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Interaction Of High Molecular Weight 1D Glutenin Subunit In Durum Wheat

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ABSTRACT

A set of three disomic substitution lines for chromosome 1D of *Aegilops tauschii* developed in 1990 in *Triticum durum* cv. PBW114 background carried genes for red glume color. Out of three, two lines (1D3597 and 1D3601) had a substitution of chromosome 1D(1A) and one line (1D3598) had a substitution of chromosome 1D(1B). The SDS-PAGE analysis of the disomic substitution line for chromosome 1D(1A) showed the presence of HMW-GS subunit of 2^t + T2 and 6+8 belonging to 1D, 1B chromosomes and substitution line for chromosome 1D(1B) showed the presence of 2^t + T2 glutenin subunit belongs to 1D chromosome. Micro SDS-sedimentation test analysis of disomic substitution lines (3597 and 3601) for chromosome 1D(1A) had increased nearly two-fold in SDS-sedimentation value than to the parent durum cultivar and substitution line 3598 for chromosome 1D(1B) slightly decreased than to the parent durum cultivar PBW114. The present study describes the interactions of 1D chromosomes in the *Triticum durum* cultivar PBW114 and their effect on bread-making quality. The analysis suggests that chromosome 1B is a slightly more important subunit than chromosome 1D and in the combination of both chromosomes 1B and 1D important allele to improve bread-making quality in durum wheat.

Keywords: Durum wheat, Glutenin, *Aegilops tauschii*, Micro SDS-sedimentation, HMWGS

Introduction

Among cereals, hexaploid wheat (*Triticum aestivum*, 2n = 6x = 42, AABBDD) represents the cereals with 2nd largest production along with tetraploid wheat (*Triticum durum*, 2n = 4x = 28, AABB). Bread wheat accounting up with 95% of total wheat production and the remaining 5% represents durum wheat worldwide. The genomic constitution of bread wheat has three genomes (AABBDD) with 42 chromosomes while durum wheat contains two genomes (AABB) with 28 chromosomes. Common wheat is also known as bread wheat because of its baking properties and protein composition. Durum wheat is also known as pasta wheat because of its high carotenoid and protein content. D genome of common wheat plays a major role in determining the end-use quality of wheat flour due to the long arm of the 1D chromosome of bread wheat carries some beneficial high molecular weight subunits 5+10, 2+12, and 2^t+T2 important for bread-making quality. Due to the lack of the D genome from the durum wheat, both durum, as well as bread wheat, has different physical properties for gluten to their respective flour, due to this reason common wheat traditionally has been used for making bread, and durum wheat is used for pasta, semolina, etc.

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There is a predominant perspective that durum wheat lacks extensibility, high carotenoid, high starch content, high water absorption capacity, and is viscous as par with common wheat with lesser loaf volume therefore they are used for making flat bread. However, the research on the bread-making quality of pasta wheat has improved over time. Wheat homeologous group 1 chromosome carries genes for seed storage proteins: High molecular weight (HMW) glutenin present on the long arm of group 1 homeologous chromosome and low molecular weight (LMW) glutenin and gliadin α, β, γ, and δ gliadin on the short arm of group 1 homeologous chromosome (Bietz et al. 1975; Lawrence and Shepherd 1980; Li et al. 2020; Shepherd 1968; Wrigley and Shepherd 1973; Zhang et al. 2018). In the absence of the 1D chromosome and the silencing of the long arm of the *Glu-A1* locus, most durum wheat cultivars possess poor gluten-strength dough extensibility and poor bread-making quality (Bustos et al. 2000; Lafiandra et al. 1997; Sharma et al. 2020). There is a lot of evidence that chromosome 1D has a great impact on both dough extensibility and elasticity. The introgression of gluten proteins encoded by genes of the 1D chromosome of bread wheat is associated with the improved baking performance of durum wheat (Lukaszewski and Curtis 1994; Kiszonas et al. 2021; Morris 2021; Ruiz et al. 2021; Vitellozzi et al. 1997).

