Abyss. J. Sci. Technol. Vol. 8, No. 1, 2023, 41-49.
ISSN 2616 – 4728 (Online); 2616 – 471X (Print).
DOI: https://doi.org/10.20372/ajst.2023.8.1.937
Publisher: Wollo University, Dessie, Ethiopia

Abyssinia Journal of Science and Technology

Diversity and Quality Analysis of Tetraploid Wheat Farmer Varieties from Ethiopia with HPLC and Pheno-quality Parameters

Faris Hailu[a,b], Maryke Labuschagne[c], Helena Persson-Hovmalm[b], Angeline Van Biljon[c], and Eva Johansson[b]

[a]Department of Biology, College of Natural Science, Wollo University, Dessie, Ethiopia
[b]Department of Plant Breeding, Swedish University of Agricultural Sciences, SE-230 53 Alnarp, Sweden
[c]Department of Plant Sciences, University of the Free State, Bloemfontein 9300, South Africa

ABSTRACT

Tetraploid wheat, including landraces, is a traditional and important food crop in Ethiopia and its protein composition determines the quality and usefulness of the crop. A total of 49 landraces of tetraploid wheat was obtained from Ethiopian Biodiversity Institute and grown on farmland of Dessie Zuria and Kutaber districts. The landraces were evaluated for diversity in protein composition via size exclusion-high performance liquid chromatography, reverse phase-high performance liquid chromatography and pheno-quality traits were determined in the field. The result showed a high level of variation among the landraces evaluated both for pheno-quality traits and for protein composition, indicating opportunities to use the landraces directly for crop production or in breeding for quality varieties. Accessions containing HMW and LMW glutenin alleles known to correlate with quality characters were present in the material, as were accessions with similar or higher values for grain protein concentration and gluten strength (obtained from SE-HPLC) as compared to commercial varieties. Results from RP-HPLC indicated a closer correspondence in variations of wheat from Ethiopian regions situated close to each other, although a similar pattern was not seen for the quality evaluation by SE-HPLC. Crop development time was found correlating with SE-HPLC quality parameters. Thus, the study showed the presence of wide variation in Ethiopian tetraploid wheat landraces with high quality traits being promising candidates to be used for pasta production.

Keywords: Durum Wheat, Gel Electrophoresis, High Performance Liquid Chromatography, Landraces.

INTRODUCTION

Wheat is among the most widely cultivated crops, being distributed almost all over the world (FAO, 2017; Pena-Bautista, et al., 2017). Hexaploid bread wheat (*Triticum aestivum* L.), mainly used for various bread and baked products, and the tetraploid durum wheat (Triticum turgidum L. subsp. durum) mainly used for pasta products, are the two most commercially important types of wheat (Ali, et al., 2013; Siahbidi, et al., 2013). Wheat is an important crop in Ethiopia; in 2012, 78% of crop production was cereals and of these cereals, wheat production area was 13% and yield was 15% (Hailu et al., 2010). Ethiopia is the largest wheat producer in sub-Saharan Africa and durum wheat constitutes about 40% of the total wheat area (Belay et al., 1995).

The tetraploid durum wheat of Ethiopia is known to be morphologically different from durum wheat in other origins, with more uncompact spikes and smaller and darker seeds (Pecetti, et al., 1992). Therefore, the Ethiopian durum wheat has been classified into its own subspecies, i.e. *T. durum* subs. *Abyssinicum* or *T. aethiopicum* (Hailu et al., 2010; Belay et al., 1995) and Ethiopia has been classified as a 'center of wheat diversity' (Vavilov, 1992). Ethiopia has been suggested as a center of diversity for emmer wheat, the ancestor of durum wheat, due to the high variability being available in the region (Takenaka, et al., 2010). Furthermore, recent studies applying modern molecular techniques have indicated the possibility of Ethiopia also being center of origin for durum wheat (Kabbaj, et al., 2017).

***Corresponding author:** markhmets@yahoo.com;
Received: 14-08-2022, **Accepted:** 28-05-2023, **Published:** 30-06-2023

Journal homepage: https://abjol.org.et/index.php/ajst/index
Citation: Hailu, F., Labuschagne, M., Persson-Hovmalm, H., Van-Biljon, A., & Johansson, E. (2023). Diversity and Quality Analysis of Tetraploid Wheat Farmer Varieties from Ethiopia with HPLC and Pheno-quality Parameters, *Abyssinia Journal of Science and Technology*, *8*(1), 41-49.

The wheat improvement work in Ethiopia started in the 1930's using indigenous collections together with introduced varieties from Europe and later from Kenya (Hailu & Merker 2008). Later, a hybridization program was initiated in Northern Ethiopia in 1956 with the objective of transfering stem rust resistance from the local to exotic varieties. As a result, a few wheat cultivars were released between 1956 and 1966. Since then, the program has carried out more than 2000 single, threeway, double and back crosses involving hundreds of parents.

