Heliyon 8 (2022) e10715

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Role of tryptophan content in determining gluten quality and wheat grain characteristics

Rupinder Kaur [a], Harinderjeet Kaur [a], Puja Srivastava [b,*]

[a] Department of Biochemistry, Punjab Agricultural University, Ludhiana 141001, India
[b] Department of Plant Breeding and Genetics, Punjab Agricultural University, Ludhiana 141001, India

ARTICLE INFO

Keywords:
Bread wheat
Protein
Gluten strength
Gluten index
Tryptophan
Wheat quality characteristics

ABSTRACT

Gluten protein makes 75–80% of the wheat protein and is of utmost importance because of its unique visco-elastic properties which have significant role in development of various food products especially the baked ones. The quality of gluten protein is determined by its subunit (glutenin and gliadin) number and composition. To determine the effect of amino acid tryptophan on gluten strength and other quality parameters, a set of 34 bread wheat genotypes consisting of 19 lines and 15 checks were estimated in the present study. As per the results obtained in this study, tryptophan, although present in low amounts in gluten, accorded positively with gluten strength. The study also highlights the relation between wheat grain protein, gluten strength and tryptophan content.

1. Introduction

Wheat is one of the world's most commonly consumed cereal grains. Bread wheat also called common wheat, is the leading species of wheat which is consumed on daily basis. 100 grams of wheat provides more than 20% of DV (Daily Value) of multiple essential nutrients such as protein (13% of wheat's dry weight), dietary fiber and niacin-vitamin B3 (a tryptophan derived vitamin) (Shewry et al., 2002; Shewry, 2019). About 75–85% of the total protein in bread wheat is gluten (Shewry et al., 2002; Shewry, 2019). It is a water-insoluble protein which consists of over 60 different polymorphic polypeptides (Rydz et al., 2018). Gluten is a major wheat endosperm protein, comprising of two prolamin groups, gliadins and glutenins and is responsible for the unique property of elasticity and stickiness of wheat dough which make it of prior use in breadmaking and other wheat-based foods including pasta, noodles and semolina. Gluten protein has a prominent role in determining the baking quality of wheat as it confers cohesiveness, viscosity and elasticity of the dough and also affects its water absorption capacity (Wieser, 2007; Wall, 1979; Ortolan and Steel, 2017). Many scientists have specified the importance of gliadins in bread making the quality of wheat (Wrigley et al., 1982). Experiments on gluten fractionation and reconstitution specifies that gluten is responsible for variations in bread making performances (Veraverbeke and Delcour, 2010; Barak et al., 2022). Strength of the gluten protein informs the baker about the quality of flour thus helps in developing recipes for various products. The elasticity as well as strength of gluten in flour can be measured by estimating gluten index as well as SDS-sedimentation values. Significant genotypic differences are observed in gluten strength of wheat varieties which may be the result of the variations in its structure, size distribution, and subunit composition (Veraverbeke and Delcour, 2010). Glutamic acid and proline are of significant structural importance in the gluten proteins as they together make up one half or more of the peptide-bound amino acids in it whereas tryptophan content is the lowest of all amino acids profiled in gluten protein (Woychik et al., 1961; Norton et al., 2012). The nutritional value of gluten protein is quite low due to the low levels of essential amino acids such as lysine and tryptophan. Thus significant steps are needed to improve the nutritional quality of wheat by increasing its essential amino acid content to combat the malnutrition prevailing in the wheat consuming population. In maize, this problem is being resolved by carrying out genetic mutations like *Opaque-2* mutation which almost doubles the content of lysine and tryptophan in maize (Wu et al., 2010). Only a handful of studies, if any, have been done to investigate the feasibility of a more balanced amino acid profile of wheat (Hoisington, 2002). The biggest challenge is the wide usage of this cereal in baking industry. Since, quality of flour is greatly dependent on the grain's protein composition therefore, can be greatly altered even with slight modifications. Amino acid composition of wheat should be carefully studied for knowing their role in determining grain quality.

* Corresponding author.
E-mail address: pujasrivastava@pau.edu (P. Srivastava).

https://doi.org/10.1016/j.heliyon.2022.e10715
Received 1 April 2022; Received in revised form 21 May 2022; Accepted 15 September 2022

Since wheat is staple food for a significant population therefore the studies on factors affecting its end-product utility and nutrition quotient should be a priority of researchers. Along with gluten strength, grain hardness or endosperm texture and protein content are considered as the foremost determinants of the quality of wheat. The present study is thus, directed towards studying the relation between wheat grain tryptophan content (an essential amino acid) and major wheat quality parameters.

