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Wheat Grain Protein Composition—A Screening Tool to Be Used in Plant Breeding for Improved Tajik Food Security

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Abstract: Non-satisfactory bread-making quality in wheat, a Tajik staple, hampers food security in Tajikistan and calls for plant breeding efforts. Here, methods were searched for to study grain protein composition, which is of use for Tajik plant breeding to improve bread-making quality. Size-exclusion high-performance liquid chromatography (SE-HPLC) was used to determine protein composition in 22 wheat varieties and breeding lines grown in two locations, which were then compared with the specific protein composition evaluated using electrophoresis and previous results from Tajik breeding and farmer-grown wheat. As Tajik wheat generally showed a large variation in high-molecular-weight glutenin subunit (HMW-GS) composition, with several allelic variants in the same line, single-seed selection was required when using this methodology in breeding for improved bread-making quality, and such an evaluation willalso result in more homogenous lines for protein composition. SE-HPLC was found to be a suitable tool to evaluate protein composition in the current Tajik wheat material with a heterogeneous protein composition, which might be advantageous for adaptation to the local and future climate. However, more easy-to-handle and high-throughput methods, e.g., marker-assisted selection, could be preferable alternatives for studying protein composition in wheat and for use in breeding for increased bread-making quality to increase food security in Tajikistan.

Keywords: bread; gluten strength; HMW-GS; SE-HPLC; wheat

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1. Introduction

Plant breeding, i.e., changing traits to produce plants that benefit humanity [1,2], has been adopted in most crops but with higher intensity in the major ones [3,4]. Wheat is one of the three major crops around the globe (together with maize and rice), which is produced in a wide array of environments and used for a variety of products including bread, pasta, noodles, etc. [5]. A major goal of plant breeding is to increase yield, resulting in food security for the growing human population and a higher economic return to the producer [4,6], and therefore, obtaining resistance to major diseases has been a target for securing harvests [7]. However, in wheat, bread-making quality has also been a clear target as bread is a major product from wheat [8]. Bread-making quality is globally one of the most important and required quality parameters of bread wheat, although this parameter is highly dependent on the end use of wheat and the processing technology used to obtain the wheat product [9]. Furthermore, the bread-making quality of wheat flour is largely dependent on the wheat genotype and growing conditions [10,11].

The bread-making quality of wheat is determined by a wide array of techniques, including baking tests (standardized or specific to local conditions), physicochemical tests (e.g., by alveograph, mixograph, extensograph, mixolab, etc.) and component analyses (e.g., of protein content and composition) [12–15]. The latter (component analyses) are advantageous in breeding programs due to the fact that these methods require a small amount of samples (the determination of the composition of storage proteins using electrophoresis can even be carried out on half grains, and then the germ of the selected grains can be utilized for plant propagation and crossings, as shown in previous studies [16]), which results in opportunities for selection in early generations. The specific composition of storage proteins, and the amount and size distribution of polymeric proteins are known as two traits that require a small sample size for their analyses and they have strong correlations to bread-making quality [11,12,17]. Of these traits, the specific composition of wheat storage proteins (gluten proteins) is a genetically determined trait, although these proteins are known to be among the most complex types of proteins, due to their different components, sizes and variation [11–15,18]. Among the specific gluten proteins, high-molecular-weight glutenin subunits (HMW-GSs) have been shown to have the highest impact on bread-making quality [10,11]. The amount and size distribution of monomeric and polymeric proteins are known to be genetically determined although they are also impacted by the environment and genotype x environment interactions [10,11]. Thus, the specific protein composition, e.g., HMW-GS, influences the amount and size distribution of monomeric and polymeric proteins, but also, factors such as cultivar determined development time, soil conditions and the temperature of the growing environment have been found to have an impact on the amount and size distribution of monomeric and polymeric proteins [10].

In Central Asian countries, including Tajikistan, the consumption of wheat products is clearly above the global average, making common bread wheat an extremely important crop for their populations [19,20]. The products of wheat constitute the staple food in Tajikistan and other countries in the region and contribute significantly to the food consumed daily for the majority of people [19,21]. Thus, food security in Tajikistan and also in other countries in the region is highly dependent on wheat and specifically on their local production [22]. Tajikistan is a country with a high degree of undernourishment among its population, and changes targeting hunger are slow [23]. According to the World Food Program, 30% of the Tajik population was classified as moderately food-insecure by the end of 2022, and 18% of the children in Tajikistan are stunted, which is the highest rate in the Central Asian and European region [24]. The current wheat production in Tajikistan is facing two major problems: low yield and poor baking quality [19,23]. Thus, to secure food for the population in this region, breeding for an increased yield and improved baking quality of Tajik common bread wheat should be a major target. According to the National Development Strategy 2030 of the Government in Tajikistan, the strengthening of food security and giving agriculture a more productive, commercial and sustainable footing are major goals [24]. Increasing wheat yield has been a goal within the breeding programs of Tajikistan and the surrounding region in recent years [19]. In particular, combatting the high prevalence of weeds, ending the practice of using non-certified seeds and improving the knowledge of farmers have been suggested as measurable improvement methods needed to increase yield [19]. Furthermore, breeding for resistant genotypes to prevailing diseases and pests is the target within the breeding programs to increase yield performance [23]. The poor baking quality of Tajik wheat has rarely been targeted in breeding programs [23]. However, a recent study showed that most of the currently grown cultivars and breeding lines contain a high proportion of HMW-GS 5+10, known to contribute to high gluten strength, while the same study showed heterogeneity in Tajik wheat when it comes to HMW-GS composition [23]. Studies focusing on understanding the limitations and opportunities of the utilization of the wheat grain protein content and composition to improve the bread-making quality of Central Asian and, in particular, Tajik wheat have until now been scarce.