Wheat can grow in the Ethiopian highlands, which are situated 6° - 16°N and 35° -42°E at an altitude ranging from 1500 - 3000 m; however, the most suitable altitude zones for wheat fall between 900 and 2700 m above sea level (Hailu & Merker, 2008). In Ethiopia, the wheat grain is used in a number of food products consumed by different individual groups in a variety of forms such as different pasta products, roasted wheat, and in some areas it is preferred for bread or home-made liquor and other beverages (Belay, et al., 1995). Tetraploid wheat including landraces is a traditional and especially important food crop in Ethiopia.

Landraces are still widely present and cultivated in Ethiopia (Hailu & Merker, 2008). A landrace is defined as a balanced population that is variable, in equilibrium with both environment and pathogens and genetically dynamic; the result of millennia of natural and artificial selections. As landraces are eco-geographically distinct populations with diverse genetic composition, adequate evaluation is needed to facilitate their use as genetic resources. Various researchers (Belay, et al., 1995; Hailu, et al., 2006) have reported the uniqueness of the Ethiopian tetraploid wheat germplasm for different useful traits. Further, both physiological and quality traits as well as gliadins composition, have been used to classify the Ethiopian wheat genotypes as related to their genetic diversity (Murat, et al., 2013). Genetic diversity of plants determines their potential to be used in breeding for improved yield and quality, thereby contributing to a possible enhancement of food production (Hailu, et al., 2006; Siahbidi, et al., 2013). However, despite the fact that a range of investigations have been carried out as related to diversity of Ethiopian tetraploid wheat, limited information is available as to its genetic variation in quality traits. For such evaluations, analyses by SE- and RP-HPLC is particularly useful since several quality traits are linked to amount and size distribution of polymeric and monomeric proteins (Johansson, et al., 2013).

The proteins of the wheat grain, being of relevance for quality traits consists of the gliadins and the glutenin subunits (Gupta, et al., 1993; Shewry, et al., 2003). The glutenin subunits, consisting of high molecular weight (HMW-) and low molecular weight glutenin subunits (LMW-GS), are in their native state known to form large protein polymers connected by intermolecular disulphide bonds (Johansson, et al., 2013). Gliadins in their native state are mainly monomeric, having only intra-molecular disulphide bonds, but during processing are also contributing in various degrees in building the network (Johansson, et al., 2013). The formation of these large polymeric networks are known as the major determinants of pasta and bread-making quality of wheat flour (Shewry, et al., 2003; Martinez, et al., 2004; Johansson, et al., 2013).

Due to a lack of information on protein quality and polymerization behavior in the Ethiopian germplasm, the objective of this study was to assess variation in amount and size distribution of polymeric and monomeric protein as well as of protein composition determined by SE-HPLC, RP-HPLC and SDS-PAGE in Ethiopian tetraploid durum wheat landraces accessions. Further, pheno-quality assessments were carried out on the wheat accessions to understand relationships between protein and pheno-quality parameters.

MATERIALS AND METHODS

Plant materials:

Forty-nine landraces of tetraploid wheat were obtained from Ethiopian Biodiversity Institute and grown on farmland in Dessie Zuria and Kutaber districts and used in the study (Table 1). The wheat varieties were sown in the 2009, 2012, 2015 and in 2017 main cropping season. All samples collected in different year were used for further analysis.

Experimental design and pheno-quality evaluation:

For the field experiment a simple lattice square with eight replications were used. Each variety was sown in 1.5 m^2 (1.5 m × 1 m) plots. Each plot contained four rows with inter-row spacing of 20 cm. The distance between replications was 1m. The layout and randomization were done as per the standard procedure set by Cochran and Cox (1957). Standard management practices were exercised, seed rate was adjusted on the basis of 150 kg ha-1. The pheno-quality classes of the traits were recorded as recommended by Hailu, et al. (2010).

Analysis of HMW and LMW glutenin subunits:

The gliadin and glutenin subunits were extracted from individually ground grains and the proteins were separated according to the method of Hailu, et al. (2006) on 10% polyacrylamide gels in the presence of sodium dodecyl sulphate (SDS–PAGE). Then, in order to classify the gluten

Table 1. Plant materials used for the study and their allelic composition at Glu-A1, Glu-B1, Glu-A3, Glu-B3, and Glu-B2 for 50 accessions in terms of region of origin