2. Material and methods

2.1. Plant material

The breeders of Wheat Section of Punjab Agricultural University (PAU), Ludhiana, India conducted crosses of PBW 550 with a set of tall superior *chapatti* quality wheat varieties (C 273, C 306, C 518 and C 591). The set had total of 34 genotypes consisting of 19 bread wheat lines (BWL 1663, BWL 1664, BWL 3498, BWL 3500, BWL 3502, BWL 3504, BWL 3560, BWL 5228, BWL 5229, BWL 5230, BWL 5232, BWL 5429, BWL 5461, BWL 5463, BWL 6209, BWL 6249, BWL 6250, BWL 6251 and BW 9023) and 15 checks (H L-1, C 273, C 306, C 518, C 591, WG 357, PBW 1 Zn, PBW 175, PBW 550, PBW 644, PBW 660, PBW 725, PBW 758, PBW 761 and PBW 762). These agronomically improved and tested lines along with high grain protein lines (Table 1) carrying grain protein content gene-*Gpc-B1*, were tested for their protein content, tryptophan content, gluten content and gluten index.

Table 1. Parentage of bread wheat genotypes.

	ENTRY	PARENTAGE
High Zn variety	PBW 1 Zn	T.DICOCCON CI 9309/Ae.sq(409)/3/ Milan/S. 87230//BAV 92/4/2*MILAN/ S 87230//BAV 92
Genetic Stocks	BWL 9023	MILAN/AMSEL
	BWL 1663	GLUPRO/3*PBW 568
	BWL 1664	GLUPRO/3*PBW 568
C-derivatives, PBW-175 derivatives	BWL 3498, 3500	WL 711-Ae. Ovata/CS(S)//WL 711 NN/ 3/3* C 306
	BWL 3502, 3504	WL 711-Ae. Ovata/CS(S)//WL 711 NN/ 3/3* PBW 175
C-lines Hybridization between landraces	C 306	REGENT 1974/3*CHZ//2*C 591/3/P 19/C 281
	C 273	C 591/C 209
	C 518	TYPE 9/8A
	C 591	TYPE 9/8B
	WG 357	PV 18*C 273
Rainfed released	PBW 175	HD 2160/WG 1025
	PBW 644	PBW 175/HD 2643
	PBW 660	WG 6761/WG 6798
PBW 550 derivatives	BWL 5228, 5229, 5230, 5232	WL 711-Ae. Triuncialis IL/4*PBW 550/ 4/WL 711-Ae.ovata/CS(S)//WL 711 NN/3/4*PBW 550
	PBW 761	PBW 550//Yr15/6*Avocet/3/2*PBW 550
	PBW 762	Yr15/6*Avocet//2*PBW 550
	BWL 5429, 6209	PBW 550//Yr15/6*Avocet/3/2*PBW 550/4/GLUPRO/3*PBW 568//3*PBW 550
	BWL 6251	IITR 67/4*PBW 550
	PBW 550	WH 594/RAJ 3856//W 485
	BWL 5461, 5463	ARRINO/HD 3027
PBW-621 derivatives	BWL 3560, PBW 725	PBW 621//GLUPRO/3*PBW 568/3/ PBW 621
	PBW 758	HD 3027/PBW 621
	BWL 6249	C 306/4*PBW 621
	BWL 6250	PBW 621/1EL (1AS)//5*PBW 621
Local from HP	HIMACHAL LOCAL-1	

2.2. Estimation tryptophan content

The tryptophan content was estimated using Hernandez and Bates (1969) method. The samples were de-moisturized and defatted before extraction procedure. Finely ground wholemeal flour was wrapped in small packets of whatman filter paper and immersed in petroleum ether for 72 h (40 °C). Dried de-fatted sample (0.1 g) was taken into glass vials. 4 ml of freshly prepared papain solution was added to capped glass vials which were placed in incubator at 65 °C overnight after proper shaking. The following reagents were used.

i. Reagent A: 270 mg of $FeCl_3.6H_2O$ (high purity) dissolved in 0.5 ml of distilled water and volume made up to one litre with glacial acetic acid
ii. Reagent B: 15 N Sulphuric acid.
iii. Reagent C: Volume to volume mixture of reagent I and II
iv. Papain solution: Papain (Merk Lifescience Private Limited) (171 μM enzyme dissolved) in 0.1 M sodium acetate buffer, pH 7.0

Hydrolyzed samples were left to cool at room temperature and a clear supernatant was aspirated. To 1 ml of supernatant 4 ml of freshly prepared (1 h before using) reagent C was added. After shaking the samples were kept in an incubator at 65 °C for 15 min. Absorbance against the blank was recorded at 545 nm (Spectro UV-Vis-2505 LaboMed Inc., United States). Standard curve of DL-tryptophan (Merk Lifescience Private Limited) was prepared at a concentration range of 0–35 μg/ml and expressed tryptophan as g per 100 g of protein. The results are expressed as g per 100g of protein.