The aim of the present study was to evaluate the opportunities and challenges of the use of various analytical methods to evaluate the protein content and composition of wheat to reveal suitable methods to be used in plant breeding to improve bread-making quality in a developing country such as Tajikistan. Measurements of protein composition were carried out on Tajik wheat, and the results were used as an example of how wheat breeding can be carried out to improve baking quality parameters in developing countries in the regions of Central Asia and North Africa.

2. Materials and Methods

2.1. Plant Material

The results from two previous studies [19,22] based on farmer-grown wheat on 21 production farms and 210 wheat fields over two and three years, respectively, were used as a baseline in the present study. Additionally, a plant material consisting of 22 different bread wheat (*Triticum aestivum* L.) varieties/lines, selected from the national wheat breeding program of Tajikistan [23], previously investigated for the specific protein composition [23], was evaluated here for its amount and size distribution in order to understand opportunities and challenges with different analytical methodologies in breeding for improved bread-making quality. These varieties/lines were grown in 2009, in multi-location yield trials for the Tajik national wheat breeding program, and the lines used were selected as these were the ones present in the last step of selection before the lines are going to official variety testing, meaning that they should be in their final stage of plant breeding. The lines were grown in two geographical locations of Tajikistan. One location is situated in the central–western part of the country in Hisor Valley (38°31′16 N; 68°34′21 E, 788 masl), while the second location is situated in the north-eastern part of the country in the Isfara district (40°09′ N; 70°43′ E, 822 masl). The distance between the two locations is 400 km. The samples of the varieties/lines were harvested at maturity, and a representative collection of 500 g grains per sample was sent to Sweden for analyses. Upon arrival in Sweden, the samples were stored in a seed storage room at 4 °C until analyses were carried out (SDS-PAGE in 2010 and SE-HPLC in 2011).

2.2. Experimental Methods

2.2.1. Size-Exclusion High-Performance Liquid Chromatography (SE-HPLC)

Protein composition (amount and size distribution of monomeric and polymeric proteins) was analysed in triplicate samples from each of the 22 varieties/lines from each location by size-exclusion high-performance liquid chromatography (SE-HPLC), basically following the two-step extraction method developed by Gupta et al. [25] with slight modifications [26]. All chemicals were of analytical grade. Water was purified by a Milli-Q system (Millipore Corporation, Billerica, MA, USA). HPLC-grade acetonitrile and dithiothreitol (DTT) were acquired from VWR BDH Prolabo (VWR Chemicals, Sweden). To obtain the samples, grains from each variety/line were milled using a laboratory analytical mill (Yellow line, A10, IKA-Werke, Staufen, Germany). Flour was thereafter transferred to a −80 °C ultrafreezer and lyophilized (Cool SafeTM, Scanvae, Denmark), and 16.5 $\pm$ 0.05 mg flour was weighed into Eppendorf tubes, to which 1.4 mL of sodium dodecyl sulphate (SDS) phosphate buffer (0.5% SDS, 0.05 M NaH_2PO_4, pH 6.9) was added. The samples were vortexed on a Whirli VIB2 (Labassco, Sweden for 10 s, shaken for 5 min at 2000 rpm on a IKA-VIBRAX VXR (IKA, Germany) and centrifuged for 30 min at 10,000× *g* on a Sorvall, Legend Micro 17 (Thermo Scientific, Sweden) to obtain the supernatant protein. The supernatant protein was transferred to a vial for further SE-HPLC analyses. For the second extraction, 1.4 mL of the same extraction buffer was added to the pellet, and the sample was thereafter sonicated for 30 s at an amplitude of 5 using a Soniprep 150 (Tamro, Sweden). The samples were centrifuged for 30 min at 10,000× *g* on a Sorvall, Legend Micro 17 (Thermo Scientific, Sweden), and the obtained supernatant was transferred to a vial for

SE-HPLC analyses. The SE-HPLC separation of the proteins was conducted using a Waters 2690 Separation Module (Waters, Milford, USA) with a Waters 996 Photodiode Array Detector (Waters, Milford, USA). Separation was carried out for 30 min by injecting 20 µL into a Biosep-SEC-S4000 Peak Phenomenex Column (Phenomenex, Califirnia, USA) with an eluent of 50% acetonitrile, 0.1% trifluoroacetic acid (TFA) and 50% H_2O—Millipore water, at isocratic flow at a rate of 0.2 mL/min. The proteins were detected through UV absorbance at 210 nm, and the data of the runs were extracted using Empower Pro (Waters, Milford, USA). Depending on molecular size, the SE-HPLC chromatograms were integrated into four arbitrary sections: large polymeric proteins (LPPs), small polymeric proteins (SPPs), large monomeric proteins (LMPs) and small monomeric proteins (SMPs). Following previously developed methodologies, the total SDS-extractable proteins (TOTE) were calculated as TOTE = eLPP + eSPP + eLMP + eSMP (e = SDS-extractable), and the total SDS-unextractable proteins (TOTU) were calculated as TOTU = uLPP + uSPP + uLMP + uSMP (u = SDS-unextractable). Furthermore, the percentages of the total unextractable polymeric proteins in the total polymeric proteins (%UPP) and the percentages of large unextractable polymeric proteins in the total large polymeric proteins (%LargeUPP) were calculated [26].

2.2.2. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The analyses of the specific HMW-GS composition of these wheat varieties/lines were previously described in Husenov et al. [23] and were carried out utilizing 10% sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE), following a methodology previously described by Payne et al. [27–29]. Thus, the total wheat proteins were extracted from eleven individually crushed grains from each wheat sample using a 0.5 mL extraction buffer (0.06 M Tris, 2% SDS, 2% DTT, pyronin). The extracted samples were kept in a refrigerator before incubation at 90–95 °C for 5 min just prior to the loading of the samples on the gel. The gels were stained with Coomassie brilliant blue R250 solution (0.2%, *w*/*v* Coomassie brilliant blue R-250, 10% *v*/*v* ethanol and 8%, *w*/*v* trichloroacetic acid) for 24 h and destained with distilled water. After destaining, the gels were scanned using an Epson Perfection V200 Photo (Epson Co, Japan). The presence of protein subunits was evaluated according to the description of Payne and Lawrence [30].