		HMW glutenin subunits			LMW glutenin subunits			
Accession number	**Region of origin**	**Hm/Ht**	**Glu-A1**	**Glu-B1**	**Hm/Ht**	**Glu-A3**	**Glu-B3**	**Glu-B2**
5487	Gojam	Ht	0	7+8/20	Hm	a	g	b
5546	Gojam	Hm	2****	20	Ht	a/e	c/i	b
5632	Tigray	Hm	0	7+8	Hm	a	h	a
5634	Shewa	Hm	0	20	Hm	a	g	b
5668	Shewa	Hm	2****	7+8	Ht	b	b/g	b
5716	Gonder	Hm	2****	7+8	Ht	a	b/g	a/b
5982	Shewa	Hm	2****	7+8	Hm	b	i	b
6212	Shewa	Hm	0	7+8	Ht	a/b	a/f	a/b
6842	Gonder	Ht	2****/0	7+8	Hm	a	f	b
6980	Gojam	Ht	0	7+8/20	Hm	a	g	a
7119	Shewa	Ht	2****	7+8/20	Hm	b	a	b
7282	Gonder	Hm	0	20	Hm	a	i	b
7302	Gonder	Hm	2****	7+8	Hm	a	g	b
7362	Wollo	Ht	2****	7+8/20	Ht	a/e	e/g	a/b
7370	Wollo	Hm	2****	7+8	Hm	b	i	b
7472	Wollo	Hm	0	7+8	Ht	a/e	b/g	a/b
7503	Wollo	Ht	0	7+8/20	Ht	a	a/g	a/b
7927	Shewa	Hm	0	20	Hm	a	a	a
7943	Tigray	Ht	0	7+8/20	Hm	a	a	b
7955	Tigray	Hm	0	7+8	Hm	a	a	b
8241	Wollo	Ht	2****/0	7+8	Ht	a/e	e/g	a/b
8356	Gojam	Hm	2****	7+8	Hm	a	g	b
203760	Gojam	Hm	2****	20	Hm	a	g	b
203922	Tigray	Hm	0	20	Hm	a	c	b
203942	Shewa	Hm	0	20	Hm	a	g	a
203958	Shewa	Hm	2****	7+8	Hm	a	g	b
204378	Gojam	Ht	0	7+8/20	Ht	a	g	a/b
		Ht	2****/0	7+8/14+1	Ht	a/b	f/g	a/b
208127	Gojam			5/20				
208212	Gojam	Hm	2****	7+8	Ht	a	b/d	b
212649	Wollo	Hm	0	14+15	Hm	a	g	b
212652	Wollo	Ht	0	7+8/20	Hm	a	g	a
214263	Gonder	Hm	0	20	Hm	b	i	b
214508	Wollo	Ht	2****/0	7+8/20	Ht	a/e	b/g	a/b
214512	Gojam	Hm	2****	7+8	Hm	a	g	b
214588	Wollo	Hm	2****	20	Hm	a	g	b
222196	Shewa	Hm	0	7+8	Hm	a	g	b
222503	Gonder	Ht	2****/0	7+8	Ht	a/e	g	a/b
222533	Gonder	Ht	2****	7+8/20	Ht	a	b/f	b
222627	Gonder	Hm	2****	20	Ht	a/e	b/g	a/b
222704	Wollo	Hm	2****	20	Ht	a	f/g	b
226119	Gonder	Ht	0	7+8/20	Hm	a	g	b
226198	Tigray	Hm	0	20	Hm	a	g	a
226207	Gonder	Hm	0	7+8	Hm	a	g	b
226233	Tigray	Hm	0	7+8	Hm	a	g	a
231558	Shewa	Hm	0	7+8	Hm	a	g	b
231620	Gojam	Hm	0	7+8	Hm	b	g	a
238135	Tigray	Ht	2****/0	7+8/20	Ht	a	b/g	b
238136	Tigray	Ht	2****/0	7+8/20	Hm	a	b	b
238137	Tigray	Hm	2****	20	Hm	g	b	b

Hm-homogenous; Ht-heterogenous).

composition, the gels were stained with Coomassie Brilliant Blue R-250 solution at least overnight according to Johansson, et al. (1993) and de-stained in 8% (w/v) trichloroacetic acid (TCA) for a day and finally the nomenclature or designations of Nieto-Taladriz, et al. (1997) was used for the

HMW and LMW glutenin subunits, respectively. A total of 11 individual seeds were evaluated for protein composition for each of the accessions.

Preparation of flour:

An IKA A10 experimental mill was used to mill tetraploid wheat at a maximum speed of 20,000 rpm. The whole wheat flour was then used for protein extraction and analysis by High Performance Liquid Chromatography (HPLC).