2.3. Estimation of protein content

The protein content of bread wheat grains was analyzed by a non-destructive method. Whole grain analyzer Infratec 1241 supplied by M/S Foss Analytical AB, Sweden was used for the purpose. The instrument is based on the principle of using near-infrared light transmitted through the grains which is pre-calibrated with protein concentration and moisture levels (Kaur et al., 2020). The results were displayed as percent protein (14% moisture basis).

2.4. Gluten content and gluten index

Perten Glutomatic® 2100 System was used to measure the content of gluten protein and determine gluten index. 10 g of wheat flour was mixed with water and kneaded into a ball. After a rest of about 20 min, gluten protein was extracted from it using AACC (2000) method. The wet gluten obtained was then centrifuged in Gluten Index Centrifuge to get strong and weak gluten fractions, which were weighed and gluten index was calculated. The complete gluten fraction was then dried in the Glutork 2020 gluten drier and the weight of dried gluten fraction was recorded. The gluten index was expressed as the percent wet gluten retained inside the centrifuge cassette.

2.5. Sedimentation value

The sedimentation value of whole grain flour was measured using Axford et al. (1979) method. Six g of ground sample was dispersed in 50 ml of water. Then, 50 ml of a solution containing 0.002 g ml^{-1} of SDS (Sodium Dodecyl Sulphate) and 0.002 g ml^{-1} of lactic acid was added and samples were repeatedly inverted after set time intervals. The samples were allowed to stand for 20 min. The sedimentation volume was recorded and contents were expressed in ml.

2.6. Grain hardness

Hardness of bread wheat grains was measured by using the grain hardness tester supplied by M/S Ogawa Seiki Co. Ltd., Japan. 10 grains of

each line were taken randomly and crushed one by one. The mean force (N) needed to crush a single grain was calculated.

2.7. Hectoliter weight

Hectoliter weight also known as test weight was determined using an apparatus developed by the Indian Institute of Wheat and Barley Research (IIWBR), Karnal. The weight of grains required to fill the container to the full of its capacity was recorded and the hectoliter weight was expressed in kg/hl.

2.8. Grain appearance score

The genotypes were scored based on grain size, shape, colour and lustre. A subjective score was given to each genotype out of a maximum score of 10 for each attribute and a combined weighted score was finally calculated out of 10. The subjective scoring was done by a panel of expert breeders and cereal analysts to a set of randomly placed grains of all genotypes (in triplicates).

2.9. Statistical analysis

The Pearson correlation coefficients of all the parameters studied are listed in Table 2. The data recorded for all the parameters was the average of triplicates with standard deviations depicted in graphs. The data obtained was analyzed for variance (ANOVA) using Completely Randomized Design (CRD) factorial. The CD values at 5% level of significance were calculated by analyzing the mean values of triplicates and inferences were derived accordingly. The critical differences in the values due to Environment, Genotype and Environment × Genotype interactions are listed in Table 3.

3. Results

3.1. Protein and tryptophan content

The protein content of the whole set ranged from 8.80% (PBW 644) to 13.88% (BWL 1663) during Rabi season 2017–18 (Figure 1a). The average protein content of the set was 10.61%. The whole set was categorized into three groups viz. low (<9%), medium (9–11%) and high (>11%). Three genotypes viz. PBW 644, BWL 6249 and PBW 175 were having low protein content, 22 genotypes had medium protein content and 9 genotypes showed higher content of protein in their seeds.

The tryptophan content ranged from 0.8 g per 100 g protein to 2.4 g per 100 g protein during 2017–18 and from 0.4 to 1.8 g per 100 g protein during 2018–19 with a mean value of 1.4 g per 100 g protein and 0.8 g per 100 g protein respectively (Figure 1b). Lower values of tryptophan content in second year indicate the effect of environment as indicated by the ANOVA (Table 3). Protein content correlated negatively with tryptophan (**−0.403***) (Figure 1b) (Table 2). The tryptophan content was found to be strongly correlated with gluten index i.e. the cultivars having higher content of tryptophan showed greater gluten strength (**0.422***). On the contrary, higher tryptophan content was accompanied by reduced dry gluten (**−0.411***) and total protein content (**−0.403***) (Table 2). Thus, indicating tryptophan's role in determining the grain protein quality rather than quantity. Both grain appearance score (**−0.375***) and hectolitre weight (**−0.405***) correlated negatively with tryptophan content. It also showed negative but non-significant correlation with grain hardness (**−0.255**). PBW 550 derivatives showed higher values of tryptophan content followed by current commercial varieties.

Table 3. Analysis of variance.

Factors	Try	GI	DG	SDS	GH	GA	HW
Environment	0.10	0.37	0.64	0.41	NS	0.08	0.23
Genotype	0.40	1.54	2.64	1.70	1.23	0.31	0.97
Genotype X Environment	0.50	2.18	NS	2.40	NS	0.44	1.37

NS-Non significant.
Values are significant at 5% ($P \leq 0.05$) level of significance.
Note: Try-Tryptophan; GI-Gluten Index; DW-Dry Gluten; SDS-Sedimentation; GH-Grain Hardness; GA-Grain Appearance; HW-Hectolitre Weight.

3.2. Quality characteristics

To evaluate the quality of the set, it was estimated for gluten index, dry gluten content and SDS-sedimentation value. Gluten index (GI) provides information on textural the properties of gluten. Values above 80 indicate strong gluten with high ratio of glutenin to gliadin. The GI of the set ranged from 49.22 to 95.12 with mean value of 75.98 (Figure 2). BWL 3560, BWL 5230, BWL 5429, BWL 5461, BWL 6209, PBW 1 Zn, PBW 550, PBW 725, and PBW 761 were significantly superior and stable during both years. GI was found to be congruent with SDS-sedimentation values even at 1% level of significance (Table 2), which is as per the expectations as both the parameters indicate gluten strength. The commercial varieties and PBW 550 derivatives were having higher GI and SDS-sedimentation values than other groups.

Dry gluten content gives the dry matter of gluten protein present in flour. It ranged from 9.2 to 18.5% (Figure 3). BWL 1663 being a protein genetic stock was significantly superior during both years. Dry gluten and protein content associated positively with each other (**0.803****).

To evaluate wheat quality for breeding, the gluten strength is usually determined by SDS sedimentation value (Brites and Carrillo, 2001). Measurement of sedimentation is based on the fact that gluten protein absorbs water. The Sedimentation value of the whole set ranged from 31.0 (HL 1) to 58.5 ml (BW 9023) (Figure 2). BWL 1663, BWL 3560, BWL 6209, BW 9023, PBW 1 Zn, PBW 725, PBW 758 were significantly superior in terms of their SDS-sedimentation values and were stable during

Table 2. Correlation coefficients.

	Try	GI	DG	SDS	PC	GH	GA	HW
Try	1							
GI	**0.434***	1						
DG	**-0.411***	-0.320^{NS}	1					
SDS	0.193^{NS}	**0.422***	0.039^{NS}	1				
PC	**-0.403***	-0.193^{NS}	**0.803****	0.253^{NS}	1			
GH	-0.255^{NS}	0.023^{NS}	-0.063^{NS}	**-0.359***	0.091^{NS}	1		
GA	**-0.375***	-0.125^{NS}	0.030^{NS}	-0.201^{NS}	-0.016^{NS}	**0.344***	1	
HW	**-0.405***	-0.190^{NS}	-0.060^{NS}	-0.264^{NS}	-0.026^{NS}	**0.546****	**0.698****	1

NS- Non-significant.
Note: Try-Tryptophan; GI-Gluten Index; DW-Dry Gluten; SDS-Sedimentation; PC-Protein Content; GH-Grain Hardness; GA-Grain Appearance; HW-Hectolitre Weight.
* Significant at 5% level of significance ($P \leq 0.05$).
** Significant at 1% level of significance ($P \leq 0.01$).