2.3. Statistical Analyses

An evaluation of the data was carried out using the statistical package SAS (2004) [31] and MS Excel. Spearman rank correlation analyses were carried out in order to evaluate the relationships between different protein factors. Analysis of variance (ANOVA) followed by Duncan's post hoc test were carried out in order to understand the influences of the genotypes of varieties/lines and locality on the amount and size distribution of proteins, and to evaluate differences in protein amount and size distribution for various varieties/lines and localities. Principal component analysis (PCA) were carried out to understand the variation in the amount and size distribution of monomeric and polymeric protein between locations. Also, a comparison of differences in TOTE and %UPP between the two locations, and a comparison of %UPP and the specific protein composition were conducted to understand whether varieties/lines varied similarly in both environments.

3. Results and Discussion

3.1. Plant Breeding to Improve Bread-Making Quality of Tajik Wheat

Despite the unsatisfactory bread-making quality of Tajik wheat, focusing on plant breeding to improve this characteristic has been limited [23]. Plant breeding is a useful tool for changing characteristics in crops, not least being verified by the 74% yield increase it has contributed to crops grown in the Western world since the millennium shift [32]. Due to the importance of bread-making quality in wheat, obtaining improvement in this characteristic has been an important target in plant breeding programs across the globe

[10]. This has also resulted in substantial improvements for this trait in wheat grown in major bread wheat production areas [33,34].

In Tajik common bread wheat, the strength of thegluten is generally too low to suit the traditional bread-making procedure, utilizing clay-made ovens in which bread dough is pasted (Figure 1) [23]. Additionally, improvements in the bread-making quality of Tajik wheat have shown limited progress, e.g., recently released varieties from the Tajik wheat breeding program do not have better quality than the standard varieties of Tajikistan [22]. Thus, to increase the food security of Tajikistan, the use of plant breeding to improve the bread-making quality of wheat is important. Protein content and composition are well known as major contributors with a significant impact on the gluten strength of wheat dough [11,17,35]. Thereby, changes in the protein content and composition of Tajik wheat can contribute to the strengthening of gluten in wheat dough. Various methods can be utilized in breeding to change the protein composition of wheat, including, e.g., screening using SDS-PAGE, SE-HPLC and markers. The results of the present study indicate the benefits and challenges of using SDS-PAGE and SE-HPLC and also suggest that markers connected to the amount and size distribution of polymeric and monomeric proteins should be developed.

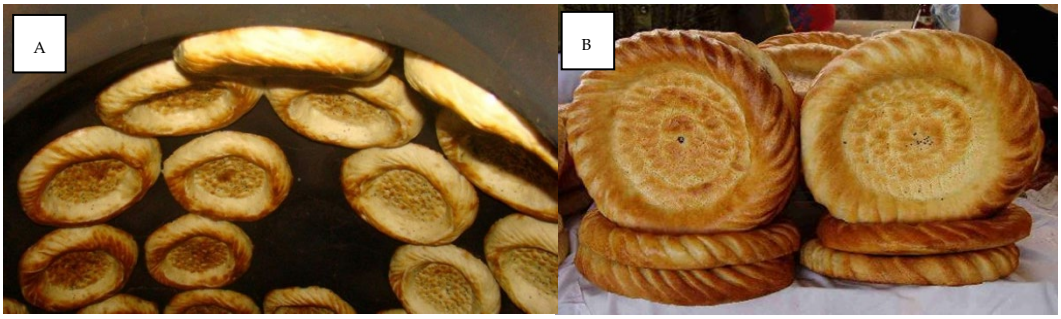

Figure 1. Traditional Tajik bread-making (**A**) in clay oven and (**B**) final bread product.

Despite the fact that starch and protein accumulation in wheat grain are known as separate events governed by specific genes [36], a negative relationship between these two characteristics has been verified in many studies [37]. Furthermore, a negative correlation has been reported in several studies between grain protein content and gluten strength [17]. Tajik wheat diverges from these results, as it generally has a low yield and relatively low protein content and gluten strength [22]. Thus, a better understanding of how to change the protein content and composition of Tajik wheat is important for improving the bread-making quality of this wheat through breeding.

3.2. Protein Composition as a Breeding Tool for Increased Food Security for the Tajik Population

The specific composition of grain proteins, e.g., HMW-GS, has been used in breeding programs to improve the gluten strength and thereby the bread-making quality of wheat [12,38,39]. However, most Tajik wheat contains a high proportion of HMW-GS 5+10, and the effect of HMW-GSs on gluten strength is limited [23]. In addition, Tajik wheat was found to be highly heterogeneous for the HMW-GS composition [23]. Thus, Navruz and Huavun Inia give the impression in Figure 2 of being homogenous, while CMNN82A.1294/2*Kauz// shows a highly heterogeneous pattern. However, a total of 44 grains per variety/line were analysed by SDS-PAGE [23], resulting in all of them being heterogeneous to various degrees, with the exception of Eskina-8 [23]. Still, the SDS-PAGE of the grain protein composition of Tajik wheat may be used as an efficient method of breeding for bread-making quality, as selection can be conducted on a single seed (Figure 2).