SE-HPLC:

The SE-HPLC analyses carried out basically followed the methods described in Labuschagne, et al. (2004). Accordingly, HPLC analyses were performed on a Shimadzu LC-20AT system, with a UV/VIS photodiode array detector, on-line degasser and column oven CTO-10AS VP. For SE-HPLC the system was equipped with a BIOSEP SEC-4000 column and separation was achieved in 15 min by loading 20 µl of sample into an eluant of 50% (v/v) acetonitrile and water containing 0.1% (v/v) trifluoroacetic acid (TFA) at a flow rate of 0.4 ml/min. Proteins were detected by UV absorbance at 210 nm. Areas of different peaks were calculated with the chromatography data system class-VP 6.14 SP1 software. Relative amount of total SDS-extractable protein (TOTE) and total SDS-unextractable protein (TOTU) was calculated directly from the total area under each of the obtained chromatograms. The protein fraction separated by SE-HPLC before and after sonication was further divided into four peaks in which the first represents the large polymeric protein (LPP), the second small polymeric protein (SPP), the third represents large monomeric protein (LMP) and the last one small monomeric protein (SMP). Polymeric protein (PP) was calculated from each of the two polymeric protein areas as was the monomeric protein (MP) from the two monomeric protein areas according to Johansson, et al. (2008). The percentage of total unextractable polymeric protein in the total polymeric protein and the percentage of large unextractable polymeric protein in the total large polymeric protein were calculated according to Gupta, et al. (1993) and Johansson, et al. (2008). Samples were prepared and analyzed in duplicates.

RP-HPLC:

The extraction procedure of proteins for RP-HPLC analyses as used by Hailu, et al (2016) was used with some modification. In this procedure, the proteins were extracted stepwise to isolate gliadins (Gli) and glutenin (Glu) subunits as described in Hailu, et al. (2016). Samples were prepared and analyzed in duplicates.

Statistical Analyses:

The SAS software program (SAS, 2004) was used to evaluate the variation as well as the relationships for pheno-quality characters and the composition of grain protein parameters from HPLC data. Spearman rank correlation analyses, analyses of variance followed by mean value calculations and separation of means using the Duncan post hoc analysis was applied as was also principal component analysis (PCA).

RESULTS

Pheno-quality:

Large variation was obtained among both region of origin of the samples as well as among accessions for all pheno-quality characters evaluated. The accessions, in the present study, showed considerable variation in yield related parameters although they were of different origin but grown in the same environment in the farmers' field in Dessie Zuria and Kutaber districts.

Specific protein composition:

A large variation was noted in the allelic composition at the Glu-A1, Glu-B1, Glu-A3, Glu-B3 and Glu-B2 loci of the tetraploid wheat landraces analyzed (Table 1). The 2**** subunit from Glu-A1 was commonly found, i.e. 18 accessions were found with the 2**** subunit and seven accessions were heterozygous for the 2****. However, the null allele was the most commonly found Glu-A1 encoded allele present in 24 accessions, but was also present in the seven heterozygous samples (Table 1). The most frequent subunits at Glu-B1 were 7+8 present homogenously in 42.9% of the accessions and heterogeneously in 28.6%, followed by the subunit 20 in the rest of the accessions and the least common Glu-B1 encoded subunits were 14+15 in only two accessions. A total of 15 alleles of LMW glutenin subunits were found in the material, and of these, four, nine and two alleles were encoded by the Glu-A3, Glu-B3 and Glu-B2 loci, respectively.

Amount and size distribution of polymeric protein by SE-HPLC:

All protein factors measured and evaluated by SE-HPLC were found to vary significantly among both region of origin of the samples as well as among accessions (Table 2). A large variation in amount of various protein factors, e.g., TOTE, TOTU and %UPP were found among the accessions but also in relation to regions of origin (Table 3). Accessions originating from the evaluated wheat cultivation areas of this study also showed a broad variation in amount of various protein factors. In general, material originating from Wollo and Gondor showed the highest levels of both TOTE and %UPP in this study (Table 3).

Table 2. Mean Square from ANOVA of SDS-extractable and SDS-unextractable proteins and %UPP obtained from peak areas of SE-HPLC chromatograms.

Source	Degrees of freedom	SDS-extractable (10^{13})			SDS-unextractable (10^{13})			%UPP
		PP	MP	TOTE	PP	MP	TOTU	
Accessions	48	0.59***	16.62***	18.91***	0.25***	0.70***	1.56***	82.59***
Region of Origin	4	1.49 *	80.83 **	85.78 **	1.33 ***	3.43 ***	8.5 ***	253.4 *
Error	192	0.517	10.78	12.83	0.16	0.46	0.93	67.06

Polymeric protein (PP), monomeric protein (MP), total SDS-extractable protein (TOTE), total SDS-unextractable protein (TOTU), percentage of unextractable polymeric protein in the total polymeric protein (%UPP)

*, **, *** significant at $p<0.05$, $p<0.01$ and $p<0.001$ level, respectively.

Table 3. Ranges of content of different protein fractions from SE-HPLC analyses in Ethiopian durum wheat landraces (this study) and in released varieties of durum wheat (originating from Ethiopia and Spain but cultivated in Ethiopia; Hailu et al., 2016) and in the landraces of different origin.

