over resulting in differences in the copy number of the core nucleotide sequence. Data in Table 4 indicated that the six Egyptian bread and wild wheat were resistant for most traits especially for Fusarium head blight, Stripe rust resistance and Stem rust resistance. The results also showed that these genotypes have high protein content (HGPC). While in some traits such as pre harvest sprouting, all the genotypes were susceptible, on the other hand both resistant and susceptible were showed for drought tolerance, aluminum tolerance and leaf rust resistance (Table 4). These kinds of data can be very important for wheat breeders in Egypt.

The advantage of molecular markers for researchers is that they can test for a particular trait as early as in seeds of plants before they are planted. There is no longer a need for the plant to develop to a stage at which the trait can be observed, delay that in some cases can take many months. DNA markers have gradually been integrated into breeding programs, not as a big revolution replacing conventional breeding, but as an additional tool. This integration is only possible through a close interaction between breeders and molecular laboratory so that there is a mutual understanding of what is required for an optimised use of markers within the breeding schemes (Reynolds and Borlang*,* 2006). Several studies of molecular assisted-selection markers on wheat using different methods such as Abdel Tawab *et al*. (2003) detected five positive and negative RAPD markers for drought tolerant in Egyptian bread wheat. Moreover, the results are in line with those reported by Bruckner and Fraberg, (1987), Abdel–Bary *et al*. (2005), Rampino *et al.* (2006) and Alan, (2007) who assigned RAPD markers to drought stress tolerance in wheat genotypes using molecular markers. The present results also agree with those of Rashed *et al.* (2010), who indicates that there are potential markers to be used as marker assisted selection to improve drought stress tolerance by molecular breeding. Marker-assisted selection based on genotype mean performance will greatly increase breeding efficiency (Manavalan *et al.* 2009; Irada *and Samira,* 2010). Data in Figure (1) showed with using Glu-A3 marker for screening about the Gluten strength (Low Molecular Weight) for five Egyptian wheat and one wild type that Egypt 1, Gemmeiza 9 and 11 showed the specific peak at 108 bp comaring with with the published work. While with using Glu-A3d/Glu-A3 marker for Gluten strength (Low Molecular Weight) detect the specific peak on 128 bp for Egypt 1, Sakha 93 and gemmeiza 9 & 11, except the wild wheat Ae*. verntricosa* (Figure, 1). According to the data in Figure (5) with GWM0614/Lr17 marker, Gemmeiza 11, Sakha 93, *Ae. ventricosa* showing specific peak for leaf rust resistance at 168 bp. Gemmeiza 9 & 11, Sakha 93, *Ae. ventricosa* showed one specific peak at 363 bp with TSM0120/1RS Rye marker for Drought tolerance (Figure 2)

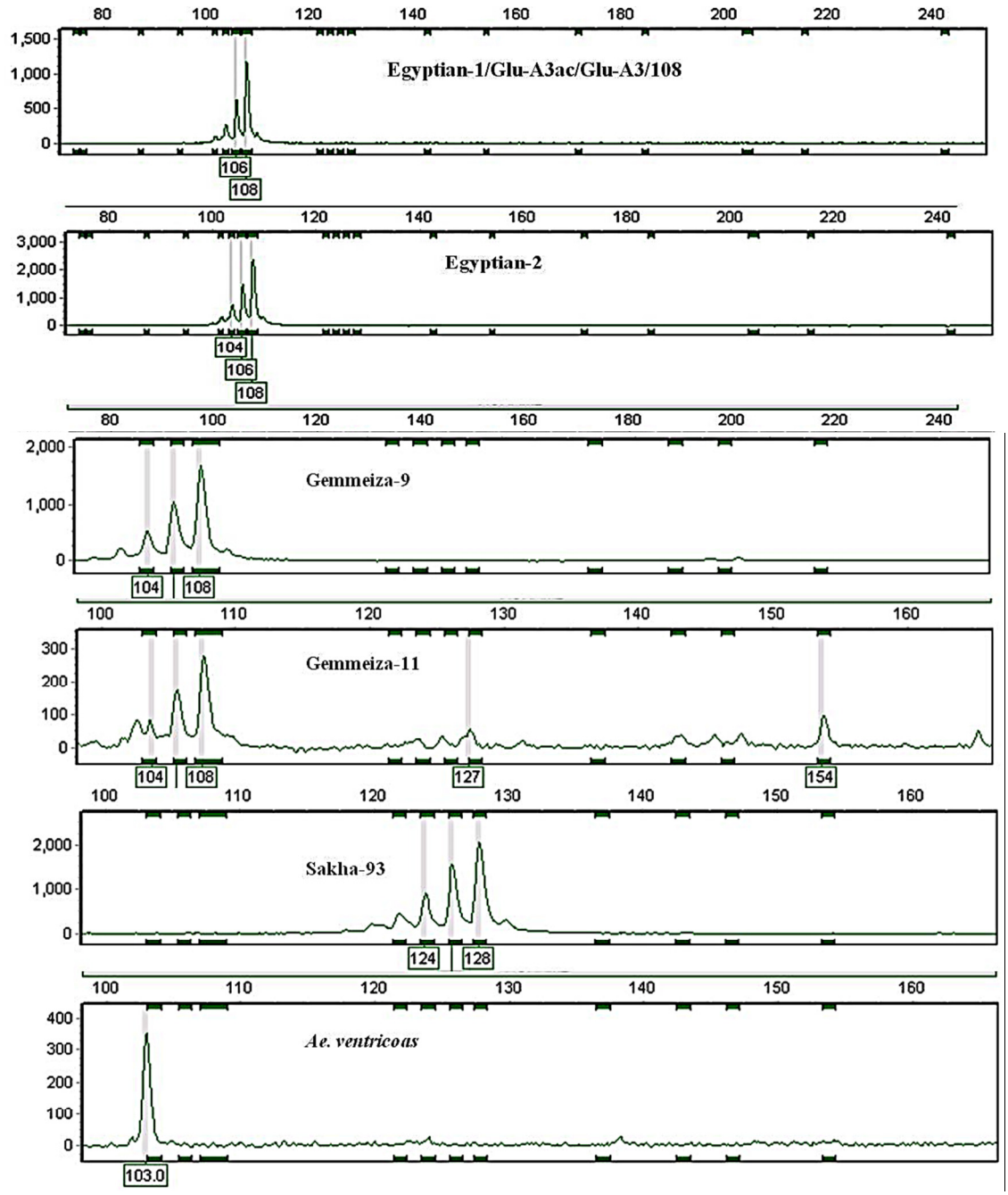

Figure (1). GeneMarker analysis for some wheat cultivars using Glu-A3 abd Glu-A3d/Glu-A3 markers showing specific peak for Gluten strength (Low Molecular Weight) and pblished in Theor Appl Genet (2004) 108:1409–1419.

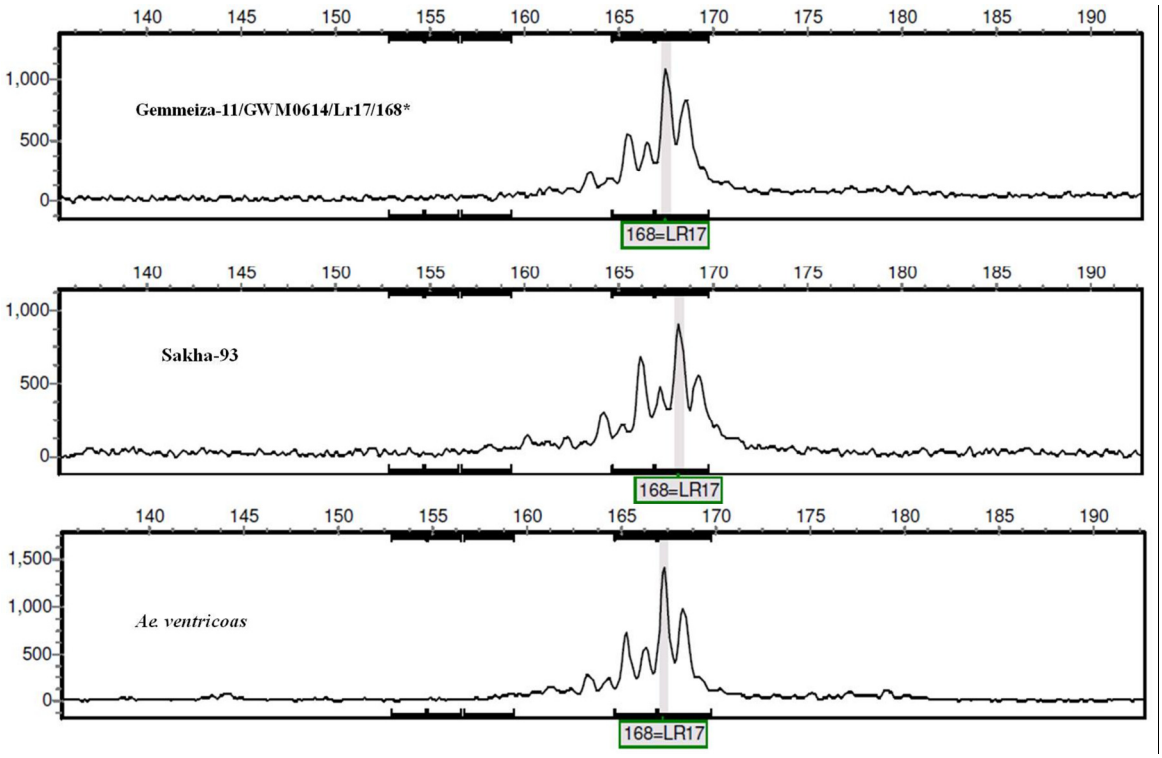

Figure (2). GeneMarker analysis for some wheat cultivar such as Gemmeiza 11, Sakha 93, *Ae. ventricosa* using GWM0614/Lr17 marker showing specific peak for leaf rust resistance and pblished in Genetics 1998,149:2007–2023.

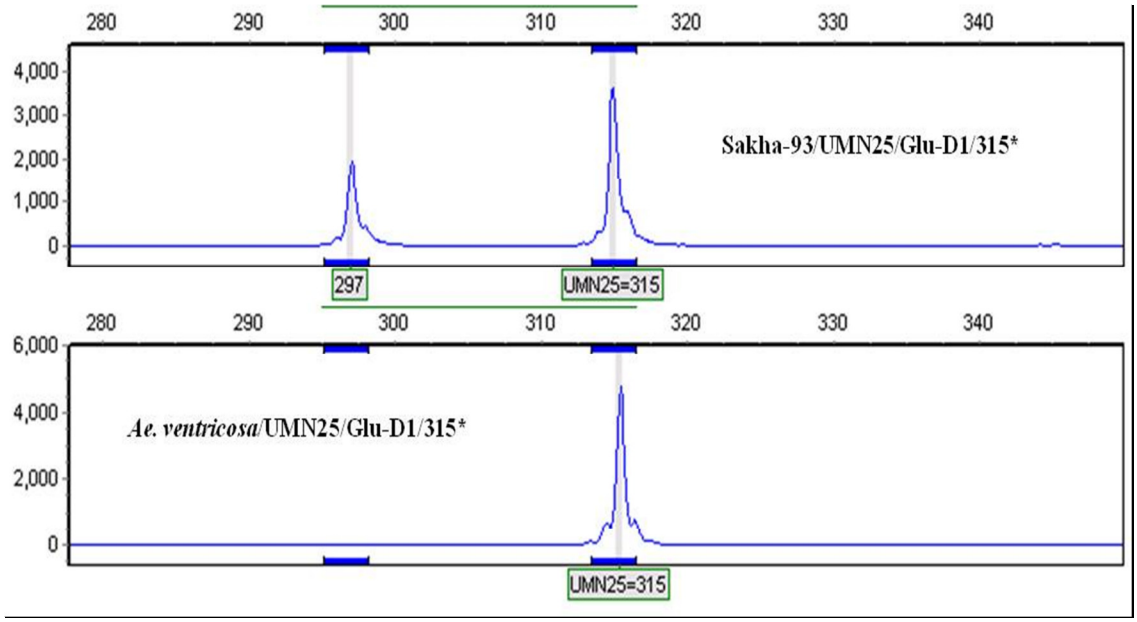

Figure (3). GeneMarker analysis for some wheat cultivar such as Sakha 93 and *Ae. ventricosa* using UMN25/Glu-D1 marker showing specific peak for Gluten strength (HMW) and pblished in Theor Appl Genet (2008) 118:177–183.

Table (3). Primers, chromosome locations, and specific SSR markers in some Egyptian wheat and their relatives

Markers	Chromosome	Size (bp)	Control	Misr 1	Sids 1	Gemeiza11	Gemeiza 9	Sakha 93	*Ae.vent
WMC0331/Al 4DL	1AS	108	108	108	108	108	108	-	-
BAR0344/Al 3BL	3BL	255-299	255	255	299	299	299	284	-
TSM0120/1RS Rye	1RS	114-363	114/363	114	114	114/363	114/363	114/363	133/363
Glu-A3ac/Glu-A3	1AS	108	108	108	108	108	108	-	-
Glu-A3d/Glu-A3	1AS	108	108	108	108	108	108	-	-
UMN19/Glu-A1	1AL	361-379	379	379	361	361	379	361	-
UMN25/Glu-D1	1DL	297-315	315	297	297	297	297	297/315	315
UMN26/Glu-D1	1DL	411-429	429	411	411	411	411	411/429	429
UHW89/Yr36	6BS	145-159	141	145	145	145	145	145	148/159
Sr35- 64A22-1/Sr35	2BS	220-235	235	220	220	220	-	220	-
Sr36-/Sr36	2BS	184-208	168	200/208	200/208	206	206	208	184
Sr28-wPt-7004/Sr28	3AL	180	180	180	180	180	180	180	180
csSr2-CAP/Sr2	3BS	188-242	188/242	242	242	242	242	188/242	-
GWM0413/Yr15	1BS	174-181	181	185	181	186	181	174	181
GWM0273/Yr15	1BS	103-128	111	108	108	108	108	128	103/108
GWM0614/Lr17	2AS	157-168	168	166	166	168	166	168	157
VENTRIUP-LN2/Lr3, Sr38,Yr17	2AS	276-276	276	-	276	276	276	276	276
BAR0055/Sr32	4AR	141-149	128/342	149	149	144	149	141	144
Lr21-214/Lr21	1DS	223-441	214	323	323	305	305	305	441
UMN10/Fhb1	3BS	247-257	249/257	249/257	249/257	249/257	247/257	247/257	247/257