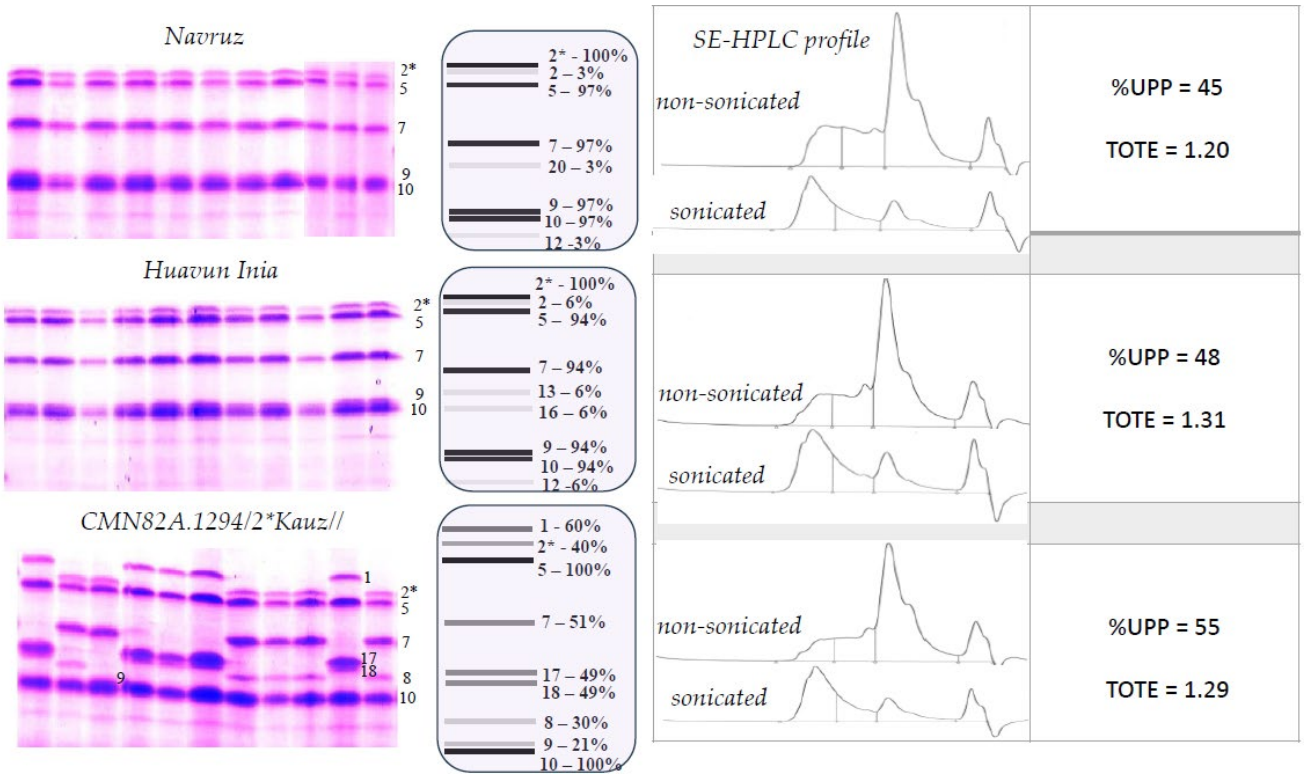

Figure 2. Examples of the protein composition of Tajik wheat. The SDS-PAGE figures show the analyses of 11 grains of three varieties/lines, where high-molecular-weight glutenin subunits are indicated by numbers. The images show the protein compositions that would have been obtained if the flour of the three varieties/lines had been analysed by SDS-PAGE. This would have resulted in some of the bands being strong and others being faint as a result of the relative presence of proteins, as indicated with percentages. The representative HPLC chromatograms of the same varieties/lines show their profiles of non-sonicated and sonicated proteins with their calculated %UPP and TOTE values. TOTE is the relative area under the chromatogram of the non-sonicated sample, and %UPP is the relationship between the polymeric proteins in the sonicated and non-sonicated chromatograms (see Materials and Methods for an additional description).

Half of the grains can then be used to screen for their protein composition, and the rest (containing the germ) of the grains with the preferred protein composition can be selected, planted and then used for propagation or crosses. A question regarding Tajik wheat is whether such a selection will contribute to increasing bread-making quality, as there is a lack of correlation between the specific HMW-GS composition and gluten strength, as shown below. Furthermore, the SDS-PAGE analyses of the flour of Tajik wheat will result in figures, such as the schematic ones in Figure 2, with a range of bands of various strength depending on the presence of certain alleles. Thus, the results from such analyses will be unclear, and there will be an uncertain determination of protein composition, due to the presence of many bands, of which some will be very faint, due to the low presence of some alleles (e.g., 3% of 2+12 in Navruz).

The ability of gluten proteins to form polymers in wheat grain and dough has been found to be an important contributor to bread-making quality [11,40]. Thereby, this characteristic might be a useful tool in plant breeding for improved gluten strength in Tajik wheat. The present study found a significant variation in grain protein composition and the ability of proteins to polymerize, using SE-HPLC to evaluate 22 wheat varieties/lines from two locations (Tables 1 and 2). Such a result corresponds well with previous studies on the presence of cultivar variations for protein composition as measured by HPLC [41,42]. Detailed numbers on the protein parameters of SE-HPLC is given in the Supplementary Materials.

Table 1. Mean squares for combined analysis of variance, as well as mean values for different localities for protein parameters. TOTE = total SDS-extractable proteins; TOTU = total SDS-unextractable proteins (proteins extractable by sonication); %UPP = percentage of unextractable polymeric proteins in total polymeric proteins; %LargeUPP = percentage of large unextractable polymeric proteins in total large polymeric proteins.

Source	DF	TOTE (10^{14})	TOTU (10^{14})	%UPP (10^{-2})	%LargeUPP (10^{-2})
Locality	1	91.2 ***	18.1 ***	3.78 ***	3.32 ***
Variety	21	3.46 ***	1.64 ***	0.57 ***	1.46 ***
Locality*Variety	21	2.92	0.85	0.28	0.46
Error	88	0.23	0.08	0.02	0.11
Hisor Locality		1.41a	6.18a	0.58b	0.46b
Isfara Locality		1.24b	5.44b	0.61a	0.49a

*** Significant at $p < 0.005$. Mean values followed by the same superscript letter do not differ significantly from each other at $p < 0.05$ using Duncan's multiple range tests.