Source	TOTE (10^6)	TOTU (10^6)	%UPP
Landraces	29-91	6-23	10-54
Released varieties	60-82	18-39	32-50
Origin			
Gojam	34-67	7-17	20-38
Tigray	34-59	6-14	10-51
Shewa	34-51	7-22	21-38
Gonder	29-84	7-23	25-54
Wollo	34-82	7-18	23-54

Total SDS-extractable protein (TOTE), total SDS-unextractable protein (TOTU), percentage of unextractable polymeric protein in the total polymeric protein (%UPP).

Quantification of Protein Fraction by RP-HPLC:

A total of 34 gliadin peaks and 38 glutenin peaks were differentiated by RP-HPLC. Presence and absence of peaks varied among the accessions as did the number of various peaks. Previous studies have shown significant positive or negative correlation between some of the specific peaks detected by RP-HPLC and wheat quality, and thereby proven this method to be a highly efficient tool for qualitative and quantitative studies of wheat gluten proteins.

PCA based on the RP-HPLC data in the present study resulted in the first principal component explaining 18.1% of the variation and the second principal component explaining 12.2% of the variation. The released varieties were differentiated by the PCA with the generally more positive values on the first principal components (mean PCA1 of all released varieties=5.13) as compared to the landraces of the present study (Fig. 1). The accessions from Tigray, Wollo and Gonder showed the most negative values on the first principal component (mean -2.55, -1.72 and -1.62), respectively. Clustering the accessions from different regions based on the genetic distance variation also showed a proximity based trend, implying material exchange more among neighboring regions than among those further apart, thereby clustering material from Tigray, Wollo and Gonder together.

Relationships among pheno-quality data and protein parameters:

Highly positive Pearson's correlation coefficients ($p \leq 0.01$) was observed among several of the pheno-quality parameters, including among plant height (PH), awn length (AL), glume colour (GC), awn colour (AC), spike length (SL), and kernel length (KL; Table 4). Furthermore, highly negative correlation coefficients ($P<0.01$) were found with these mentioned pheno-quality parameters and lower glume shoulder width (LGSW) and shape (LGSS), spike density (SD), beak shape (BS; Table 4).

The first two components from the principal component analyses based on pheno-quality parameters and protein parameters by SE-HPLC, explained 24.2% and 16.0% of the variation, respectively. The results of the principal component analyses corresponded well with the Pearson correlation analyses, collecting PH, GC, AC, AL, SL and KL with positive values on the first principal component (PC1) indicating their

Table 4. Pearson correlation coefficients among different quality parameters of tetraploid wheat accessions

	DH	DM	PH	LGSW	LGSS	GC	GH	AC	AL	SD	SL	BS	KL	KC	SN	TOTE	TOTU
DM	.639**																
PH	.095	-.071															
LGSW	-.027	.047	-.325*														
LGSS	-.177	-.105	-.410**	.528**													
GC	.081	.090	.144	-.329*	-.289*												
GH	-.129	-.018	-.078	.045	.045	-.125											
AC	.032	.139	.291*	-.307*	-.358*	.193	-.291*										
AL	.185	.068	.345*	-.567**	-.843**	.471**	-.139	.389**									
SD	.060	.056	-.260	.160	.591**	.020	-.084	-.115	-.418**								
SL	.219	.088	.489**	-.281	-.679**	.108	-.107	.149	.692**	-.527**							
BS	-.194	-.081	-.001	.334*	.611**	-.386**	.372**	-.254	-.723**	.321*	-.408**						
KL	.194	.125	.671**	-.391**	-.684**	.201	-.009	.387**	.575**	-.372**	.654**	-.273					
KC	-.196	-.196	-.167	.038	.025	-.193	.056	.028	.028	-.198	.146	-.003	-.188				
SN	-.063	-.161	-.082	.131	.139	-.067	-.188	-.262	-.266	.052	-.179	.149	-.217	-.085			
TOTE	.236	.249	.166	.137	-.141	.062	-.078	.153	.179	.003	.122	-.180	.163	-.178	-.285*		
TOTU	.462**	.161	-.171	.237	.183	-.005	.038	-.106	-.017	.110	-.029	.137	-.153	-.150	.091	.032	
%UPP	.062	-.175	-.034	.095	.209	-.107	.138	-.254	-.150	.094	-.013	.339*	-.082	-.119	.263	-.434**	.556**

Days to heading (DH), days to maturity (DM), plant height (PH), lower glume shoulder width (LGSW), lower glume shoulder shape (LGSS), glume colour (GC), glume hairiness (GH), awn color (AC), awn length (AL), spike density (SD), spike length (SL), beak shape (BS), kernel length (KL), kernel colour (KC), seed texture (SN), total SDS-extractable protein (TOTE), total SDS-unextractable protein (TOTU), percent of unextractable polymeric protein in the total polymeric protein (%UPP),

*, **, significant at $p<0.05$, and $p< 0.01$, respectively.

positive relationships, while LGSW, LGSS, SD and BS were assembled with negative values on PC1, showing a positive relation with each other but a negative relation with the previously mentioned factors. DH, DM and TOTE all showed the highest positive values on PC2, indicating their positive relationship (Fig 2).

