Genetic diversity of bread wheat genotypes in Iran for some nutritional value and baking quality traits

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Abstract Genetic variation among 78 irrigated bread wheat genotypes was studied for their nutritional value and baking quality traits as well as some agronomic traits. The experiment was conducted in a randomized complete block design with three replicates under normal and terminal drought stress conditions in Kermanshah, Iran during 2012–2013 cropping season. The results of combined ANOVA indicated highly significant genotypic differences for all traits. All studied traits except grain yield, hectoliter weight and grain fiber content were significantly affected by genotype × environment interaction. Drought stress reduced grain yield, thousand kernel weight, gluten index, grain starch content and hectoliter weight and slightly promoted grain protein and fiber contents, falling number, total gluten and ratio of wet gluten to grain protein content. Grain yield by 31.66% and

falling number by 9.20% attained the highest decrease and increase due to drought stress. There were negative and significant correlations among grain yield with grain protein and fiber contents under both conditions. Results of cluster analysis showed that newer genotypes had more grain yield and gluten index than older ones, but instead, they had the lower grain protein and fiber contents. It is thought that wheat breeders have bred cultivars with high grain yield, low protein content, and improved bread-making attributes during last seven decades. While older genotypes indicated significantly higher protein contents, and some of them had higher gluten index. We concluded from this study that it is imperative for breeders to pay more attention to improve qualitative traits coordinated to grain yield.

Keywords Drought stress · Falling number · Fiber · GGE-biplot · Gluten · Protein

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Introduction

Wheat (*Triticum aestivum* L.), due to its wide consumption as a staple food, is an important cereal grain worldwide with its unique nutritional and economic properties. Like many other countries, wheat-grain products constitute the major food in Iran (Amiri et al. 2015). Bread wheat is unique among cereals due to its baking property; hence, wheat is the major source of mankind's favorite food. Therefore, enhancing the quantity and quality of wheat grain has an important impact on food security, nutritional status and human health, particularly in developing countries (Amiri et al. 2015). Improving end-use quality has become of increasing importance to wheat breeders over the past few decades and nowadays, improving grain quality is becoming a high preference topic in crop sciences (Kong et al. 2013).

In bread making process, grain and flour proteins have a major role in dough visco-elastic properties and governing the technological and rheological properties of wheat flour (Simsek et al. 2014). Moreover, it is widely known that both protein content and protein composition are important factors to have a good baking process (Zhao et al. 2010). Wheat grain protein content varies from 8 to 17%, depending on genotype and environmental factors (Peña et al. 2002).

Some other traits such as gluten, starch, fiber contents and alpha amylase activity (falling number), play an important function in processing and end-use quality of wheat-based products, especially bread (Peña et al. 2002). The preliminary criteria of flour quality are the relative amounts of mentioned compounds, their composition, and their interactions (Souza et al. 2002). Gluten content which constitutes about 80% of endosperm proteins (Peña et al. 2002) is a very important trait for flour quality assessment. The visco-elastic property of gluten, for end-use purposes, affects the quality of bread gluten and is commonly known as dough strength (Matos and Rosell 2015). Starch as the major storage carbohydrate of cereals is an important part of human nutrition. It is the main component of wheat endosperm and generally contributes 65–80% of final dry weight. It is hypothesized that grain yield will increase by increasing starch accumulation and promoting dry matter remobilization (Yang et al. 2014). As alpha-amylase activity has a major role in determining of flour starch hydrolyzing, higher and lower amount of activity causes a negative effect on baking dough properties (Peña et al. 2002). Falling number, as an important chemical parameter, measures both alpha-amylase activity (sprout damage) and specific starch value (Baeckström et al. 2004). High falling number shows a good viscosity and processing of flour quality, whereas its low amount indicates a high quantity of amylases present in grain (Ashraf 2014); therefore a proper value of falling number represents a suitable alpha-amylase activity in dough processing.

Genotype is known as the most important factor affecting the wheat quality indicators (Hristov et al. 2010). Therefore, identification of the genotypes with high-quality grain productions may be an effective way to improve bread quality. Thus, screening germplasm should be one of the major wheat breeder's task to improve grain quality parameters through selection of crossing parents (Zhang et al. 2004). Likewise, wheat grain quality is affected by environmental conditions such as drought and heat stress (Ashraf 2014). In Iran, water scarcity and high temperatures during grain filling stage are two important factors that restrict grain production and cause large fluctuations in grain composition and quality (Ashraf 2014; Balla et al. 2011).