Table 2. Mean values of protein fractions of 22 wheat varieties/lines from two locations. TOTE = total SDS-extractable proteins; TOTU = total SDS-unextractable proteins (proteins extractable by sonication); %UPP = percentage of unextractable polymeric proteins in total polymeric proteins; %LargeUPP = percentage of large unextractable polymeric proteins in total large polymeric proteins.

Variety/line	Hisor Location				Isfara Location			
	TOTE (10^8)	**TOTU (10^7)**	**%UPP**	**%LargeUPP**	**TOTE (10^8)**	**TOTU (10^7)**	**%UPP**	**%LargeUPP**
1. Navruz	1.34fgh	6.09defg	45efg	56fgih	1.05i	5.30ef	45k	50m
2. Alex	1.63a	6.52bcde	41ijk	49ghi	1.18gh	5.23efg	48ghi	58hji
3. Jagger	1.60a	5.05jk	41ijk	52fghi	1.27ef	4.99efgh	47ijk	58jkl
4. TNMU/Munta	1.50b	6.84ab	49bcd	62abc	1.36bc	5.21efg	48efghi	60fghi
5. Prinia/Star	1.37fgh	6.61bcd	47cdef	57cdef	1.27e	5.29ef	48fghi	63cd
6. Shark/F4105W2.1	1.40defg	5.55hijk	45fgh	62abc	1.31cd	5.20efg	49defgh	65b
7. Vorona/Kauz/1D13.1/MLT	1.49bcd	5.96efgh	44ghi	61abcd	1.25e	4.86ghi	48ghi	61defg
8. Tam200/Kauz	1.41cdefg	6.86ab	50bc	67a	1.14h	5.36de	53b	56c
9. 1D13.1/MLT/TUI	1.35efgh	6.03efgh	48cde	60abcd	1.16gh	4.96fghi	18hji	60efgh
10. Arilw Pronghorn	1.38efg	5.61ghi	42hij	52fghi	1.16gh	5.76c	52bc	63cd
11. Eskina-8	1.41cdefg	4.92k	39kl	45i	1.28def	4.61hij	48ghij	56kl
12. YN/3NPM/VOS83	1.24ij	6.70abc	54a	67a	1.06i	5.22efg	50cdef	60efgh
13. Pastor/3/Vorona/CN079	1.28ij	6.03efgh	46defg	59bsdef	1.22fg	4.29j	43l	54l
14. Skauz BV 92	1.22j	6.27cdef	51ab	65ab	1.42ab	6.27b	51bcde	62ed
15. Vorona SN079	1.42bcdef	6.50bsde	45fgh	53fgh	1.46a	5.75c	46jk	57ijk
16. Soroka	1.39efg	6.55bcde	48cdef	56cdefg	1.34cd	6.03bc	48ghi	59ghij
17. Otus Toba 97	1.44bcde	6.53bc	46defg	55cdefg	1.14h	4.58ij	46jk	56kl
18. Kauz2/Chew//BCN/3Milan	1.49bc	6.88ab	46defg	58defg	1.25ef	5.99bc	51bcd	61def
19. Chen/Aegilops Squ…/RAV	1.41cdefg	5.23ijk	37l	45hi	1.31cde	6.04bc	47ijk	57jk
20. CBRD/Kauz	1.40efg	5.33ijk	40jkl	53defg	1.16gh	5.76c	51bcd	65b
21. Huavun Inia	1.33ghi	6.42bcdef	47cdefg	60abcde	1.30cd	5.71cd	50defg	65bc
22. CMN82A.1294/2*Kauz//	1.41cdefg	7.12a	51ab	66a	1.17gh	7.08a	59a	72a

Mean values followed by the same superscript letter do not differ significantly from each other at $p < 0.05$ using Duncan's multiple range test.

No clear relationship was found between the specific protein composition determined by SDS-PAGE [23] and the amount and size distribution of proteins revealed with SE-HPLC (Table 3) in the wheat lines evaluated here. As shown in a previous study, Tajik wheat material was highly heterogeneous, e.g., most of the studied wheat breeding lines have more than one HMW-GS pattern [23]. The heterogeneity of the material might be one factor behind the lack of correlation found in the present study between the specific HMW-GS and gluten strength as measured by SE-HPLC. However, even for the homogenous lines, no clear relationship was seen between the specific protein composition (i.e., HMW-GS 2+12 normally associated with weak gluten or 5+10 normally associated with strong gluten) [43–45] and %UPP as measured in this study. The present study revealed that several factors hampered the opportunity to use SDS-PAGE to evaluate the breeding lines for improved bread-making quality in Tajik wheat: i) the specific protein composition in the wheat genotypes varied as much within as between genotypes; ii) most of the genotypes contain HMW-GS 5+10, even if the gluten strength varies considerably, making it difficult to judge gluten strength from the specific protein composition; and iii) factors other than the specific protein composition (e.g., the cross-linking behaviour of the proteins and/or heat shock proteins) seem to be involved to a high extent in determining the bread-making quality of Tajik wheat. Thus, our results indicated that SE-HPLC is a more reliable method than SDS-PAGE for determining the bread-making quality of Tajik wheat due to these facts. Therefore, selection for the bread-making quality of wheat lines in a breeding program to secure food (bread) for the Tajik population should be based on analytical methods, allowing for the selection of breeding lines with high gluten strength, e.g., SE-HPLC (or physicochemical tests, taking into account dough extensibility and gluten strength, such as alveograph, which results have been shown correlating with SE-HPLC results [13]) and not on the specific protein composition.

Table 3. A comparison of the specific HMW-GS composition from the *Glu-D1* allele, determined by SDS-PAGE [23], and the amount and size distribution of the proteins obtained from SE-HPLC.

#	Variety/Line	HMW-GS from Glu-D1 (SDS-PAGE)	%UPP (SE-HPLC)
1	Navruz	5+10/2+12	45
2	Alex	5+10/2+12	48
3	Jagger	5+10/2+12	47
4	TNMU/Munta	5+10/2+12/4+12	48
5	Prinia/Star	5+10/2+12	48
6	Shark/F4105W2.1	5+10/2+12	49
7	Vorona/Kauz//1D13.1/MLT	5+10/2+12/4+12	48
8	Tam200/Kauz	2+12	53
9	1D13.1/MLT//TUI	5+10/2+12	48
10	Arilw Pronghorn	5+10	52
11	Eskina-8	4+12	48
12	YN/3NPM/VOS83	5+10	50
13	Pastor/3/Vorona/CN079	5+10/2+12/4+12	43
14	Skauz BV 92	5+10/2+12	51
15	Vorona SN079	5+10/2+12/2+10	46
16	Soroca	2+12/5+10	48
17	Otus Toba 97	2+12/5+10	46
18	Kauz2/Chew//BCN/3Milan	5+10/2+12	51
19	Chen/Aegilops Squarosa/Taus/RCN//3/RAV	2+10/5+10/2+12	47
20	CBRD/Kauz	5+10	51
21	Huavun Inia	5+10/2+12	50
22	CMN82A.1294/2*Kauz//	5+10	59

3.3. Tajik Wheat and Its Protein Composition as an Example of Opportunities for Wheat Breeding to Improve the Baking Quality of Wheat in Developing Countries in the Regions of Central Asia and North Africa

Using Tajikistan as an example of a developing country in the Central Asian and North Africa regions, the present study clearly showed opportunities to improve food security through breeding for the improved bread-making quality of Tajik wheat. Bread is the most important staple food for the Tajik population, and therefore, both the quantity of wheat produced and the quality of bread are important aspects that contribute to food security for Tajikistan [20,21]. The wheat samples in this study showed considerable variation in the amount and size distribution of polymeric and monomeric proteins (Table 1), indicating variations in protein concentration, gluten strength and bread-making quality among the samples. However, the observed variation among the investigated varieties/lines was not consistent between locations (Table 2). The impact from cultivation environments was generally large on the amount and size distribution of the polymeric and monomeric proteins of Tajik wheat (Table 2), which has also been reported in other studies [11,46]. No genotype x environment interactions were noted for the wheat material studied. Previous studies with the lack of genotype x environment interactions have described these phenomena as being a result of genetically alike genotypes being homogenous in various environments or genotypes clustering in their reaction to different environments [47]. However, these results were obtained from field trials in two locations over one year, and additional locations/years might produce other results. Investigations on the amount and size distribution of polymeric and monomeric proteins in wheat from farmers' fields in Tajikistan indicated lower values of TOTE specifically [22] as compared to those in the breeding material, further verifying the impact of growing conditions on the bread-making quality of Tajik wheat. Also, previous studies from other parts of the world have shown that environmental conditions have large impacts on the quality of the wheat produced [10,48–50]. Tajikistan is a country with significant differences in altitudes, cultivation conditions, farm sizes, mechanisation, etc. [19,22]. The found differences in the protein quality of the wheat grown in different locations complicate the issue of breeding for increased food security in terms of bread-making quality in Tajikistan. The fact that different genotypes perform the best in various growing locations within this country indicate the need for the breeding of certain varieties for specific localities within Tajikistan. Such a need will require a breeding program focused on several geographically different areas within a country, with each listing their specific needs, and will limit multi-environment breeding. As shown by a principal component analysis (PCA), where the first and second principal components explained 56.6 and 32.5% of the variation, respectively, higher TOTE and TOTU values were found in wheat grown in Hisor, while higher %UPP and %LargeUPP values were found in wheat grown in Isfara (Table 2; Figure 3).

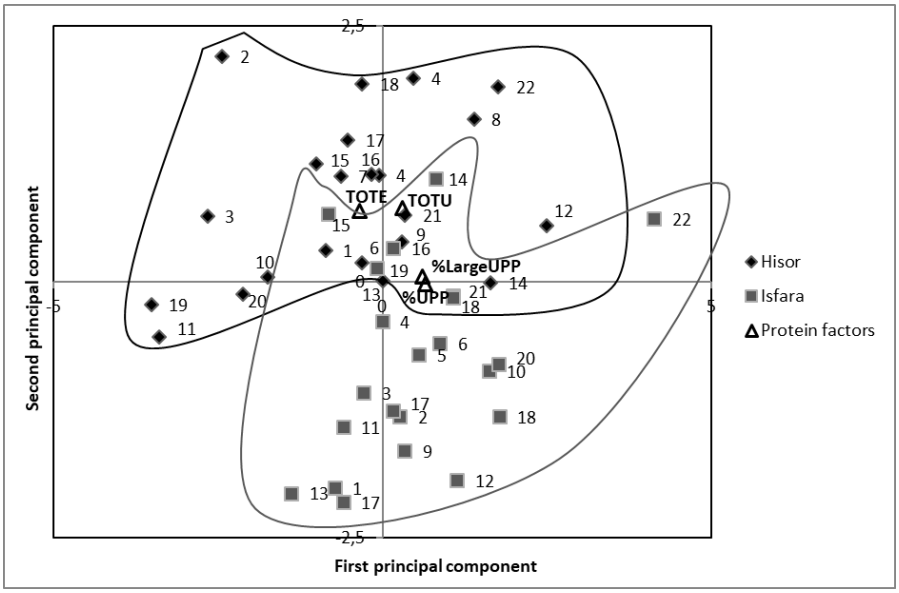

Figure 3. Principal component analyses (combined loading and score plot) showing variation along the principal components for the different genotypes grown in two locations based on the protein factors TOTE, TOTU, %UPP and %LargeUPP. Numbers indicate the various varieties/lines as mentioned in Table 2.