DISCUSSION

With regard to specific protein composition similarly as in previous investigations on Ethiopian wheat (Hailu, et al., 2006), the 2**** subunit from Glu-A1 was commonly found. Thus, the HMW-GS composition in the present study corresponded well with variation in HMW-GS in Ethiopian wheat reported previously (Hailu, et al., 2006). As the storage proteins are highly polymorphic and a large variation is present, their composition is a useful tool to estimate variation among wheat accessions. Previous studies on durum wheat from other countries have indicated a similar amount of variation (Nieto-Taladriz, et al., 1997) as was found in the Ethiopian landraces evaluated here. However, both a different composition of alleles and different frequencies of alleles have been reported in other studies (Nieto-Taladriz, et al., 1997; Igrejas, et al., 1999) as compared to what was found in the Ethiopian landraces. Generally, a larger variation was seen in the composition of LMW-GS in the present wheat material as compared to what was reported in wheat materials of other origin, which also corresponds to previous investigations on Ethiopian wheat (Hailu, et al., 2006). Certain LMW-GS alleles have in previous studies been correlated to pasta quality (Martinez et al 2004). The landraces of this study were not found homogenous in their composition of storage proteins, which may contribute variation in the quality performance of a wheat material (Husenov, et al., 2015).

The previous study on amount and size distribution of polymeric protein by SE-HPLC has clearly shown the correlation between both TOTE and TOTU with grain protein content (Malik, et al., 2011). Specifically, TOTE is correlated with grain protein concentration in wheat (Malik, et al., 2011), while for barley, the correlation between TOTU and grain protein concentration has been found more pronounced (Holm, et al., 2018). The landraces evaluated in the present study were found to have a broader range of TOTE as compared to Ethiopian and Spanish varieties cultivated in Ethiopia (Hailu, et al., 2016). This indicates that lines with higher as well as lower grain protein concentration may be found among the landraces as compared to currently grown varieties for pasta production in Ethiopia. Grain protein concentration is well known to impact the bread volume of wheat (Finney & Barmore, 1948; Johansson, et al., 2013) and is also known to impact pasta quality (Johansson et al., 2013). Thus, despite low grain protein concentration being more common feature in the landraces evaluated here, some of the accessions showed high levels and should therefore be evaluated for use in breeding for high grain protein concentration varieties.

The %UPP has in previous investigations been related to gluten strength in wheat (Malik, et al., 2013) although %UPP during mixing has also been found to change independently as related to content in the mature grain (Hussain, et al., 2012). A high %UPP is an important character for formation of a suitable network during the pasta drying process to produce pasta of good quality (Bruneel, et al., 2010). Similarly, as for TOTE, a large variation was found for %UPP in the tetraploid wheat accessions in the present investigation. In particular accessions with low %UPP were found among the landraces although also accessions with higher %UPP than the variety with the highest value on this character.

With respect to quantification of protein, similar findings have been reported also in previous studies (Hailu, et al., 2005; Hailu, et al., 2016). However, in the present study, the PCA was able to differentiate the landraces from Tigray by the second principal component (mean of 3.12), with two accessions showing PC2 values above the limits of 4, accessions from the other regions and the released varieties all having lower values (means close to zero for varieties and below zero for accessions from other regions).

The previous studies on Ethiopian tetraploid wheat have shown correlations among several pheno-quality parameters (Bechere, et al., 2002; Hailu & Merker, 2008), similar to the present study. A couple of previous studies have indicated the effect of plant development on amount and size distribution of polymeric and monomeric proteins in wheat (Malik, et al., 2013). Those studies have primarily identified a positive significant correlation between delayed maturation time in wheat with increased green biomass accumulation and thereby to increased starch accumulation with a decrease in grain protein concentration (significantly positively correlating primarily to TOTE) as a result (Malik, et al., 2013). The results of the mentioned studies are primarily on hexaploid wheat grown in a Nordic European context. Results on barley show primarily a positive correlation among TOTU and grain protein concentration (Holm, et al., 2018). In the present study, days to heading (DH), as a measurement of plant development time, was significantly and positively correlated ($p<0.01$) with TOTU, indicating a relationship between plant development and grain protein concentration in terms of unextractable type. Furthermore, a positive correlation ($p<0.01$) was found between days to heading (DH) and dry matter (DM), indicating a similar reason of accumulation of biomass with a resulting higher starch accumulation in the grain as the main reason for the increase in TOTU. Also, a negative correlation was found between TOTE and %UPP, simultaneously as reported in previous studies (Malik, et al., 2013).