Following the above facts and regarding lack of reports on wheat germplasm screening for wheat grain

composition and quality in Iran, it seems there are major gaps in this research area. The aim of current study was screening of 78 bread wheat genotypes including commercial wheat cultivars and elite lines cultivated in Iran, under normal and terminal drought stress conditions. This study could help to select genotypes tolerant to drought stress and with high economically important traits such as quality attributes. Also, the study will give new cues for future wheat breeding efforts.

Materials and methods

Seventy-eight irrigated bread wheat genotypes with a wide range of genetic backgrounds (73 commercial cultivars released from 1942 to 2012 and five superior elite lines listed in Table 1) were obtained from Seed and Plant Improvement Research Department, Kermanshah Agriculture and Natural Resources Research and Education Centre, AREEO, Kermanshah, Iran. This investigation was conducted during 2012–2013 cropping season at the Research Farm of Razi University, Kermanshah located on the western part of Iran (latitude 34° 21' North, longitude 47° 9' East, altitude 1319 m. above sea level). The rainfall at the cropping season of the experiment was 393 mm.

Experimental layout design was based on Randomized Complete Block Design (RCBD) with three replicates at two adjacent sites as control and drought stress. Seed sowing was done by hand at five row plots, 1.5 m length, and 0.22 m row spacing as 400 seeds per square meter density. Terminal (end-season) drought stress was imposed on May 20, 2013 at stressed plots. Meanwhile, non-stressed plots were irrigated twice more. Chemical fertilizers, herbicides and pesticides were not used at both sites. At full physiological maturity, two middle rows of each plot were harvested by hand to determine studied traits.

Grain protein, fiber and starch percent were determined on a whole grain dry weight basis by Near Infrared Reflectance spectrometer (NIR), using a “Pertin Instruments DA-7200” instrument (Osborne et al. 2007). To determine falling number and gluten-related traits, the whole grains were milled by a laboratory miller (Pertin 3100, Sweden). The falling number values were determined based on alpha-amylase activity through standard method AACC, 65-81B (AACC 2000a), by Falling Number set (1500-Pertin Instruments, Huddinge, Sweden). Total and strong glutes were determined based on the Glutomatic Gluten Washer (Pertin—GM 2200, Sweden) and Gluten Index Centrifuge (Pertin—CF 2015, Sweden) and measured by Glutomatic System (AACC 2000b). Gluten index was calculated as: (strong gluten/total gluten) × 100. Wet gluten was calculated as: total gluten × 10. Falling number and gluten-related traits were measured for two replicates of each site.

Table 1 Details of genotypes used in the study

No.	Genotype	Year of release	No.	Genotype	Year of release	No.	Genotype	Year of release
1	Karaj-1	1973	27	Soisson	1994	53	Darab-2	1995
2	Karaj-2	1973	28	Shahryar	2002	54	Atrak	1995
3	Karaj-3	1976	29	Tous	2002	55	Chamran	1997
4	Azadi	1979	30	Pishgam	2008	56	Star	1995
5	Ghods	1989	31	Mihan	2010	57	Dez	2002
6	Mahdavi	1995	32	Oroom	2010	58	Vee/Nac	1995
7	Niknejad	1995	33	Zaree	2010	59	LineA	–
8	Marvdasht	1999	34	Inia	1968	60	Aflak	2011
9	Pishtaz	2002	35	Khazar-1	1973	61	Baaz	2009
10	Shiraz	2002	36	Mughan-1	1973	62	Shahpasand	1942
11	Sepahan	2006	37	Mughan-2	1974	63	Omid	1956
12	Bahar	2007	38	Mughan-3	2006	64	Roshan	1958
13	Parsi	2009	39	Golestan	1986	65	Tabassi	1951
14	Sivand	2009	40	Alborz	1978	66	Sholleh	1957
15	M-85-7	–	41	Kaveh	1980	67	Sorkhtokhm	1957
16	WS-82-9	–	42	Rassoul	1992	68	Adl	1962
17	Sirwan	2012	43	Tajan	1995	69	Sabalan	1981
18	DN-11	–	44	Shiroudi	1997	70	Spring B.C. of Roshan	1998
19	Bezostaya	1969	45	Darya	2006	71	Winter B.C. of Roshan	1998
20	Navid	1990	46	Arta	2011	72	Cross of Shahi	1995
21	Alamout	1995	47	N-85-5	–	73	Maroon	1991
22	Alvand	1995	48	Arvand	1973	74	Kavir	1997
23	Zarin	1995	49	Chenab	1975	75	Hamoon	2002
24	MV-17	1993	50	Bayat	1976	76	Bam	2006
25	Gaspard	1994	51	Falat	1990	77	Akbari	2006
26	Gascogne	1994	52	Heirmand	1991	78	Sistan	2006