With the known correlations between TOTE and grain protein concentration versus %UPP and gluten strength [17], this study indicates that there are higher grain protein concentrations in Hisor and higher gluten strength in Isfara. Increased protein concentration is often a result of decreased nitrogen availability and increased temperature before grain filling [11]. The climatic data from Tajikistan show that the average daily mean temperature varied between 17 °C and 30 °C, with a slightly higher temperature for Isfara than that for Hisor during the grain filling period (May–June), as shown in Figure 4. With the predicted climate change, the mean temperature might increase, which would then have an impact on the protein concentration and composition of wheat [51–53]

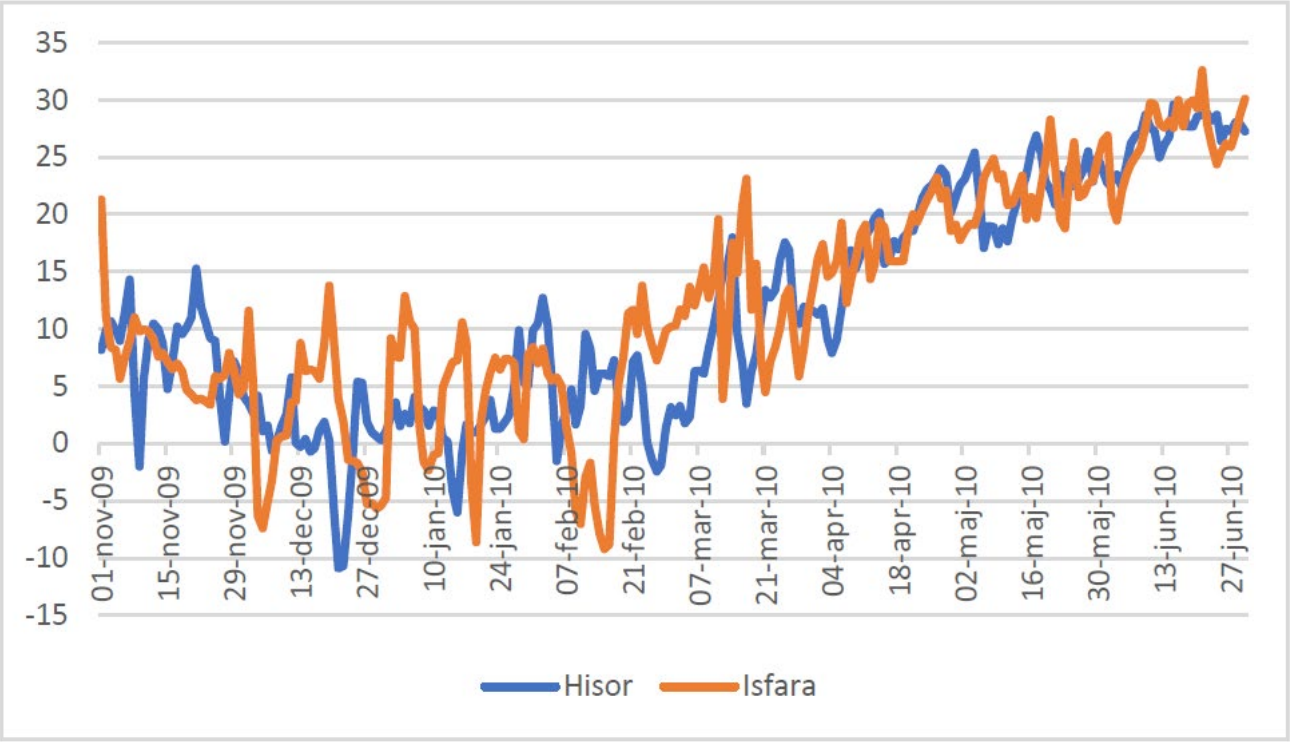

Figure 4. The average temperature of Hisor and Isfara over the growing season of 2009 to 2010.

The obtained results of this investigation are of importance for the wheat breeding program for improving the baking quality of wheat in Tajikistan. The wheat material of the present study originates to a great extent from the international germplasm from CIMMYT or other international programs [23]. This material is supposed to have broad adaptation to various environments of Tajikistan and Central Asian countries. However, to improve the bread-making quality of Tajik wheat, it seems suitable to genetically enhance local adaptation. Locally adapted material has been shown to be specifically important for organic wheat production [54]. Due to the limited use of fertilizers and pesticides in Tajikistan [19,22], cultivation conditions may resemble organic production, and also, cultivation locations vary largely due to altitude, farm size, mechanization, etc. Despite the eventual need for local adaptation, the Tajik wheat material is currently not homogeneous [23], which might be a target trait to work on for Tajik wheat breeders. Additionally, the introduction of novel genotypes with increased gluten strength into breeding schemes conducted under Tajik cultivation conditions may be an important task. However, non-homogenous crops might be beneficial in low-input systems [55,56]. Furthermore, a non-homogenous material might be valuable for cultivation under the predicted climate change scenarios, due to the fact that such a material might have multiple genes for various traits, including those that might be useful for tolerance to abiotic stresses. Recent studies have indicated an effect of the future climate, with drought stress, on quality parameters in wheat and that specific lines are available that have the potential to contribute both high yield and good quality despite abiotic stresses [51]. A negative correlation is known to exist between yield and grain protein concentration in wheat, but no such relationship has been described for yield and gluten strength [57]. Thus, despite the fact that there is a weak negative correlation between grain protein concentration and gluten strength in wheat [17], breeding towards improved protein composition and thereby better bread-making quality should have no negative impact on yield. If non-homogenous materials should be used further in breeding programs, HPLC is a better selection tool for protein composition than SDS-PAGE for specific protein composition. However, other