In conclusion a large diversity of quality related traits is present in Ethiopian landraces of durum wheat in some of the Ethiopian landraces of durum wheat show high grain protein concentration with high gluten strength, characteristic of high value for commercial use of the wheat for pasta production. Genetic diversity in general, as for e.g. phenotypic traits and specific protein composition, seemed to prevail with more resemblance between regions of Ethiopia that were closely situated, while such a pattern did not seem to prevail for protein quality (TOTE and %UPP) traits.

Acknowledgements:

The plant material was obtained from Ethiopian Institute of Biodiversity and the field and other experiments was supported by Wollo University Research and community service office and UD-40 project (an initiative from the Ministry of Foreign Affairs in Sweden administered through the Swedish University of Agricultural Sciences) for which we are very grateful.

Funding:

The Wollo University, Dessie, Ethiopia and Swedish University of Agricultural Sciences, Sweden financially supported this research for write up. The funders had no role in the study design, data collection and analysis, decisions to publish, interpretation of the data, and preparation of the manuscript for publication.

Data availability statement:

Data are available from the corresponding author on reasonable request.

Declaration:

Ethics approval and consent to participate:

Not applicable.

Consent for publication:

The authors have provided their consent for publication.

Competing interests:

The authors declare that they have no competing interests.

References:

Ali, S., Khan, G., Zia Kumar, B., Yousuf, M., Roomi, T., & Ahmad, M. F. (2013). Estimation of genetic diversity in genetic stocks of common wheat (*Triticum aestivum* L.) using SDS-PAGE. *European Academic Research*, *1*, 1861-1871.

Bechere, E., Pena-Bautista, R. J., & Mitiku, D. (2002). Gluten composition, quality characteristics, and agronomic attributes of durum wheat cultivars released in Ethiopia. *African Crop Science Journal*, *10*, 173–182.

Belay, G., Tesemma, T., Bechere, E., & Mitiku, D. (1995). Natural and human selection for purple-grain tetraploid wheats in the Ethiopian highlands. *Genetic Resources and Crop Evolution*, *42*, 387-391.

Bruneel, C., Pareyt, B., Brijs, K., & Delcour, J. A. (2010). The impact of the protein network on the pasting and cooking properties of dry pasta products. *Food Chemistry, 120*, 371–378.

Cochran, W. G., & Cox, M. (1957). Experimental Designs. John Wiley and Sons, Inc. New York.

FAO. (2017) Food and Agriculture Organization of the United Nations. FAOSTAT Database. Rome, Italy: FAO.

Finney, K. F., & Barmore, M. A. (1948). Loaf volume and protein content of hard winter and spring wheats. *Cereal Chemistry*, *25*, 291-312.

Gupta, R. B., Khan, K., & Macritchie, F. (1993). Biochemical basis of flour properties in bread Wheats I. Effects of variation in quantity and size distribution of polymeric protein. *Journal of Cereal Science*, *18*, 23-41.

Hailu, F., & Merker, A. (2008) Variation in gluten strength and yellow pigment in Ethiopian tetraploid wheat germplasm, *Genetic Resources and Crop Evolution*, *55*, 277–285.

Hailu, F., Merker, A., Belay, G., & Johansson, E. (2005). Molecular diversity and phylogenic relationships of tetraploid wheat species as revealed by inter-simple sequence repeats (ISSR) from Ethiopia. *Journal of Genetics and Breeding*, *59*, 329–338.

Hailu, F., Johansson, E., Merker, A., Belay, G., & Harjit-Singh, Z. H. (2006). Composition of and variation in high- and low molecular weight glutenin subunits, and omega gliadins in Ethiopian tetraploid wheat germplasm. *Plant Genetic Resources*, *4*, 134-143.

Hailu, F., Johansson, E., & Merker, A. (2010). Patterns of phenotypic diversity for phonologic and qualitative traits in Ethiopian tetraploid wheat germplasm. *Genetic Resources and Crop Evolution*, *57*, 781–790.

Hailu, F., Labuschagne, M., Van Biljon, A., Persson Hovmalm, H., & Johansson, E. (2016). Quality assessment with HPLC in released varieties of tetraplod (*Triticum durum* Desf.) wheat from Ethiopia and Spain, *Cereal Research Communications*, *44*, 617-627.

Holm, L., Malik, A. H., & Johansson, E. (2018). Optimizing yield and quality in malting barley by the governance of field cultivation conditions. *Journal of Cereal Science*, *82*, 230-242.

Husenov, B., Makhkamov, M., Garkava-Gustavsson, L., Muminjanov, H., & Johansson, E. (2015). Breeding for wheat quality to assure food security of a staple crop: The case study of Tajikistan, *Agriculture and Food Security*, *4*, 9.

Hussain, A., Larsson, H., Kuktaite, R., Prieto-Linde, M. L., & Johansson, E. (2012). Towards the understanding of bread-making quality in organically grown wheat: Dough mixing behavior, protein polymerization and structural properties. *Journal of Cereal Science*, *56*, 659-666.