Combined analysis of variance was performed over trials using SAS software (ver. 9.1). Least Significant Difference (LSD) values among mean treatments were calculated at 5 and 1% probability levels. The SPSS software ver.16 was used for: (1) Pearson correlation, (2) Cluster analysis using Ward's method and Squared Euclidean Distance measure and (3) Multivariate tests to determine the optimal number of acceptable clusters. Principal Component Analysis was performed by Genotype $\times$ Environment Interaction-biplot (GGE-biplot) software version 6.3

Results

Environmental and genotypic effects

Results of combined analysis of variance for 78 bread wheat genotypes indicated high significant genotypic differences for all traits (data not shown) suggesting the potential for selecting the best genotypes in terms of grain quantity and quality. Effect of environment (drought stress)

was significant on grain yield, thousand kernel weight, grain protein content, total gluten, gluten index and the ratio of wet gluten to grain protein content. In this experiment, stress intensity (SI) was 0.32 which is mild. Drought stress reduced half of studied traits including grain yield, thousand kernel weight, gluten index, grain starch content and hectoliter weight by 31.66, 9.88, 8.13, 0.83 and 0.74%, respectively. Increase of grain protein content, grain fiber content, falling number, total gluten and the ratio of wet gluten to grain protein content was less than 10% under drought stress, meanwhile falling number had the highest increases rank by 9.20%. It should be noted that these variation were genotype-dependent.

All studied traits except grain yield, hectoliter weight and grain fiber content were significantly affected by genotype $\times$ environment interaction (data not shown). It means these genotypes had no similar reaction to drought. Therefore, a summary for simple analysis of variance and mean comparison under control and drought stress conditions have been presented separately at Table 2.

The results of simple ANOVA revealed highly significant genotypic differences for all traits under both

conditions, except grain yield under drought stress (Table 2). Grain yield ranged from 4602 to 11,642 kg ha⁻¹ and from 2497 to 7950 kg ha⁻¹ under control and drought stress conditions, respectively. Genotypes 13 (Parsi), 17 (Sirwan), 60 (Aflak) and 32 (Oroom) by 11,642, 11,207, 11,201 and 11,017 kg ha⁻¹, and genotypes 47 (N-85-5), 30 (Pishgam), 8 (Marvdasht) and 12 (Bahar) by 7950, 7565, 7379 and 7371 kg ha⁻¹ were the superior genotypes based on grain yield under non-stress and drought stress conditions, respectively. The genotypes 62 (Shahpasand), 65 (Tabassi), 66 (Sholleh) and 69 (Sabalan) had the high grain protein content and the genotypes 74 (Kavir), 77 (Akbari), 76 (Bam), 6 (Mahdavi) and 51 (Falat) were the low grain protein content genotypes, respectively which were common under both conditions.

The common genotypes under both conditions with maximum falling number were 71 (Winter B.C. of Roshan), 65 (Tabassi), 68 (Adl), 64 (Roshan) and 63 (Omid). Under non-stress conditions, the highest gluten index was obtained by genotypes 27 (Soisson), 5 (Ghods), 49 (Chenab) and 40 (Alborz) with more than 80%. The maximum gluten index under drought stress condition was recorded by genotypes 24 (MV-17), 17 (Sirwan), 30 (Pishgam), 52 (Heirmand), 27 (Soisson), 23 (Zarin) and 4 (Azadi) by 60–72%. The mean values, ranges and statistical information of the other studied traits are shown in Table 2.

Correlation analysis

Under non-stress condition, grain yield was highly significant and positively correlated with thousand kernel weight, hectoliter weight and grain starch, while a negative and significant correlation was observed between grain yield and grain protein, grain fiber, falling number, total gluten and the ratio of wet gluten to grain protein content values. At stress condition, grain yield was positively significantly correlated with hectoliter weight and grain starch, but the correlation was highly significant and negative between grain yield and grain fiber as well as grain yield and grain protein. Therefore, grain protein and fiber contents had significantly negative correlations with grain yield under both conditions.

Grain starch and protein contents as well as grain starch and fiber contents were found to be highly inversely correlated under both conditions. Moreover, negative correlation was observed between grain starch and total gluten (Table 3).