physicochemical tests such as the use of an alveograph, mixograph, etc., might be preferable in breeding programs due to their higher simplicity [13]. Furthermore, the wide array of emerging novel breeding tools, including the use of algorithms and big data together with the development of more and more specific primers, opens new opportunities for more specific selections of the most appropriate lines to be used in breeding programs [58,59]. These developments also have the potential to benefit Tajik wheat breeding programs. Currently, markers to be used for marker-assisted selection in breeding programs to improve bread-making quality are primarily available for specific protein subunits (mostly HMW-GSs). The present study clearly shows that it might be beneficial to develop suitable markers such as kompetitive allele-specific PCR (KASP) markers that can identify gluten strength in wheat lines based on correlations to SE-HPLC data. Such markers can then be used in breeding programs in Tajikistan and beyond, adapting high-throughput marker-based selection to improve bread-making quality and thereby food security. However, before developing KASP markers, loci associated with TOTE (correlated to grain protein content) and %UPP (correlated to gluten strength), as determined by SE-HPLC, need to be identified by, e.g., genome-wide association studies (GWASs). When the genes behind these traits have been identified in these loci, KASP markers should be used to label these candidate genes in breeding programs.

3.4. Wheat Grain Protein Composition as a Screening Tool in Plant Breeding for Improved Baking Quality

The present study compared two different methods to evaluate protein-based characteristics that have an impact on bread-making quality. One benefit of evaluating the protein composition instead of using physicochemical tests in breeding for improved bread-making quality is the significantly lower amount of seeds needed for evaluation. Both protein determination using SDS-PAGE and that using SE-HPLC, as utilized in the present study, only need a few grains for analyses, which is also the case for the suggested marker-based analyses. Thus, wheat breeding lines can be screened during early generations, allowing for the early selection of the bread-making quality trait, as shown in the flowchart in Figure 5. Early selections for traits of interest result in savings in terms of money and time (years in a breeding program). However, using physicochemical tests is beneficial since they are often both easier to carry out and understand than the analyses of protein composition. The present study evaluated the protein composition on a grain basis using SDS-PAGE (Figure 2), which might be useful in breeding programs. Analyses can be carried out on separate half grains, where the other part of the grain, holding the germ, can be used for the production of the plant, which can thereafter be used for crosses (Figure 5). However, if there is a desire to use non-homogenous wheat materials, such as the Tajik one, to breed wheat with improved bread-making quality, the SDS-PAGE results become difficult to use, as visualized in Figure 2. As there are many different alleles with different frequences in flour from a non-homogenous material, the gel picture becomes messy, with a range of different bands of different strength (Figure 2). Thus, selections within breeding programs based on this information are difficult to conduct. In such a material, SE-HPLC-based evaluations becomes more reliable. However, one issue with this type of analysis is that it requires reliable equipment with trained personnel. Also, the results are dependent on the equipment and column used, which means that an exact number of preferences to use in breeding programs does not exist. Instead, numbers have to be compared and be relative. Thus, %UPP is positively correlated to gluten strength [17], but the exact value for obtaining the best baking performance depends both on the HPLC equipment utilized and the baking method used. Marker-based analyses are easier and cheaper to perform than SE-HPLC and might therefore be the future solution [60,61], if markers correlated to SE-HPLC-based protein composition factors can be developed.

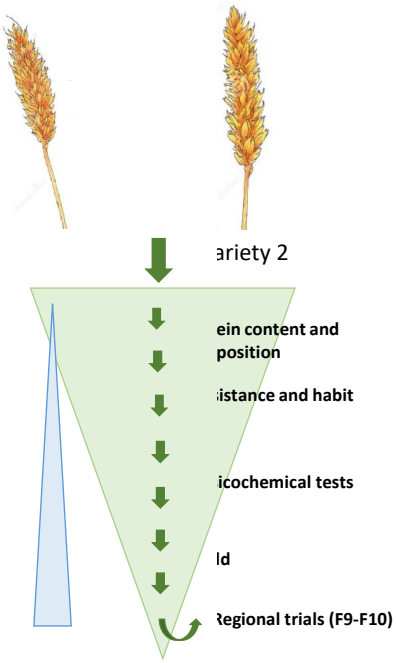

Figure 5. A flowchart of the plant breeding of a new wheat variety, indicating suitable generation for different screening and selection procedures, as modified from Koebner and Summers [62].

4. Conclusions

Tajik wheat varieties and breeding lines have a suitable amount of variation in protein composition to allow for breeding for improved bread-making quality to secure food (bread) for the Tajik population. The locality of wheat production is a major determinant of the obtained quality of Tajik wheat grain, and local adaptation was a prerequisite for the production of high-quality bread wheat in Tajikistan. Thereby, wheat breeding for quality in Tajik wheat calls for the breeding of locally adapted lines. The use of SE-HPLC to analyse the amount and size distribution of mono- and polymeric proteins is a more reliable method for selecting bread-making quality than analysing the specific protein composition with SDS-PAGE (primarily due to the homogeneity of the specific protein composition in the material and the high proportion of HMW-GS 5+10 that do not correlate to gluten strength). However, for wheat breeding programs in a developing country such as Tajikistan, a simpler and more high-throughput methodology would be beneficial. Emerging marker-based selection methods might be upcoming solutions for such breeding programs to improve the bread-making quality of Tajik wheat and simultaneously increase food security. Thus, the analyses of the specific protein composition, e.g., HMW-GS 5+10 or 2+12, might be useful if the selection of the most suitable grains for breeding is conducted on a single-grain analysis basis. However, if the most suitable populations, which are non-homogenous, as in the Tajik case, should be selected for, SE-HPLC-based analyses of protein composition might be more useful, especially if GWAS can be used to identify loci in the genome that are associated with protein composition traits as measured by SE-HPLC Then, these candidate genes should be labelled through the development of suitable markers such as KASP markers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/crops4040046/s1.

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editing, M.M., B.H., L.G., H.M., R.K., M.P., E.J.; visualization, R.K.; supervision, L.G., R.K., E.J.; project administration, H.M. and L.G.; funding acquisition, E.J. All authors have read and agreed to the published version of the manuscript

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