In the past several substitution lines have been developed for chromosomes 1D(1A) and 1D(B) by transferring the 1D chromosome of hexaploid wheat into durum wheat cultivars (Xu et al; 2005). Some previous work has been done on the baking performance of durum wheat by developing a complete set of disomic substitution lines of D-genome carrying *Dx2+Dy12* allele from Chinese Spring was prepared in a durum wheat cultivar 'Langdon' which showed that 1D chromosome in place of 1A has a great effect on gluten characteristics in terms of SDS sedimentation volume, mixing time and alveograph parameter positively associated with improved bread making performance (Joppa et al. 1998). Latter Liu and co-workers produced a substitution line for chromosome 1D(1B) by transferring the 1D chromosome into the durum cultivar 'Edmore' with superior dough mixing properties (Blanco et al. 2002; Lukaszewski et al. 2003; Saini et al; 2022). PBW114 is a superior durum variety and resistant to Karnal bunt. A set of three *Aegilops tauschii*-1D chromosome disomic substitution lines were developed into a durum wheat cultivar, 'PBW114' using donor parent *Ae. squarrosa* (DD) accession 3754 (Dhaliwal et al; 1990; Garg et al; 2007). In the present study interaction of high molecular weight glutenin1D subunit into durum wheat cultivar, PBW114 and their effect on bread-making quality have been observed.

Materials and methods

Plant Materials

Selfed seed of disomic substitution lines of chromosome 1D(1A) (1D3597, and 1D3601) and chromosome 1D(1B) (1D3598) along with recipient durum cultivar 'PBW114' were used in the present study. These lines were evaluated for molecular marker analysis, SDS-PAGE, nutritional, and bread-making characteristics and were grown in Eternal University experimental farm (2019-2021) with the recommended agronomic practices for the cultivation of wheat.

Morphology, moisture content, and yield characteristic

In the present study seven morphological characteristics of all disomic substitution lines (1D3597, 1D3598, and 1D3601) along with recipient durum cultivar 'PBW114' were measured. Spike morphology, glume characteristic, thousand-grain weight, tillers number per plant, spikelet number per spike, spike density, and spike length were measured. Five plants

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were randomly selected from each cultivar for the measurement of the traits. The spike length (cm) was measured without an awn. The following formula was used for the estimation of spike density.

Spikelet density = spikelet number per spike /spike length

Molecular marker analysis

Genomic DNA was isolated from the leaf samples using the CTAB method (Murray et al. 1980). To amplify genes for short and long arms of group 1 homeologous chromosome SSR and one dominant marker were used according to their relative map position. The sequence of the primers was retrieved by using grain genes from Xgwm (Röder et al. 1998), Xbarc (http://www.wheat.pw.usda.gov/GG2/ index.shtml), and Xwmc (Somers et al. 2004). PCR reactions were carried out according to the method of Röder et al. (1998) with some minor modifications. The DNA from the parents was extracted and further analyzed by SSR group 1 homeologous marker for chromosomes (1AS, 1AL, 1BS, 1BL, and 1DL) and one dominant marker was used for the short arm of chromosome 1D.

Electrophoretic separation of glutenin, SDS-sedimentation volume and grain protein content

Extraction of glutenin protein was carried out from the three durum wheat substitution lines 1D(1A) (1D3597, and 1D3601) and 1D(1B) (1D3598) along with their parent cultivar PBW114. The sodium dodecyl sulfate (SDS-PAGE) was performed using 12% acrylamide according to the method described by Singh et al. (1991). The gel profiling of the high molecular weight glutenin subunit (HMW-GS) was analyzed according to Smith and Payne (1984), using three wheat and two cultivars as a control Chinese Spring, WL711, PBW343, PDW233**.** Total grain protein content was estimated by using the standard procedure described by Kjeldahl (1883). The SDS-sedimentation volume was measured on small scale using three replications in 1g of wheat flour dissolved in lactic acid and SDS solution by using the standard protocol followed by Dick and Quick (1983).

Cytological analysis

To study the meiotic chromosome behavior, spikes were fixed in Carnoy's fixative solution of a ratio of (ethanol/chloroform/acetic acid = 6v/3v/1v) for 1 day and then transferred into a 70% of ethanol solution. The anthers of pollen mother cell squashed in 2% acetocarmine solution. Chromosomal number and pairing behavior of the pollen mother cell was observed at meiosis-I by using a compound microscope (Magnus, New Delhi, India) and the image was captured using a digital camera (12J875).

Loaf volume

The bread was made by using whole wheat flour (100 g) protocol followed by Food Technology Laboratory at Eternal University. Whole wheat flour, bread improver, sugar, salt, and activated yeast are some principal components used for preparing the dough. The dehydrated yeast was activated by adding it to the warm water (temperature of 28°C and 80% relative humidity) for 10 min. It was added to the sample flour and mixed for 2 min at 60 rpm followed by 5 min at 95 rpm. The dough was kept for 15 min at a temperature of 28°C and 80% RH. The dough was put for kneading or punched to reduce excessive CO_2 throughout the mass the process was known as punching or knocking back, and then dividing the dough into equal pieces which were then rounded and molded by using hands. After that, the rounded dough pieces were transferred to the pre-greased pans and then all dough pieces

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were put into a proving cabin which was kept at a temperature of 35°C, 80% RH for 60 min, and then they were transferred to the oven and baked for 20 min at the temp of 210°C. The bread was allowed to rest for 1 h at room temperature and loaf volume was measured by the seed displacement method (Greene and Bovell 2004).