A regression line of gluten index on wet gluten/protein resulted in negative and positive associations under control and drought stress conditions, respectively (Fig. 1). The estimated reduction of gluten index was 18.08% per unit, calculated as ratio of wet gluten to grain protein content

under non-stress condition. While, this regression line under drought stress condition showed a small improvement equal to 2.13% per unit of mentioned ratio.

Principal component analysis

The first two principal components justify about 60.0 and 54.7% of total data variation set under non-stress and stress conditions, respectively. The polygon of “which is best for what” (Fig. 2) shows the best genotypes for each trait. At non-stress condition, eight rays divided the biplot into eight sections. The peak genotype for each quadrant was the one that give the highest amount for traits fall within the quadrant. Genotype 17 (Sirwan) was the best based on grain yield, thousand kernel weight and hectoliter weight. Genotype 65 (Tabassi) located as the top genotype in regard to grain protein content, falling number and total gluten. Genotypes 62 (Shahpasand) followed by 2 (Karaj-2) were located in the quadrant which belonged to grain fiber content. Genotype 5 (Ghods) was the best based on gluten index. Under drought stress condition, seven rays divided the biplot into seven sections. Genotype 74 (Kavir) was the best based on grain yield and grain starch content. Genotype 24 (MV-17) had a high amount of gluten index. Genotypes 62 (Shahpasand) was located in the quadrant which belonged to grain protein and fiber content, falling number and total gluten. The highest thousand kernel weight and hectoliter weight were observed for genotype 54 (Atrak).

Cluster analysis

Cluster analysis grouped the considered genotypes in three and four clusters under non-stress and stress conditions, respectively (Data not shown). These number of clusters were the optimal acceptable clusters which revealed the highest “*F*” value (within-group variance was less than between-group variance) (Mohammadi and Prasanna 2003) according to Wilks’ Lambda, Lawley-Hotelling and Pillai’s Trace “*F*” tests (Fig. 3 and Table 4).

Under non-stress condition, the first cluster contained 27 genotypes which had low grain yield, grain starch content and gluten index, but were high in thousand kernel weight, grain protein content, grain fiber content, falling number and total gluten. Moreover, these genotypes were not suitable in regard to optimum ranges of falling number and gluten quality. Only five of 27 genotypes in first cluster, had been released or introduced after 2000, while the rest had been released between 1942 and 1998. The second cluster included 14 genotypes which had the lowest grain protein content, total gluten, falling number, hectoliter weight and thousand kernel weight and had highest grain starch content and gluten index. Thirty-seven genotypes

Table 2 Summary of simple analysis of variance and mean comparison in the 78 wheat genotypes under non-stress and drought stress conditions