Igrejas, G., Guedes-Pinto, H., Carnide, V., & Branlard, G. (1999). The high and low molecular weight glutenin subunits and ω-gliadin composition of bread and durum wheats commonly grown in Portugal. *Plant Breeding*, *118*, 297–302.

Johansson, E., Henriksson, P., Svensson, G., & Hennen, W. K. (1993). Detection, chromosomal location and evaluation of the functional value of a novel high Mr glutenin subunit found in Swedish wheats. *Journal of Cereal Science*, *17*, 237- 245.

Johansson, E., Prieto-Linde, M. L., & Gissen, C. (2008). Influences of weather, cultivar and fertiliser rate on grain protein polymer accumulation in field-grown winter wheat, and relations to grain water content and falling number, *Journal of the Science of Food and Agriculture*, *88*, 2011–2018.

Johansson, E., Malik, A. H., Hussain, A., Rasheed, F., Newson, W. R., Plivelic, T., Hedenqvist, M. S., Gällstedt, M., & Kuktaite, R. (2013). Wheat gluten polymer structures: The impact of genotype, environment and processing on their functionality in various applications. *Cereal Chemistry*, *90*, 367-376.

Kabbaj, H., Sall, A. T., Al-abdallat, A., Geleta, M., Amri, A., Filali-Maltouf, A., Belkadi, B., Ortiz, R., & Bassi, F. M. (2017). Genetic diversity within a global panel of durum wheat (*Triticum durum*) landraces and modern germplasm reveals the history of alleles exchange. *Frontiers in Plant Science*, *8*, 1277.

Labuschagne, M. T., Koen, E., & Dessalegn, T. (2004). Use of Size-Exclusion High-Performance Liquid Chromatography for wheat quality prediction in Ethiopia. *Cereal Chemistry*, *81*, 533-537.

Malik, A. H., Prieto-Linde, M. L., Kuktaite, R., Andersson, A., & Johansson, E. (2011). Individual and interactive effects of genetic background and environmental conditions on amount and size distribution of polymeric proteins in wheat grain. *Czech Journal of Genetics and Plant Breeding*, *47*, 5186-5189.

Malik, A. H., Kuktaite, R., & Johansson, E. (2013). Combined effect of genetic and environmental factors on the accumulation of proteins in the wheat grain and their relationship to bread-making quality. *Czech Journal of Genetics and Plant Breeding*, *57*, 170-174.

Martinez, M. C., Ruiz, M., & Carrillo, J. M. (2004). New low Mr glutenin subunit alleles at the Glu-A3, Glu-B2 and GluB3 loci and their relationship with gluten strength in durum wheat. *Journal of Cereal Science*, *40*, 101–107.

Murat, O., Imren, K., Ayter, N. G., Ozcan, Y., & Zekiye, B. (2013). Determination of genetic diversity in bread wheat (*T. aestivum* L.) by agronomic and quality traits and SDS-PAGE method. *Global Journal of Science Frontier Research*, *13*, 975-987.

Nieto-Taladriz, M. T., Ruiz, M., Martinez, M. C., Vazquez, J. F., & Carrillo, J. M. (1997). Variation and classification of B low molecular weight glutenin subunit alleles in durum wheat. *Theoretical and Applied Genetics*, *95*, 1115–1160.

Pecetti, L., Annicchiarico, P., & Damania, A. B. (1992). Biodiversity in a germplasm collection of durum wheat. *Euphytica*, *60*, 229-238.

Pena-Bautista, R. J., Hernandez-Espinosa, N., Jones, J. M., Guzman, C., & Braun, H. J. (2017). Wheat-based foods: Their global and regional importance in the food supply, nutrition and health. *Cereal Foods World*, *62*, 231-249.

SAS (2004). SAS/STAT TM Users guide. SAS Institute Inc., Cary, NC, USA

Siahbidi, M., Aboughadareh, A. G. T., Teymoori, M., & Jasemi, A. (2013). Evaluation of genetic diversity and interrelationships of agro-morphological characters in durum wheat (*Triticum Durum* Desf.) lines using multivariate analysis. *International Journal of Agricultural Research and Reviews*, *3*, 184-194.

Shewry, P. R., Halford, N. G, & Lafiandra, D. (2003). Genetics of wheat gluten proteins. *Advanced Genetics*, *49*, 111-184.

Takenaka, S., Mori, N., & Kawahara, T. (2010). Genetic variation in domesticated emmer wheat (*Triticum turgidum* L.) in and around Abyssinian Highlands. *Breeding Science*, *60*, 212-227.

Vavilov, N. I. (1992). Origin and geography of cultivated plants. The academy of sciences of the USSR. (Translated by D Löve), Cambride University Press. 498 pp.

Publisher's Note: