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Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2017 14(2): 370-379. **OPEN ACCESS**

Variation among some Egyptian wheat genotypes for HMW glutenin and quality traits.

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High molecular weight glutenin subunits (HMW-GS) affected greatly the quality of wheat flour due to visco-elastic properties of wheat dough. The searching for encoded genes of HMW-GS in adapted landraces may be valuable for improving wheat. Thus these studies planned to explore variation among eleven Egyptian land races (LRs) along to five improved varieties of bread wheat for HMW-GS composition using PCR based DNA. Three markers included UMN19 (with two alleles, Ax2*, Ax1 or Ax null) at *Glu-A1*in addition toUMN25, UMN26 two markers (with four alleles Dx2, Dx5, Dy10 and Dy12in locus *Glu-D1*) were used. Samples of grain and flour of genotypes were used for determine particle size index % (PSI), falling number/sec. (FN), wet gluten % (WGL) and total protein percentage (PRO).PCR amplification of HMW genes in all genotypes was polymorphic by using UMN19 and UMN26 markers. Allelic variation of Ax2* was detected in all cultivars and other three races (LR1, LR10 and LR 11). LR2 possesses an unique band of Ax1 allele in all genotypes. Index of quality (IQ) and selection index (SI) based on HMW-GS and phenotypic performance, respectively were used for assessing the potentiality of wheat genotypes. The ranks of genotypes for both indices were positively significant correlation. Only FN showed positive significant rank correlation with both indices. The utilized markers may be useful for determining HMW-glutenin allele (s) for improving bread making quality of studied genotypes. The investigated wheat landraces could be of great benefit for breeding new cultivars possessed high flour quality.

Keywords: Landraces, HMW glutenin, Quality and Selection indices

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered one of the most cereals globally grown due to grains flour properties. In Egypt, the limitation of acreage and losses due to storage conditions affected negatively the production efforts and quality. Wheat landraces have advantage over wild relatives of being more easily crossed with cultivated genotypes and enrolling varietal improvement efforts (Ayala et al. 2013; Caballero et al. 2010).Bread wheat quality components reported to be governed by major genes as complex traits, which affected by glutenin properties specially the high molecular weight (HMW) subunits of storage proteins in grains **(**Anjum et al.2007).The HMW-GS are encoded by Glu-1 loci (GluA1, GluB1 and GluD1) and expressed to highly visco-elastic of dough characteristics (Liu et al. 2008). Therefore, glutenin subunits affect protein fraction and generally positively related to total protein content of wheat flour (Perten et al. 1992 and Duska et al. 2001).Previous studies were accomplished the screening of glutenin subunit via electrophoresis SDS-PAGE (Payne et al. 1981; Shewry et al. 1992). On the other hand using polymerase chain reaction (PCR) approach based on DNA glutenin genes considered an alternative method which

seemed to be easy, quick and non-destructive procedures for screening genotypes with proper bread making quality as reported by Ahmad (2000).PCR-based functional markers developed from gene sequences provide accurate and high throughput data for determining allelic compositions in breeding materials (Bagge et al. 2007; Liu et al. 2012).Moreover, Marker assisted techniques may be used indirectly to asses wheat quality traits and independent of environmental conditions(Gale, 2005).

Six selection indices were suggested to evaluate the genetic potentiality of wheat lines for quality (Branlard et al.1991). Three of which established to evaluate dough strength of Chopin alveograph, whereas the other three ones measure loaf volume according to French bread-making standard. They calculated a Index of quality IQ from the allelic effects of HMW subunits of glutenin, and is similar to the gluten score suggested by Payne et al. (1987).The exploring of an ideotype possesses genes for productivity, quality and tolerate various stresses is the wise strategy for building wheat varieties (Gomaa 1999).The determination of the targeted characteristics of available landraces to inclusion in variety building program depended greatly on précising and accelerating in evaluation processes.Therefore, the present investigation was planned to evaluate five Egyptian improved bread wheat genotypes along to eleven landraces for different baking quality traits in relationship to some functional markers of HMW subunits via specific PCR based DNA.

MATERIALS AND METHODS

Plant materials and procedures of bread quality traits

Sixteen Egyptian bread wheat genotypes including five improved cultivars and eleven landraces were studied. The improved (varieties included Misr1, Misr2, Seds1, Gemmeiza10 and Sakha93 were obtained from Agricultural Research Center (ARC), Giza. However, the eleven land races were kindly provided by National Gene Bank (NGB), which were collected mostly from desert regions of Egyp Two field trials were conducted at the Agricultural Experiments and Research Station, Faculty of Agriculture, Cairo University, Giza, during 2013/2014 and, 2014/2015 seasons. At harvest, random duplicate-samples of grains and result flour were studied for quality traits and considered as completely randomized design (CRD).The four quality traits were determined using duplicate 200 g of harvested grains. Such analyses were kindly carried out at Chemical and Cereal Technology Laboratory, Food science Department, Faculty of Agriculture, Cairo University. Particle size index (PSI %) indicates the relative hardness by grinding and sieving according to method No. 55-30 of AACC (2000). Protein content (PRO %) was determined according to the ratio of total nitrogen into grains (N × 5.7) by Kjeldahl method. Falling number (FN) or alpha amylase activity (soundness) was determined by “Falling No.1600” according to method No.02-06 of AACC (2000). Gluto-matic traits involved wet gluten (WGL %). obtained by forming dough refer to AACC (2000) No. 38-12.02.

DNA extraction and PCR procedures.

The molecular genetics procedures were carried out at Cairo University Research Park (CURP) at Faculty of Agriculture. The genomic DNA was extracted from fresh leaves by added liquid nitrogen into seedlings (after month of sowing date) of wheat genotypes, and used CTAB method according to Poresbski et al. (1997).Three specific primers (Table 1) according to Liu (2008), obtained from Vivantis Company (Selangor Darul-Ehsan, Malaysia.), were used to amplify the template DNA. Amplification reaction volumes were 25µl containing 12.5µl Master mix (MyTaq™ Mixcat no.BIO-25041 Bioline Company) 1µlprimers (10µM each), 100 ng / mg template DNA up to 25µlNuclease-Free water. PCR program for the reaction mixtures was: 95 ºC for 5 min, followed by 40 cycles (at 95 ºC during30 sec., at57 ºC 30 sec. and 72 ºC 30 sec.). This program was followed by a final extension step for 10 min. at 72 ºC. Amplified PCR products were separated and visualized on 1.5% agarose gel electrophoresis.

Statistical analyses

The obtained data of quality traits were subjected to regular analysis of variance of CRD using MSTAT-C software and Duncan's multiple range test at 0.05 level. Combined analysis over to seasons was carried out after test the homogeneity of error mean squares. Index of quality (IQ) based on HMW-GS of a wheat genotype was computed as the pooled of quality coefficients (Y_{ij}) attributed to the HMW-GS alleles belonged to the three loci *Glu-A1, Glu-B1* and *Glu-D1*of this genotype.

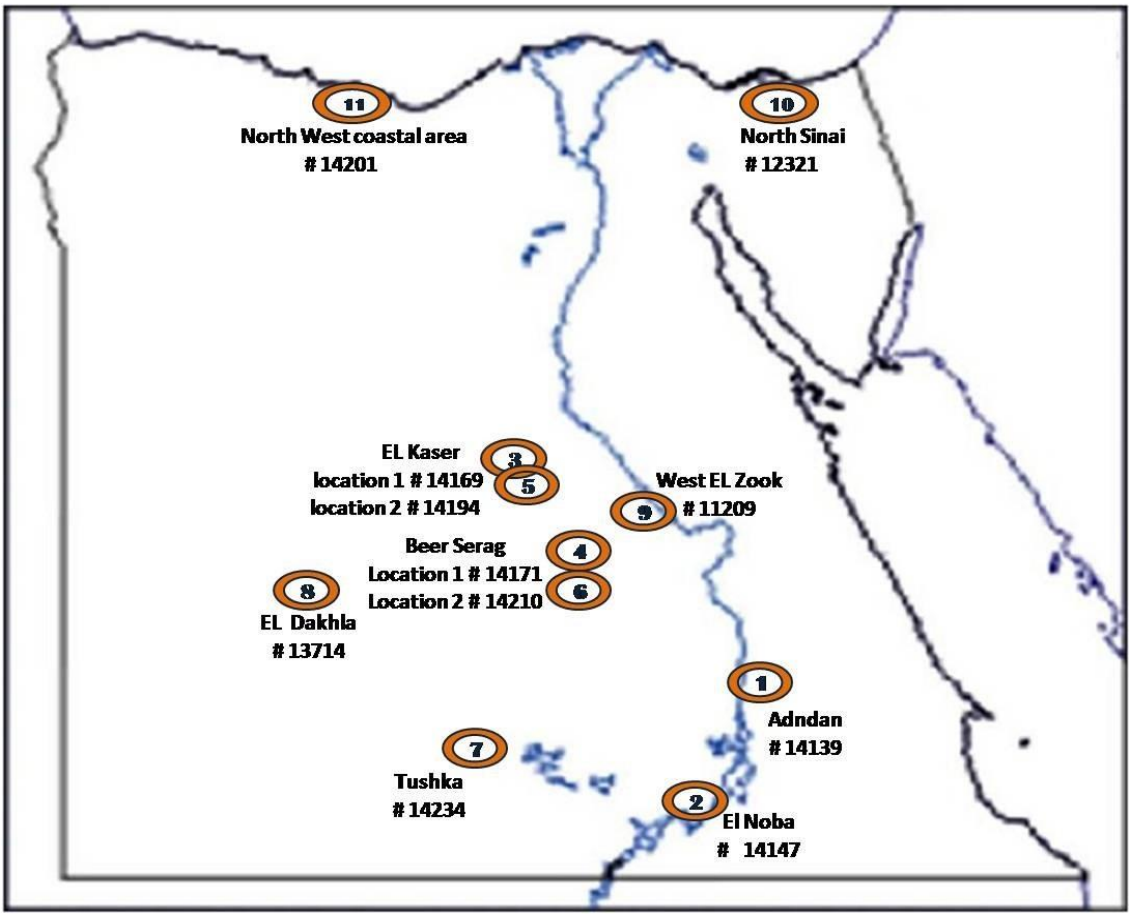


Fig.1. Sites of collection the studied eleven land races in Egypt corresponded by the accession number of NGB, but inside circles the serial number used in the article

Table 1.Loci, names and primer sequencing of DNA markers used to amplify HMW glutenin subunits for wheat genotypes (Liu 2008).

Locus	Marker	primer sequence(5'→3')	Allel name & size (bp)
Glu-A1	UMN19 F	CGAGACAATATGAGCAGCAAG	Ax2* (344)
	UMN19 R	CTGCCATGGAGAAGTTGGA	Ax1,Ax-null(362)
Glu-D1	UMN25 F	GGGACAATACGAGCAGCAAA	Dx2 (299)
	UMN25R	CTTGTTCCGGTTGTTGCCA	Dx5 (281)
Glu-D1	UMN26 F	CGCAAGACAATATGAGCAAACT	Dy10 (397)
	UMN26 R	TGCCTTTGTCCTGTGTGC	Dy12 (415)

Where $_{i:}$ the allele effect
And $_{j}$: the locus of alleles= from 1 to 3

$$IQ = \sum_{j=1}^{3} Y_{ij}$$

$$Y_{ij} = b\bar{X}_{.ij} + aj$$

Where b: the coefficients and a:and a_j is a constant corresponding to the quality value

attributed to the reference allele. For *Glu-Al* (null allele) a_1 =0 and for *Glu-Bl* and *Glu-Dl,* a_2=2 and a_3=7, respectively according to Branlard *et al*. (1991).

The mean effect $\bar{x}_{ij}$ was calculated as the mean of the4 quality traits effects as follows:

$X_{ki\,j} = (X_{ki\,j} - X_{krj})$ X 100/ Δ xk; where

$X_{ki\ j}$= the mean value of the parameter k calculated from the varieties having the allele i at the locus j

X_{kij}=the effect $X_{ki\,j}$ of the allele i from the locus j

$X_{kr\,j}$=the mean value of the parameter k calculated from the varieties lacking the allele.

$\Delta_{xk=}$ the difference between the maximal and minimal values in each parameter.

Selection index (SI)based on the phenotypic performance for the four used quality traits considering the linear index, With each selection index score *(I)* was calculated from the following formula (Baker, 1986).

$I = \Sigma biPi$

Where P_i is the phenotypic value of each trait and the weights (*bi*) were calculated as follows. Using matrix notation:

$b = P^{-1}Ga$

Smith-Hazel index (Smith, 1936; Hazel, 1943);

$b = a$

Brim-Williams index (Brim *et al.,* 1959; Williams, 1962).The phenotypic *(P)* and genotypic *(G)* variance - covariance matrices were estimated from analyses of variance and covariance for single traits and pairs of traits, respectively. Also *(a)* is relative economic values for each trait.

RESULTS AND DISCUSSION

Polymorphism of specific PCR profiles of used DNA markers for studied genes in the 16 wheat genotypes.The allelic polymorphisms in the studied wheat genotypes using HMW glutenin subunits at *Glu-A1* and *Glu-D1* locus using UMN19, UMN25 and UMN26markers are presented in Fig.2 (a, b and c). All subunits were identified in all studied wheat genotypes either landraces or cultivars using UMN19 (of *Glu-A1*) and UMN26 (of*Glu-D1*) markers. However, the bands of *Glu-D1*byUMN25 marker was absent in lane10 (LR5). Such situation may be considered a negative unique polymorphic band in the present investigation.The DNA fragment bands bp illustrated the distance between bands on gel belonged to the ladder 100bpBiolabsInc.and the predicted bands were identified by Gel- Analyzer 2010 software program (Table 2).

Zhang *et al.* (2003) reported that the development of allele specific PCR primers can to discriminate differences in sequence identity between alleles with as little as 1 bp polymorphism.

Results show that locus *Glu-D1* by UMN25 marker given two bands for Gemmieza 10 and LR3.Tentatively, the difference less than 10% between the observed (by Gel Analyzer) and the expected bands bp of each allele for the genotype may be considered an indication the presence of this allele in the genotype (Table 2). AccordinglyLR5 and LR9 wheat genotypes lacked any of studied alleles and another twelve genotypes included at least one allele. Table (2) indicated that Ax2*(344 bp) allele (by maker UMN19) was present inMisr1, Misr2, Seds1, Gemmeza10, Sakha93, LR1, LR10 and LR11wheat genotypes. However, allele Ax1 (362 bp) in the previous marker appeared only in LR2. The UMN25 marker with allele Dx2 (bp 299) was found in Seds1, Gemeza10 and LR2 wheatgenotypes. Using also the UMN25 marker, the allele Dx5 (bp 281) appeared in the improved varieties Misr 1 and Misr 2 in addition to other four land races (LR1, LR3, LR10 and LR11). The last marker UMN26 with allele Dy 10 (bp 397) appeared in seds1 and Gemmeza10as well as two land races (LR 2 and LR 3), but the allele Dy 12(bp 415) is present in four land races coded LR6 , LR7, LR 8 and LR 11 The glutenin subunits compositions varied among wheat genotypes and gene pools, and appeared to be independent of the altitude and attitude (Payne *et al.* 1987).Lagudah et al. (1987) detected allelic variations at *Glu-Al* and *Glu-BI* loci coding, whereas *Glu-DI* locus didn't show allelic differences using 60 Afghan wheat landraces. On the other hand, Cong *et al*. (2005) found that both *Glu-Al*and *Glu-BI* loci coding showed similar variation between spring and winter Chinese wheat landraces in spite of *Glu-DI* locus exhibited remarkable allelic variation only in winter ones. Such findings proved that both winter and spring Chinese wheat genotypes differed in Origin and evolution background. Analyses of wheat cultivars have shown that HMW differ in their impact on bread-making performance with subunits Ax1, Ax2 and subunits Dx5, Dy10 in particular being associated with high dough strength (Shewry *et al*., 2003).Payne *et al.* (1981), Payne *et al.* (1987) and Popineau et al. (1994) established the gluten score from 14 allele, and assigned quality scores to each of the common alleles of the Glu-1 loci.

Table2. Allelic (Ax*2, Ax1, Dx2, Dx5, Dy10 and Dy12) variation at *GLU-A1*and *GLU-D1* locus using three markers (UMN19, UMN25 and UMN26) of studied sixteen wheat genotypes.

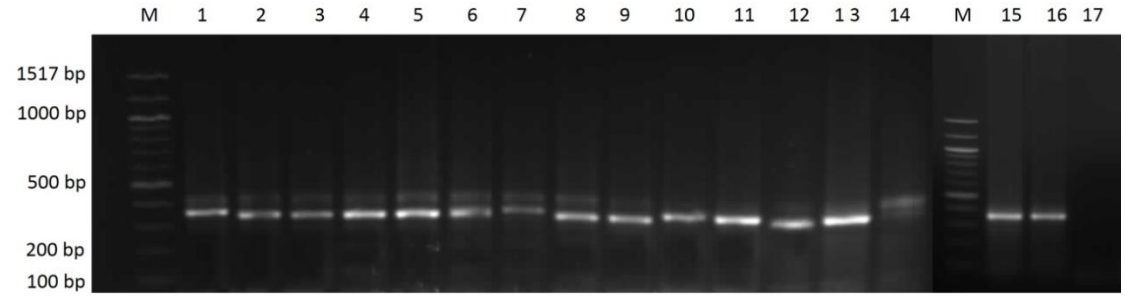
Genotype	UMN19		UMN25		UMN26	
	Ax2* (344bp)	Ax1 (362bp)	Dx2 (299 bp)	Dx5 (281bp)	Dy10 (397 bp)	Dy12 (415 bp)
Misr1	+	-	-	+	-	-
Misr2	+	-	-	+	-	-
Seds1	+	-	+	-	+	-
Gemmeiza10	+	-	+	-	+	-
Sakha 93	+	-	-	-	-	-
LR1	+	-	-	+	-	-
LR2	-	+	+	-	+	-
LR3	-	-	-	+	+	-
LR4	-	-	-	-	+	-
LR5	-	-	-	-	-	-
LR6	-	-	-	-	-	+
LR7	-	-	-	-	-	+
LR8	-	-	-	-	-	+
LR9	-	-	-	-	-	-
LR10	+	-	-	+	-	-
LR11	+	-	-	+	-	+

Table 3. Mean performance of studied wheat genotypes for quality traits, estimates of selection index (SI) and Index of quality (IQ). Combined over 2013/2014 and 2014/2015 seasons.

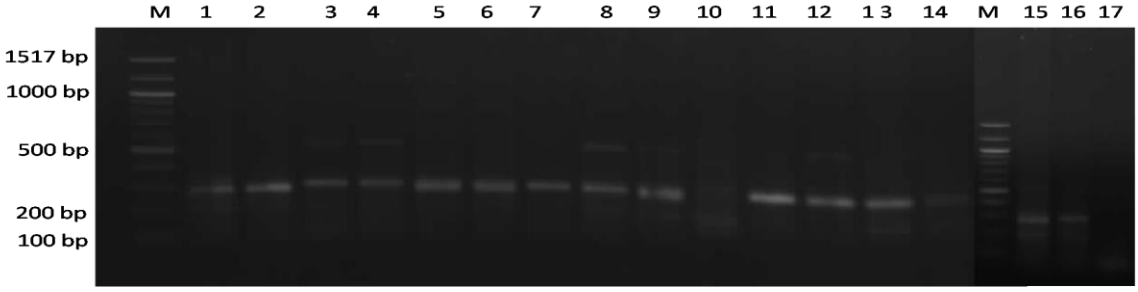
Genotype	PSI %		WGI%		PRO %		FN sec.		SI		IQ
	M	AR	M	DR	M	DR	M	DR	M	DR	
Misr1	85.0	6	36.9	11	9.1	14	400.5	1	357	1	37
Misr2	85.5	7	33.6	13	11.6	8	326.5	12	311	11	37
Seds1	87.0	9	26.8	16	10.5	10	355.5	2	329	3	165
Gemmeiza10	87.5	10	30.6	15	13.1	5	340.5	6	325	4	165
Sakha 93	85.5	8	46.6	1	12.1	6	332.5	9	318	7	46
LR1	90.0	16	36.5	12	11.7	7	328.0	10	316	10	46
LR2	89.5	13	38.2	7	14.5	2	351.0	3	337	2	502
LR3	89.5	14	37.4	10	9.6	12	346.5	5	324	5	283
LR4	88.0	11	32.0	14	11.6	9	336.5	7	319	6	0
LR5	89.5	15	39.5	4	9.4	13	265.0	15	268	15	0
LR6	84.5	5	38.1	8	13.6	4	290.0	14	290	14	25
LR7	88.5	12	39.1	6	10.1	11	262.5	16	267	16	25
LR8	79.5	1	46.2	2	15.6	1	327.0	11	317	9	25
LR9	81.5	3	41.3	3	14.0	3	310.5	13	303	13	0
LR10	82.5	4	37.5	9	8.2	15	348.5	4	318	8	37
LR11	80.0	2	39.5	5	7.5	16	333.5	8	304	12	62
LSD $_{0.05}$	2.7		1.6		0.5		40.6		--	--	--

M, AR and RD mean, ascending and descending rank, respectively; LSD= least significant difference at 0.05 level.

a- (*Glu-A1:*UMN19*)*



b- (*Glu-D1:*UMN25*)*



c- (*Glu-D1:*UMN26*)*

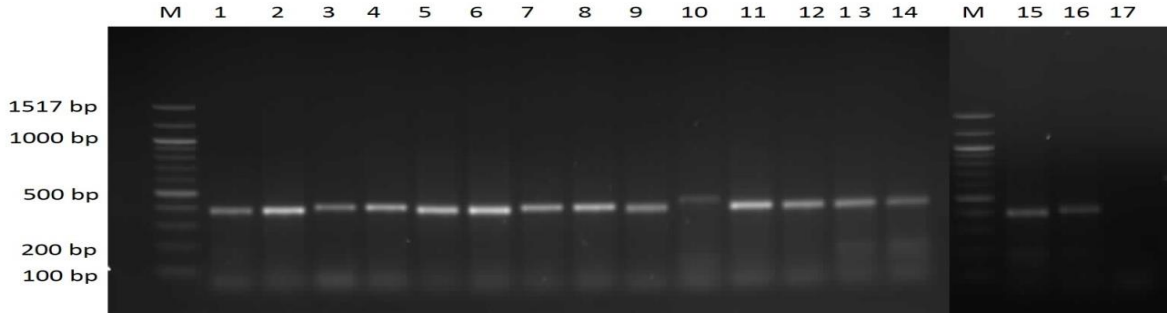


Figure 2: Agrose gel 1.5% M: ladder100bp, #1:Misr1, #2:Misr2, #3:seds1, #.4: Gemmeza 10, #5:Sakha93, #6 to #16: LR1 to LR11, respectively, #17 NTC (negative temple control free water).

Table 4. Rank correlation coefficients among studied traits and indices of selections.

	WGL%	PRO%	FN	SI	IQ
PSI%	-0.36	-0.04	0.03	0.14	0.27
WGL%		0.18	-0.47	-0.49	-0.30
PRO%			-0.22	0.03	-0.05
FN				0.94**	0.65**
SI					0.62*

***and **indicate significant at 0.05 and 0.01 levels, respectively.**

The Glu-D1 locus has the largest effect on bread making quality. Also the combination of Dx5 and Dy10 is associated with strong dough and the Glu-A1 locus, both the Ax1 and Ax2* subunits have positive effects on bread making quality. On the other hand allelic pair Dx2 + Dy12 has negative effects and the null allele has a low quality score.Payne et al. (1984) found that wheat storage proteins corresponding at 9 complex loci on six different chromosomes. The first three loci are Glu-A1, Glu-B1,Glu-D1 indicated to HMW and located in the centromere in Long arm, whereas the loci 4, 5 and 6 occurred Gli-A1,Gli- B1 and Gli-D1 in the short arm in the same previous 3 chromosomes contributed to LMW . But another three chromosomes in the telomeres A 6, B6 and D6 involved Gli-A2, Gli-B2 and Gli-D2, respectively. In addition to the recombination of genes within a locus was very rare and has so far been detected in Glub1 , and the relative quality of different alleles at each locus indicating the following order of importance Glu-1 >Gli-1>Gli-2.Shewry and Halford (2002) found that each locus of Glu-A1, Glu-B1 and Glu-D1 consist of two tightly linked HMW glutenin genes, one x-type and one y-type, respectively. Yang et al. (2005) summarized the range of the alleles at the Glu-1 loci as 3 allelic forms at the Glu-1A, 11 alleles at the Glu-1B, and 6 alleles at the Glu-1D.Radovanovic et al. (2002) estimated genetic variability for gluten strength contributed by Glu-B1 and Glu-D1 encoded HMW glutenins. Liu et al. (2008) showed Both UMN25 and UMN26 target the Glu-D1 locus and either one of them should be adequate to select for the favorable allele Glu-D1d (Dx5 + Dy10) as demonstrated above. Recombinants between the x-type and y-type genes can be identified when both markers are used for genotyping. Alleles Glu-D1b (Dx3 + Dy12) and Glu-D1c (Dx4 + Dy12) are associated with poor bread making quality. They didn't report DNA markers for the Glu-B1 locus for the subunit pairs with negative effects on bread making quality such as Bx6 + By8 or Bx20 + By20.Anjum et al. (2007) mentioned that wheat flour possessed good bread-making quality due to the presence of certain HMW subunits

Mean performance of genotypes for quality traits

Wheat genotypes varied significantly for all studied quality traits (Table 3), not only among improved varieties but also including land races. Some land races ranked better than improved varieties for studied quality traits.Particle size index (PSI) is one of protein quality parameters; particles with low value had highest hardness of grains (Pasha et al. 2010). However, PSI is influenced by protein composition which varied among genotypes (Fajerson, 1950 and Symes, 1961).The studied landraces included the two extremes of PSI%, superior group (LR8, LR11, LR9, LR10 and LR6) and inferior one (LR1, LR5, LR3, LR2 and LR7).But the improved wheat varieties performed intermediately for PSI%. Sakha93 ranked the first for WGL % (46.6%) with insignificant difference only with LR 8. Other improved cultivars showed significantly drop in gluten contents although they involved more alleles of glutamine than Sakha93. Not all wheat grain proteins belonged to gluten, there are another complicated group as gliadins which contributed in gluten strength (Schofield, 1994). LR8 is promising for PSI% (79.5%), WGL% (46.2%) and PRO% (15.6%), with poor performance in FN**.**

Bread wheat quality may be classified by protein content (PRO %) as very low (≤9.0%), low (9.1-11.5%), medium (11.6-13.5%), high (13.6-15.5%), very high (15.6-17.5%), and extra high (≥17.6%) (Williams et al., 1988).Accordingly, the studied wheat genotypes could be classified into very low (LR11 and LR10), low (Misr1, LR 5, LR 3, LR7 and Seds1) and medium (Misr2, LR4, LR1, Sakha93 and Gemmiza10).LR 9 and LR 2 may be belonged to high protein group, whereas LR 8 to very high one.

Falling number (FN) determines the activity of alpha amylase enzyme through the viscosity of flour colloidal solution (Hagberg, 1960). FN generally categorized the wheat genotypes into two major groups, those with values of 350 seconds or longer which pointed to low enzyme activity and very sound wheat quality. As the amount of enzyme activity increases, the falling number decreases and consequently values below 200 seconds indicate high levels of enzyme activity (Carl, 2006). Misr1 and Seds1 recorded 401 and 356 sec. respectively; followed by the land races LR2 which recorded 351 sec. Almost other genotypes had a reasonable values of FN. In contrast LR7 and LR 5 were approached to increase of enzyme activity and faster rate FN sec. indicated to reductions of biochemical characterizes and quality properties.

High scores of selection index could be observed by Misr1, LR2, Seds1, Gem.10 and LR3, respectively. Four land races (LR6, LR 5, LR 7and

LR 9) recorded the least selection indices of quality traits. Worthy, two of these land races (LR 6 and LR 7) have one of studied alleles , i.e Dy 12 and the other two land races lacked of any of studied alleles (LR5 and LR9). It may be observed that selection indices (SI) greatly corresponded to FN and rank correlation coefficients support this view (Table 4). Only LR2 and LR3 possessed significantly reliable indices of quality (IQ).

Although Gel Analyzer indicated that LR5 and LR9 are common in lacking the studied alleles with variable performance for quality traits. Ranks in spite of the second race ranked the better third genotype for PSI, WGL% and PRO% quality traits. On the other hand, LR5 behaved inferiorly for most quality traits.

The investigated wheat genotypes exhibited variable expressions to different quality traits, the four tabulated or not included ones. On the other hands they possessed a range of alleles either detected by used marker or mentioned in references (Liu et al, 2008). Some of such genes/alleles combinations increasing the score of glutenin quality which strengthen the expression baking traits such alveograph, farinograph ,amylograph etc

LR2 and LR3 landraces recorded the greatest significant IQ (as average of traits plus standard error) compared to all other investigated genotypes **(**Branlard et al., 1991). Five wheat genotypes show the appearance of Dy 10, i.e Seds1. Gemmeiza10, LR2, LR3 and LR4. Only the latter LR had one of these alleles (Dy10), other three genotypes (Seds1. Gemmeiza10 and LR2) included Dx2 in addition Dy 10 and Ax* or Ax1. The combinations of alleles present in the studied wheat genotypes may be play an important role in the bread quality traits. Some of these alleles specially Dx5 and Dy10 were considered as favorable ones responsible for good baking traits (see Shewry et al., 2003)

The interrelationships among studied four traits and estimated two selection indices indicated that FN only significantly positively correlated with selection criteria IQ and SI (r = 0.65** and 0.94** respectively). These indices may be recommended for selecting promising wheat genotypes along to productive criteria.

There are several alleles didn't study playing important role, and have been scores which contributed in the quality for another traits, in addition presented allele differed in genotypes and effected the performance of genotypes for FN. The FN in this study was proper indicator depended on these six allele with three markers of HMW

CONCLUSION

The obtained results proved that bread wheat landraces could be of great benefit for breeding new cultivars possessed high flour quality and adaptation to newly reclaimed desert lands, and the set of markers used here just represent a useful proceeding for screening of HMW-glutenin allele (s) for improvement of bread making quality in wheat genotypes. The FN was an important criteria that reflected into quality with selected studied alleles by IQ and SI indices.

Most of studied genotypes have some of functional marker genes, but the performances occurred differences among genotypes, so this kinds of studies need more marker alleles and the computation quality score.

CONFLICT OF INTEREST

The present study was performed in absence of any conflict of interest

ACKNOWLEGEMENT

We appreciate Mr- AbdElhay Gaber one of technical stuff of molecular genetics Laboratory in Cairo University Research Park (CURP) for his great efforts in this work.

AUTHOR CONTRIBUTIONS

The present investigation is a part of Ph.D work of I H Yacoub. DAEl-Kadi is the supervisor co-suggested the problem and reviewed the manuscript. DS Darwish acted as co-supervisor suggested statistical analyses, tabulating data, discussed results and reviewed the manuscript. REAMoghaieb: introduced advices concerning molecular markers and reviewed results and manuscript. IHYacoub conducted field and lab trials, collected and analyzed data and wrote the manuscript.

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