Site	GY (kg ha ⁻¹)	TKW (g)	HLW (kg 100Lit ⁻¹)	GPC (%)	GSC (%)	GFC (%)	FN (s)	TG (g)	GI (%)	WG/P
Non-stress conditions	Genotypes	13, 17, 60, 32, 39, 71, 48	64, 17, 65, 9, 13	65, 69, 1, 66, 68, 62	74, 76, 73, 12, 77, 67	1, 5, 2, 7, 21, 40, 39, 52	71, 65, 9, 2, 68, 64, 63, 35	65, 62, 66, 75, 61, 25	27, 5, 49, 40, 77, 15	75, 25
	Genotypes No. ^a	63, 62, 2, 26, 3	5, 4, 3, 28, 67	62, 5, 3, 2, 4, 63, 1, 10	74, 5, 77, 12, 76, 6, 51	1, 75, 65, 66, 25, 62, 69	20, 5	27, 5, 77, 78, 15, 49, 13	–	27, 5, 77, 78, 15, 13, 49
	Mean ± SE (n = 78)	8109 ± 186	39.01 ± 0.62	76.89 ± 0.32	12.01 ± 0.03	67.87 ± 0.11	2.65 ± 0.01	294 ± 2.91	57.83 ± 1.30	4.569 ± 0.04
	Minimum	4602	24.10	69.61	11.53	65.22	2.49	3.56	42.68	2.965
	Maximum	11,642	49.14	82.30	12.65	70.14	3.66	6.43	96.00	5.117
	F test	**	**	**	**	**	**	**	**	**
	C.V. %	22.35	9.46	2.93	1.30	1.12	7.38	4.10	10.62	3.85
	LSD 5%	2924	5.95	3.64	0.25	1.23	43.2	0.449	12.26	0.350
	LSD 1%	3860	7.86	4.80	0.33	1.63	57.3	0.595	16.26	0.465
	Genotypes No. ^a	47, 30, 8, 12, 26, 78	40, 17, 64, 26, 78	57, 52, 43	62, 63, 66, 65, 25, 3, 69	74, 77, 76, 78	68, 65, 64, 62, 71, 41, 63, 66, 19	21, 25	24, 17, 30, 52, 27, 23, 4	21
Drought stress conditions	Genotypes No. ^b	62, 27, 26, 24, 63	3, 4, 62, 28, 63, 5, 20	62, 3, 63, 10, 2	51, 76, 74, 77, 6, 78, 38	25, 62, 3, 1, 69	44, 20, 13	54, 60, 42, 52, 48, 74, 24	68	54, 42, 60, 52, 48, 24
	Mean ± SE (n = 78)	5542 ± 122	35.15 ± 0.54	76.32 ± 0.35	12.19 ± 0.03	67.31 ± 0.12	2.71 ± 0.01	5.85 ± 0.07	53.13 ± 0.58	4.799 ± 0.05
	Minimum	2497	24.71	66.63	11.65	64.36	2.53	3.68	43.32	3.016
	Maximum	7950	49.69	82.23	12.99	69.91	4.00	6.85	72.23	5.595
	F test	ns	**	**	**	**	**	**	**	**
	C.V. %	29.07	11.24	3.23	1.52	1.27	7.64	5.83	6.67	5.72
	LSD 5%	2599	6.37	3.98	0.30	1.38	48.8	0.68	7.06	0.546
	LSD 1%	3431	8.41	5.25	0.39	1.82	64.8	0.90	9.36	0.725
	Genotypes No. ^b	62, 27, 26, 24, 63	3, 4, 62, 28, 63, 5, 20	62, 3, 63, 10, 2	51, 76, 74, 77, 6, 78, 38	25, 62, 3, 1, 69	44, 20, 13	54, 60, 42, 52, 48, 74, 24	68	54, 42, 60, 52, 48, 24
	Mean ± SE (n = 78)	5542 ± 122	35.15 ± 0.54	76.32 ± 0.35	12.19 ± 0.03	67.31 ± 0.12	2.71 ± 0.01	5.85 ± 0.07	53.13 ± 0.58	4.799 ± 0.05

F test: ** Significant at 1% probability level, * Significant at 5% probability level, ns = not significant

GY Grain yield, TKW thousand kernel weight, HLW hectoliter weight, GPC grain protein content, GSC Grain starch content, GFC Grain fiber content, FN Falling number, TG total gluten, GI Gluten index, WG/P the ratio of wet gluten to grain protein content

^aThis row shows the genotypes with following property: (Mean + 1.5 STDEV)^bThis row shows the genotypes with following property: (Mean—1.5 STDEV)

Table 3 Pearson correlation coefficients between different traits in bread wheat genotypes under non-stress and stress conditions (n = 78)

	GY	TKW	HLW	GPC	GSC	GFC	FN	TG	GI	WG/P	Drought stress conditions
GY	1										
TKW	0.375**	1	0.223*	-0.467**	0.312**	-0.345**	-0.200	-0.092	-0.001	0.006	
HLW	0.416**	0.399**	1	-0.225*	0.309**	-0.187	0.085	-0.306**	-0.128	-0.276*	
GPC	-0.337**	0.352**	0.047	1	-0.876**	0.485**	0.455**	0.340**	-0.100	-0.229*	
GSC	0.341**	-0.183	0.117	-0.880**	1	-0.638**	-0.319**	-0.431**	0.057	-0.265*	
GFC	-0.358**	0.039	-0.269*	0.312**	-0.426**	1	0.057	0.285*	0.103	0.197	
FN	-0.274*	0.208	-0.026	0.587**	-0.500**	0.004	1	0.197	-0.215	0.113	
TG	-0.347**	0.046	0.021	0.579**	-0.617**	0.129	0.389**	1	0.147	0.977**	
GI	0.016	-0.203	-0.329**	-0.346**	0.261*	0.013	-0.186	-0.580**	1	0.188	
WG/P	-0.288*	-0.054	0.016	0.382**	-0.458**	0.052	0.272*	0.972**	-0.566**	1	

* and **: Significant at the 5% and 1% probability levels, respectively

GY Grain yield, TKW thousand kernel weight, HLW hectoliter weight, GPC grain protein content, GSC Grain starch content, GFC Grain fiber content, FN Falling number, TG total gluten, GI Gluten index, WG/P the ratio of wet gluten to grain protein content

were located in third cluster. These genotypes had average value of the most traits but had the highest grain yield and hectoliter weight but lowest grain fiber content (Table 4).

Under drought-stress condition, the first cluster included 16 genotypes. High falling number (by average of 366.6 s) was the distinctive property of this cluster. Seven genotypes were located in second cluster. These genotypes had low grain yield, grain starch content, thousand kernel weight and hectoliter weight, while they were superior based on total gluten and grain protein and fiber content. High grain yield and gluten index were the important distinctive properties of third cluster containing 24 genotypes. The last cluster included 31 genotypes with high thousand kernel weight, hectoliter weight and grain starch content, but low grain protein and fiber contents (Table 4).

In order to study the role and effect of breeding programs on improvement of different traits of wheat; the trend of grain yield, grain protein and fiber concentrations, and gluten index over the year of release are demonstrated in Fig. 4. A regression line for each trait was fitted with year of a cultivars introduction or release. As can be seen, over the past 70 years, the rate of grain protein and fiber concentrations in grains of cultivars has been decreased over the years of cultivar's introduction or release, while grain yield was increased and gluten index was almost constant. The estimated reductions of grain protein and fiber concentrations were 0.007 and 0.002 percent per year, respectively. The regression lines for grain yield and gluten index showed proper and small improvement equal to 52 kg/ha and 0.007 percent per year, respectively.

Discussion

In present study, a wide genetic variation among 78 bread wheat genotypes was found for all studied traits, suggesting the potential for selecting the best genotypes in terms of grain quantity and quality. The genotype $\times$ environment interactions were significant for all qualitative traits, except for hectoliter weight and grain fiber content. These results are supported by numerous findings which concluded that any decline in wheat quality is caused by environmental changes as well as genetic factors (Zhang et al. 2004; Balla et al. 2011; Ashraf 2014).

Nowadays, it is well known that environment has a great affect on wheat quantity and quality. In this study, environment (drought stress) reduced grain yield, thousand kernel weight, gluten index, grain starch content and hectoliter weight and slightly promoted grain protein and fiber contents, falling number, total gluten and ratio of wet gluten to grain protein content. Falling number attained the highest increase by 9.20%. Eivazi et al. (2006) reported the reduction of gluten index and increase of grain protein and

Fig. 1 The relationship between the ratio of wet gluten to grain protein content and gluten index under both **a** non-stress and **b** drought stress conditions

falling number in wheat genotypes under drought and salinity stress. Also, decline in grain yield and thousand kernel weight and increase in grain protein content under drought stress during grain filling period of wheat genotypes has been reported by Pierre et al. (2008).

The protein content particularly its composition, which is most important factor in bread making quality, is not only largely dependent on the genotype (Guarda et al. 2004), but also influenced by environment and various types of stresses (Peña et al. 2002). Generally, selection for protein is complicated due to negative association with grain yield (Peña et al. 2002). It is known that drought stress caused reduction in grain yield and consequently could increase the grain protein content (Acuna et al. 2005; Amiri et al. 2015). Most of genotypes in current study (66 of 78 genotypes) showed higher value for grain protein when exposed to drought stress. Notwithstanding the various response of wheat genotypes towards this trait, genotypes no. 20 (Navid), 62 (Shahpasand), 63 (Omid) and 28 (Shahryar) showed the highest increase. The increase of mean protein content under drought stress is mainly due to reduction of photosynthesis followed by inadequate grain filling and consequence decrease in the ratio of grain starch to protein content. In this study, genotypes with high grain yield had the lowest protein content.

Grain yield was negatively correlated with protein content under both conditions. The negative correlation between grain yield and protein content had already been previously reported (Aslani et al. 2013). The comparison of relationship between grain yield and protein content in this experiment and the relationship between them in the experiment performed in 2011–2012 (Amiri et al. 2015) indicated that environment (year) did not change the negative correlation between these two important traits. Therefore, it could be stated that the relationship between grain yield and protein content is consistent. Moreover, grain starch and protein contents as well as grain starch and fiber contents were found to be high inversely correlated under both conditions. Regarding to these correlations and high positive correlation between grain yield and starch

content under both conditions, it seems that starch accumulation increase due to photosynthesis and supply of assimilates caused reduction of the protein and fiber ratios in grain. Therefore, increase of total gluten, -major part of wheat grain protein, under drought stress in this experiment and the others (Ozturk and Aydin 2004; Noorka et al. 2009) seems reasonable.