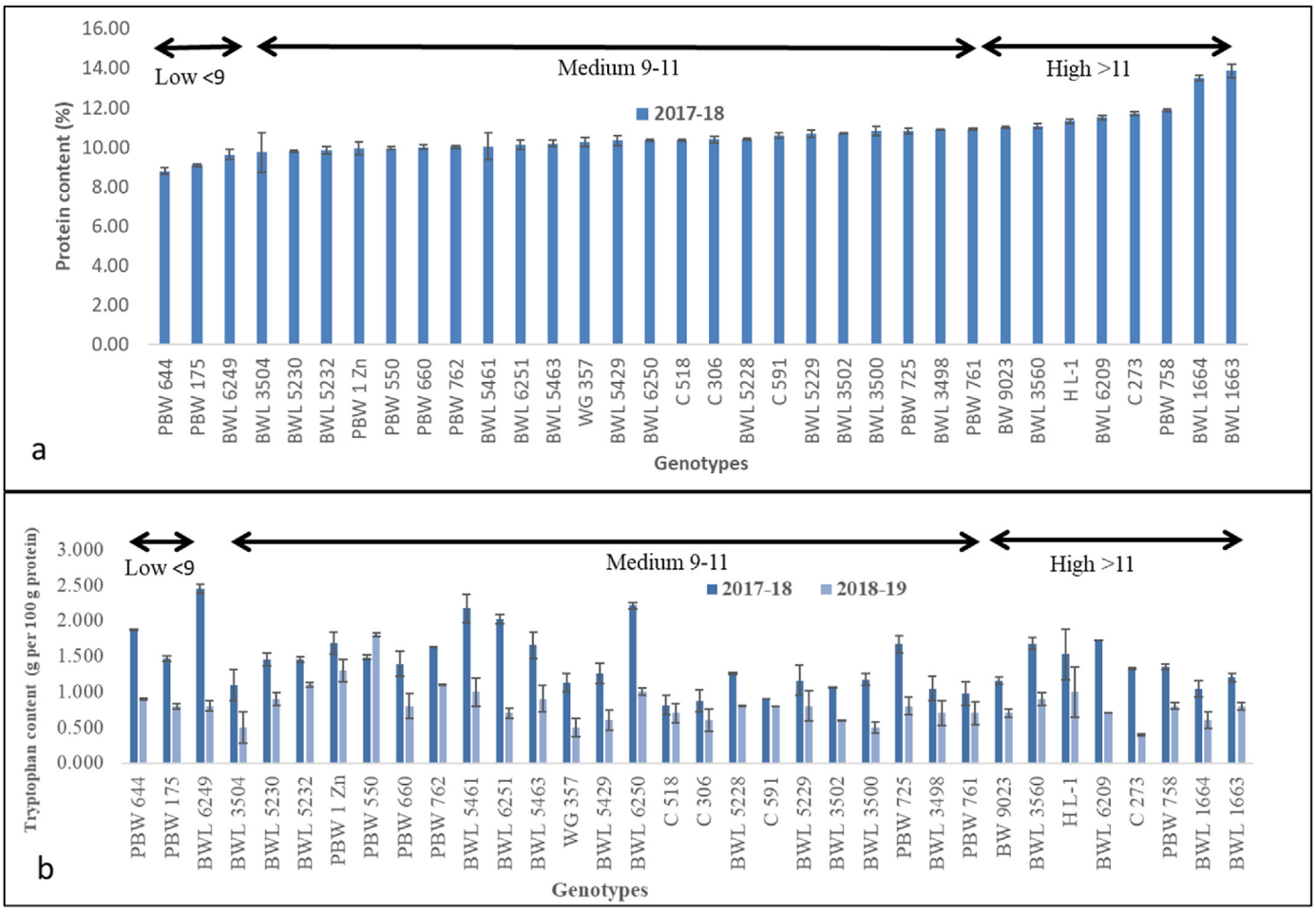

Figure 1. Characterization on the basis of protein content (a), inverse trend of tryptophan content with protein content (b). Values are mean ± SD of independent triplicates.

both years. SDS-sedimentation values were found to be negatively significantly correlated with grain hardness (**−0.359***) (Table 2).

3.3. Gain characteristics

The physical grain attributes such as hardness, appearance and hectoliter weight of the whole set was evaluated. The hardness of grains is an important and distinguishing factor in wheat (Kleijer et al., 2007). It ranged from 83.36 to 120.62 N, with mean value of 104.34 N (Figure 3). The trait was found to be independent of the environmental effect as the critical difference observed in the values of grain hardness was mainly due to genotypic factors (Table 3). WG 357 had significantly harder grains during both years. In addition to it, BWL 5232 also had edge over other genotypes in terms of grain hardness during 2018–19 Rabi season.