**Ae.vent*: *Aegilops ventricosa* Tausch

Table (4). Resistance and susceptible genotypes for some traits in Egyptian wheat.

Traits	M.1	S.1	Gem.11	Gem.9	Sa.93	*Ae.Vent.*
Drought tolerance	S	S	R	R	R	R
Aluminum tolerance	R	R/S	R/S	R/S	R/S	S
F. head blight (FHB)	R	R	R	R	R	R
Pre. sprouting	S	S	S	S	S	S
Stripe R. resistance	R	R	R	R	R	R
L. rust resistance	R/S	R	R	R	R	R/S
S. rust resistance	R	R	R	R	R	R
protein (HGPC)	R	R	R	R	R	R
G. strength (HMW)	S	S	S	S	R	R
G. strength (LMW)	R	R	R	R	S	S
Total	9	7	9	9	11	8

*R: Resistance peak *S: susceptible peak *R/s: both peaks

CONCLUSION

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

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الملخص العربى

إستخدام بعض الواسمات المتخصصة لبعض الصفات الهامة فى القمح المصرى واقربائة البرية

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قسم النبات الزراعى – كلية الزراعة سابا باشا – جامعة الاسكندرية

يعتبر القمح (*Triticum aestivum* L) المحصول الاكثر أهمية من الناحية الاقتصادية ومحصول الحبوب الإستراتيجي الاول لمعظم سكان العالم فهو يمد العالم بحوالي ٥٥% من إجمالى الكربوهيدرات و ٢٠ % من السعرات الحرارية المستهلكة، كما انة يحتل ١٧% من المساحة المنزرعة ويوفر الغذاء لاكثر من بليوني نسمة (١٠% من عدد السكان)، وعليه فقد تم تنفيذ هذة التجربة في كلية زراعة سابا باشا– جامعة الاسكندرية– مصر خلال الفترة من عام ٢٠١4 حتي عام ٢٠١6 وذلك بهدف دراسة إستخدام بعض المعلمات الجزيئية المتخصصة فى الكشف عن اهم بعض الصفات والاعراض المرضية الهامة فى محصول القمح وقد تم تجميع عينات من خمسة اصناف مصرية هي (سخا٩٣ –سدس ١– جميزة٩ – جميزة ١١–مصر١) ونوع صنف برى هو

الايجلبس فنتراكوزا وكان الهدف من البحث هو الكشف عن وجود جينات متحملة للضغوط الحيوية واللاحيوية فى الاقماح المصرية وتحديد الاوزازن الجزئيية المختلفة لكل معلم وراثى مرتبط بالجين المطلوب وتحديد درجات التحمل والحساسية فى كل صنف. اوضحت النتائج انة مع استخدام المعلم الوراثى Glu-A3 للكشف عن قوة الجلوتين فى الاقماح المستانسة والبرية فقد وجد انة كلا من جميزة ٩ و ١١ اعطوا موقع جينى متخصص عند وزن جزيئى ١٠٨ مقارنة بباقى الاصناف المستخدمة فى التجربة وأظهرت النتائج أن هناك تعدد فى الاشكال المظرية بين النوع البرى والأنواع المستأنسة بقيمه ٩٠% كما اظهرت النتائج وجود ١١٠ أليل متخصص تم تحديد ٨٩ اليل منهم خاص بكل الصفات بنسة تصل الى ٨١% أظهرت النتائج ان جميع الاصناف المستخدة لها درجة تحمل لعديد من الاعراض المرضية المدروسة كما أظهرت النتائج أيضا ان جميع الاصناف المصرية اظهرت محتوى عالى من البروتين. ومن خلال هذة الدراسة يمكن الاستفادة من الاصناف التى اظهرت درجات تحمل مختلفة فى برامج التربية المستقبلية.