Falling number is a significant trait in wheat processing quality because of its relation with pre-harvest sprouting damage (Deng et al. 2005). The optimum value of falling number, s an index of enzymatic state of the grain, is 250 s for wheat (Erekul et al. 2009). Generally, a high falling number value (above 300 s) indicates low enzyme activity and shows quality wheat flour, while the values below 200 s indicate high levels of enzyme activity and sprout damaged wheat flour leading to yield losses and poor flour quality (Deng et al. 2014). Although value of falling number was affected by drought stress, the remarkable differences among genotypes were also observed. The optimum value of falling number was exceeded by most of tested wheat genotypes under both conditions. Under non-stress condition, 28 out of 78 genotypes (36%) showed high falling number value (above 300 s) while none were below 200 s. The lowest as well as the optimum falling number was observed for genotypes no. 20 (Navid) and 5 (Ghods) by 249 and 250 s, respectively. Under drought stress condition, the falling number in 54 genotypes (69%) was above 300 s. The lowest and the optimum values of falling number belonged to genotype no. 44 (Shiroudi) by 230 s and genotypes no. 20 (Navid) and 13 (Parsi) by 249 and 258 s, respectively. Therefore genotype no. 20 (Navid) was desired genotype in regard to falling number and indicates that its flour were optimum in enzymes activity grade.

Gluten index as a better criterion of wheat processing quality is generally more appropriate than total and/or wet gluten content (Deng et al. 2005). Although, it seems that optimum range of gluten index is 60–90%, but Curic et al. (2001) reported optimum values between 75 and 90%. In this study, the mean values of gluten index were about 58

Fig. 2 Polygon view of the GGE biplot show the “which is best for what” under: **a** non-stress and **b** stress conditions. *GY* grain yield, *TKW* thousand kernel weight, *HLW* hectoliter weight, *GPC* Grain protein content, *GSC* Grain starch content, *GFC* Grain fiber content, *FN* Falling number, *TG* total gluten, *GI* Gluten index

and 53% under non-stress and drought stress conditions, respectively. Genotype no. 27 (Soisson) was ranked as high gluten index under non-stress (96%) and drought stress (62%) conditions. Amongst 78 genotypes, genotypes no. 51 (65%) and 70 (90%) showed very low gluten index by less

than 60% under non-stress and drought stress conditions, respectively.

Drought stress strongly influenced the ratio of wet gluten to protein content, as indicator of wet gluten production per protein unit ($P < 0.05$), although there was a high significant effect of genotype on the trait (Table 2). This

Fig. 3 The values of Wilks' Lambda, Lawley-Hotelling and Pillai's Trace "F" tests for each cutting point of dendrogram derived from cluster analysis of the 78 wheat genotypes under both non-stress (a) and drought stress (b) conditions

Table 4 Mean comparison of clusters for each trait in 78 wheat genotypes under non-stress and drought stress conditions

Site	Cluster	GY (kg ha ⁻¹)	TKW (g)	HLW (kg 100Lit ⁻¹)	GPC (%)	GSC (%)	GFC (%)	FN (s)	TG (g)	GI (%)
Non-stress	I	6860 ^c	39.91 ^a	76.15 ^b	12.27 ^a	66.88 ^c	2.684 ^a	315.4 ^a	5.88 ^a	53.68 ^b
	II	7783 ^b	35.44 ^b	75.20 ^b	11.74 ^c	68.88 ^a	2.669 ^{ab}	276.0 ^b	4.89 ^c	71.64 ^a
	III	9144 ^a	39.70 ^a	78.07 ^a	11.92 ^b	68.22 ^b	2.626 ^b	285.2 ^b	5.44 ^b	55.64 ^b
	F test ^a	**	*	**	**	**	*	**	**	**
	C.V. %	15.92	13.45	3.40	1.25	0.91	3.13	6.94	6.33	16.45
Drought stress	I	4885 ^b	36.86 ^a	77.12 ^a	12.38 ^b	66.74 ^b	2.738 ^b	366.6 ^a	6.07 ^a	50.60 ^b
	II	4131 ^c	29.11 ^c	70.74 ^c	12.60 ^a	65.37 ^c	2.841 ^a	315.4 ^b	6.30 ^a	53.26 ^{ab}
	III	6069 ^a	33.31 ^b	74.65 ^b	12.17 ^c	67.18 ^b	2.727 ^b	312.8 ^b	5.97 ^a	55.53 ^a
	IV	5791 ^a	37.06 ^a	78.46 ^a	12.01 ^d	68.13 ^a	2.663 ^c	305.2 ^b	5.54 ^b	52.55 ^{ab}
	F test ^a	**	**	**	**	**	**	**	**	*
	C.V. %	16.23	11.77	2.66	1.21	0.94	2.54	8.19	9.02	9.21