The grain appearance is a subjective parameter, gives a direct reflection of seed shape, size, colour, and lustre. These attributes of grains reflect environmental and genetic effects especially during the period of grain filling. The grain appearance score of the set ranged from 5.1 to 6.5, with average score of 5.7 (Figure 3). Tall landraces and C-derived varieties tend to have higher grain appearance scores than the rest of the bread wheat genotypes especially C 306, BWL 3504 and BWL 5232 were at par in terms of their grain appearance.

Hectolitre weight also called test weight is the measure of the fullness of the grain. The test weight of the genotypes had mean value 76.8 kg/hl (Figure 3) ranging from 72.4 (BWL 6250) to 80.0 kg/hl (C 306). The effect of environment was significant for all the entries but nine genotypes (BWL 3504, BWL 5229, BWL 5232, BWL 5461, BWL 5463, C 273, C 306, C 518 and WG 357) were found to be significantly superior and tolerant to environment effect as they performed consistently better during both years. The check variety C-306 was at par with the highest average hectolitre weight of 80.0 kg/hl. In fact, the whole group of C-varieties had higher values of hectolitre weight. All three of grain characteristics studied correlated highly positively with each other (Table 2).

4. Discussion

Wheat's protein content in previous studies was found to vary from 10 to 18% of the total dry matter (Zuzana et al., 2009). The protein content of commercial wheat varieties grown in India is said to range between 9% and 11% (Singh et al., 2007; Siddiqi et al., 2020). Huruskova et al. (2004) reported average protein content of wheat to be approximately 12%. The protein content of the genotypes tested in this study fell well within this range except for BWL 1663 and BWL 1664 which had significantly higher protein levels being protein genetic stocks and having *Gpc-B1* gene. The tryptophan content of the present set showed a remarkable genotypic difference (Table 3). Gafurova et al. (2002) reported similar range of tryptophan quality index in bread and durum wheat genotypes however comparatively lower values of wheat tryptophan content is reported in the literature as well (Žilić et al., 2011). The inverse relation between protein content and tryptophan content is reported in pearl millet and maize by Mathur and Mathur (1986) but in contradiction to that Olakojo et al. (2007) observed a positive correlation between both. Non-significant inverse relation between tryptophan content and grain hardness indicate the role of tryptophan in determining kernel texture. This trend is also

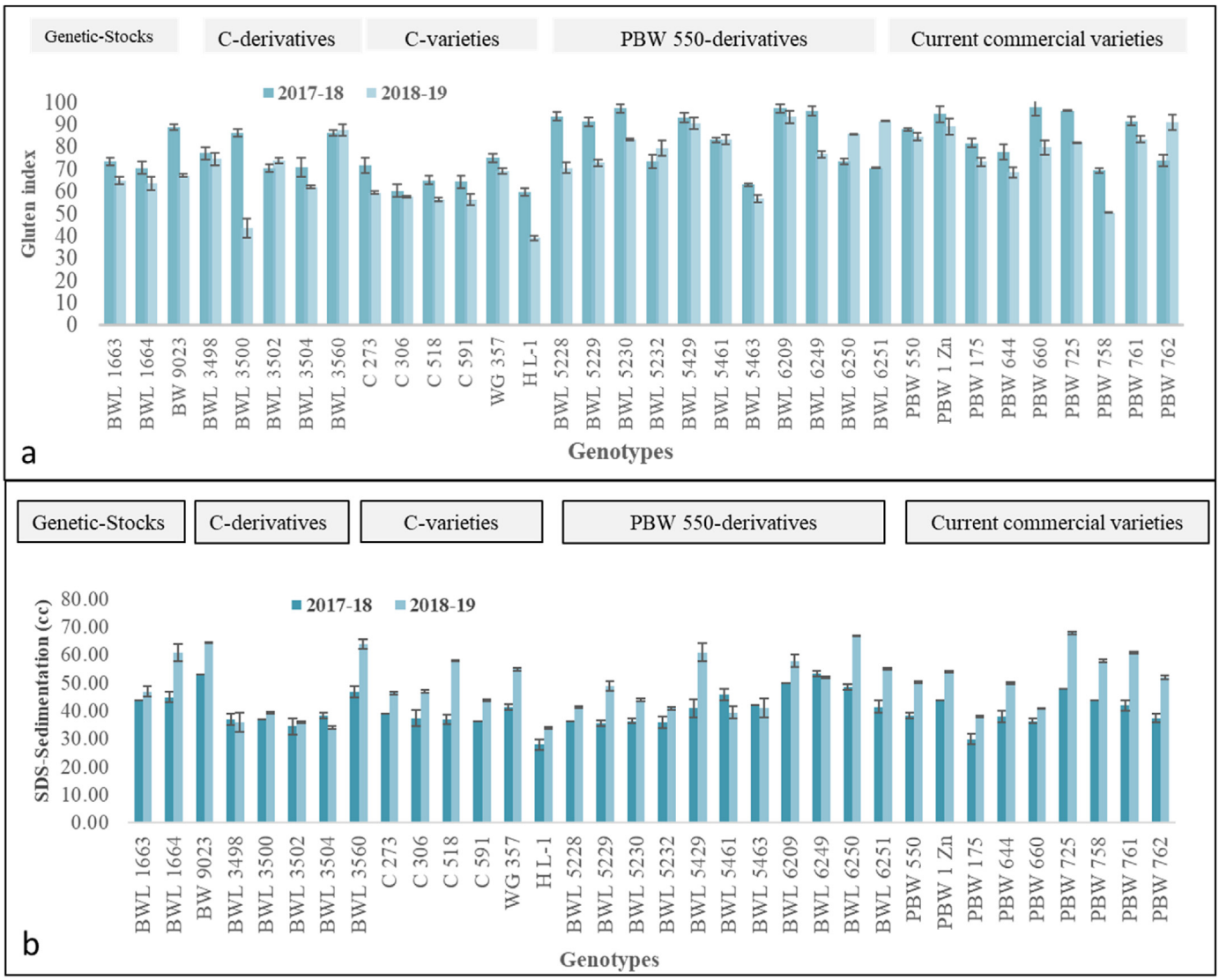

Figure 2. Graphs of gluten strength: Gluten Index (a), SDS-sedimentation (b). Values are mean ± SD of independent triplicates.

witnessed in maize as maize mutant having higher tryptophan content have chalky kernels (Vasal, 2000; Gupta et al., 2013). The GI range reported by Kaushik et al. (2015) lied between 66.25 and 75.96 which was found to be slightly lesser than the range of GI observed in the present study. The studies by Magdic et al. (2006), Kaur et al. (2020) and Ma et al. (2021) showed that the gluten index ranges from 62% to 99% which is in line with the results obtained for the tested set of genotypes. Ma et al. also deduced non-significant relation of GI with hardness index. Significant effect of environment observed on GI may be due to the conditions during the grain filling period. Although, GI and tryptophan content have been analyzed together in a study by Žilić et al. (2011) but correlation between them was not reported. Sedimentation values obtained in present study are in agreement with the values mentioned in previous studies (Supekar et al., 2005; Huruskova et al., 2004; Rittau et al., 2005; Ma et al., 2021). The significant positive correlation between SDS-sedimentation and Gluten index indicate the complimentarily of both parameters in measurement of gluten strength. Also the positive correlation between tryptophan and gluten index indicate the role of this amino acid in influencing gluten quality.

Studies reveal that the dry gluten content in wheat cultivars ranges from 8.65 to 10.35% (Kaushik et al., 2015). However, according to the results obtained by Singh and Singh (2006) dry gluten content ranged between 5.9 and 10.1%. The dry gluten content was determined to fall between 9.4 and 12.7% by Supekar et al. (2005). In present study, the effect of environment × genotype interactions was found to be non-significant on dry gluten content, evidence of which is present in the studies by Jha et al. (2012). In contradiction to the results obtained in this study, some authors reported a significant correlation between grain hardness and dry gluten content (Hruskova and Svec, 2009). Both these parameters showed negligible variations due to Genotype environment interactions, which is the reason for classifying wheat genotypes into hard, medium hard and soft on the basis of the former trait whereas the later helps to determine the application of a particular genotype in baking industry. The hectolitre weight of Indian bread wheat is said to range between 75 and 83 kg/hl by Kumar et al. (2018). The average hectoliter weight of the present set fell well within this range. The inverse relation between tryptophan content and grain hardness observed in this study is reported in cereal maize as well (Scott et al., 2004). The trait of boldness of the grains influences grain appearance, hardness and hectolitre weight such that all three parameters correlated positively and significantly in this study. The negative correlation of grain appearance and hectoliter weight with gluten index, SDS-sedimentation and tryptophan content signify that these physical grain attributes do not reflect gluten quality of wheat grains.

Just as other cereals like maize, wheat also has lower contents of essential amino acids like tryptophan FAO (2013) and FAO (2017).