Significant difference according to Duncan's multiple range tests at 5% probability level for different letters within each column

^aF test: ** = Significant at 1% probability level, * = Significant at 5% probability level

Grain yield (GY), Thousand kernel weight (TKW), Hectoliter weight (HLW), Grain protein content (GPC), Grain starch content (GSC), Grain fiber content (GFC), Falling number (FN), Total gluten (TG), Gluten index (GI)

strong environment impression on qualitative traits such as grain protein content and the ratio of wet gluten to protein content in wheat is in agreement with report by Šimić et al. (2006). However, in present study, genotypic effect was dominant for the most of the qualitative traits. The ratio for wet gluten to protein content varied within a broad range depending on genotype under both non-stress (from 2.97 to 5.12) and drought stress (from 3.02 to 5.60) conditions (Table 2). Based on the results, highly significant negative correlation ($r = -0.566^{**}$) was found between gluten index and ratio of wet gluten to protein content (Table 3) under non-stress condition which is confirmed by Fig. 1. This figure indicates that as much as wet gluten production per protein unit (WG/P) is increased; the proportion of strong gluten per total gluten (GI) (as a desirable characteristic for dough elasticity) will be decreased under non-stress condition; hence, highest gluten index was observed in lower ratio of wet gluten to protein content. This relationship was inversed under stress condition so that highest gluten index was observed in higher ratio of wet gluten to protein content. It could be concluded that dough quality

will be more desirable under stress condition with respect to gluten and elasticity property (Fig. 1).

Results of cluster analysis showed that newer genotypes had more grain yield and gluten index than older ones, but instead, they had the lower grain protein and fiber contents (Table 4). The genotypes were clustered into three clusters under non-stress condition. Genotypes on first cluster had low grain yield and starch content with high protein, fiber and gluten contents. Moreover, these genotypes were not suitable in regard to optimum ranges of falling number and gluten quality. Only five of 27 genotypes in first cluster, had been released or introduced after 2000, while the rest had been released between 1942 and 1998. The genotypes in the second and third clusters had been released or introduced more recently. They had high grain yield and rather high gluten index but had relatively modest to low quality traits. Therefore, it is thought that wheat breeders bred cultivars with high grain yield, low protein content, and improved bread-making attributes. While older one showed significantly higher protein contents, and some of them had higher gluten index which is in accordance with

Fig. 4 Relationship between grain yield, grain protein concentration, grain fiber concentration and gluten index with the year of release or introduction over 70 years (Genotypes 15, 16, 18, 48 and 60 were excluded from this analysis as they are elite lines)

Dvořáček et al. (2011). However, lower protein (especially gluten) content in some modern cultivars could be regarded as one of reasons for a lower baking quality. We concluded from cluster analysis that the newer genotypes had more grain yield and gluten index than older ones, but instead, they had the lower grain protein and fiber contents.

Therefore, in this experiment we found that grain yield was increased almost consistently during 70 years of breeding programmes, while protein and fiber concentrations were slightly decreased over the years. This leads us to believe that apparently breeders may have focused mainly on enhancing grain yield with acceptable compromise in protein and fiber content of grains, which is understandable in the national context.

Conclusions

The observed genetic diversity among studied traits might be due to different genetic resources involved and could provide opportunity to select genotypes with desirable grain quality-related traits which could be utilized to

enhance or generate new wheat genotypes through conventional and modern breeding approaches. Based on the results, drought stress positively and significantly affected grain quality attributes and response of wheat genotypes to water limited environment was not similar. Older genotypes had highest grain protein and fiber contents, but low grain yields. So, negative correlations were observed between grain yield and both grain protein and fiber contents. Most of studied genotypes had a poor constitution in regard to falling number and gluten index so that just a few genotypes showed the optimum values of these qualitative traits under both conditions despite the high yielding potential. It seems that plant breeding has played an important role in grain yield increases, irrespective of sufficient raise on bread-making characteristics. Hence, it is imperative for breeders to pay more attention to improve qualitative traits coordinated to grain yield.

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