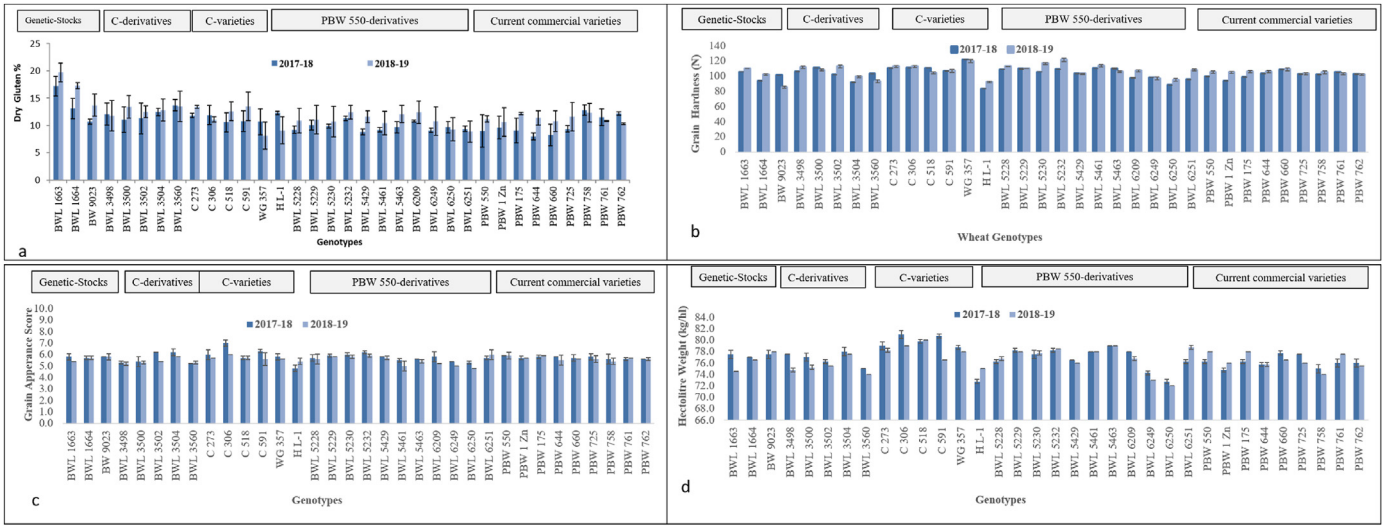

Figure 3. Dry gluten content (a), Grain characteristics: Grain hardness (b), Grain appearance Score (c), Hectolitre weight (d). Values are mean ± SD of independent triplicates.

Several different approaches have been made to enhance nutritional quality of maize protein. In early 1920s, a mutation, called *Opaque-2*, which corresponds to high concentrations of lysine and tryptophan as compared to normal maize, was first described. *Opaque-2* mutant are known to have almost double the quantities of lysine and tryptophan (4.15% and 1%, respectively) as compared to normal varieties (2.7% and 0.6%, respectively) (Wu et al., 2010). In future, if such an attempt is made for cereal wheat than it might give a perk of better processing-quality as a result of increased gluten strength. However, some pleotropic effects were observed in *opaque-2* grains as the mutated grains had dull kernel appearance with reduced grain yield which is found to be in accordance with the results of present study on wheat as grain appearance and hectoliter weight showed significant negative correlation with tryptophan content (Vasal, 2000; Gupta et al., 2013).

5. Conclusion

All the wheat quality parameters in this study showed compliance with their respective values and ranges reported in literature. In addition to that, tryptophan content showed a remarkable genotypic difference. The significant positive correlation between SDS-sedimentation and Gluten index indicate the complimentarily of both parameters in measurement of gluten strength. Grain hardness and dry gluten content showed negligible variations due to Genotype environment interactions, therefore can be used as basis of classifying the wheat genotypes into hard, medium hard and soft on the basis of the former trait whereas the later helps to determine the application of a particular genotype in baking industry. The negative correlation of grain appearance and hectoliter weight with gluten index, SDS-sedimentation and tryptophan content signify that these physical grain attributes do not reflect gluten quality of wheat grains. The observed association of tryptophan content with gluten strength (GI) in present research may help breeders to come up with varieties with increased nutritional value along with improved baking quality.

Declarations

Author contribution statement

Rupinder KAUR: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Harinderjeet KAUR: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data.

Puja SRIVASTAVA: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

Acknowledgements

We are very grateful to Dr. Dharam Paul Chaudhary, ICAR-IIMR for providing various instrument and chemical facilities through the course of research.

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