Determination of gluten content and water absorption capacity

The total gluten content was determined by using the procedure described using the AACC method (2005). The dough was prepared by using 2% NaCl solution in 40 g of wheat flour and immersed in water for 1 h because most of the starch was removed. After that, the remaining dough was washed into running water by gentle tapping by hand till the complete starch was removed from the dough. The viscoelastic mass obtained was gluten. The specific sedimentation volume/water absorption capacity of the gluten sample was determined using the method described by Sosulsk (1962). Dried gluten is crushed into a fine powder by using a metal-free grinder/cryomil (Retsch, Germany). Five hundred milligrams of gluten sample was mixed in 10 mL water shake thoroughly for 1 h and their sedimentation volume was measured.

Carotenoid pigment

Approximately 10-15 g of grain sample was crushed in a metal-free grinder cryomill to obtain a fine powder. Three color parameters (CIE L*a*b) were measured on the grounded fine flour using a Konika Minolta chromometer (R-400). The CIE L*a*b values were measured in three colors according to CIE 1976 (Commission Internationale de l'Eclairage). The CIE a* represents the redness of the flour. CIE L* values represent the brightness of the flour ranges between 0-100 (black-white) and CIE b* represents the yellow color of flour i.e., the carotenoid pigment of flour in durum wheat b represents leutin content.

Results

Grain and spike morphology of substitution lines

All the derivative substitutions of 1D for 1A and 1B have red glume color (Fig. 1). The 1000-grain weight of line 1D3598 has significantly similar to its parent durum cultivar 'PBW 114' although having fewer tillers and compact dense spikes with more spikelet. While the 1000-grain weight of the other three lines has lower than that of the parent durum cultivar (Table 1). However, the yield of line 1D3598 has a significantly much lower yield than its parent durum cultivar because of a smaller number of tillers. While the other three lines have a higher number of tillers and slightly lower yields than their parent durum cultivar. Line 3598 has a longer spike length than its parent durum cultivar and the other two derivative lines 3597 and 3601 have smaller spike lengths. Line 3598 has a higher spikelet number per spike than the parent durum cultivar while the other three lines have similar spikelet numbers to the parent durum cultivar PBW114. The spike density of line 3598 is higher while line 3597 and 3601 has slightly lesser spike density than their parent durum cultivar. The moisture content of line 1D3598 has significantly lower than its parent durum cultivar while the other lines have similar moisture content.

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Fig 1 Glume color of substitution lines for chromosome 1D(1A) and 1D(1B)

Table 1 Morphological character of substitution lines for chromosome 1D(1A) and 1D(1B) along with its parent durum cultivar PBW114

Sample ID	Plant height (cm) (Mean±SD)	Glume color	1000 grain weight (g) (Mean±SD)	Spike morphology	Number of tillers (Mean±SD)	Spikelet number per spike	Spike density (Mean±SD)
PBW114	7.0±0.56	White	51.06±2.5	Square Head	8-11	17-18	2.9±0.22
1D3597	6.7±0.59	Red	45.23±3.1	Square Head	7-10	17-19	2.5±0.17
1D3598	7.2±0.48	Red	51.06±2.0	Compact Head	4-6	21-23	3.2±0.21
1D3601	6.9±0.41	Red	42.23±1.5	Square Head	7-10	18-20	2.7±0.29

Cytological analysis and molecular analysis

The cytological analysis of lines1D3597, 1D3601, and 1D3598 had shown 26 own durum chromosomes and a pair of 1D chromosomes (Fig. 2). To examine the chromosome constitution of all derivative lines molecular analyses were conducted using two controls one is its parent durum cultivar PBW114 and 2nd one was *Ae. squarrosa* carried the D genome (Fig. 3). The PCR analysis was performed using SSR markers using the short and long arm of

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group 1 homoeologous markers and one dominant marker belonging to the short arm of chromosome 1D. Line 1D3597 and 1D3601 give amplification on the short and long arms of group-1B and group 1D chromosomes and did not give amplification on the short and long arms of group-1A chromosomes. This finding also matched with the cytological analysis and suggests that chromosome 1A substituted for 1D. Line 1D3598 had given amplification on the short and long arm of group-1A and group-1D chromosomes and did not give amplification on the short and the long arm of 1B chromosome and this finding suggests that chromosome 1B substituted for 1D.

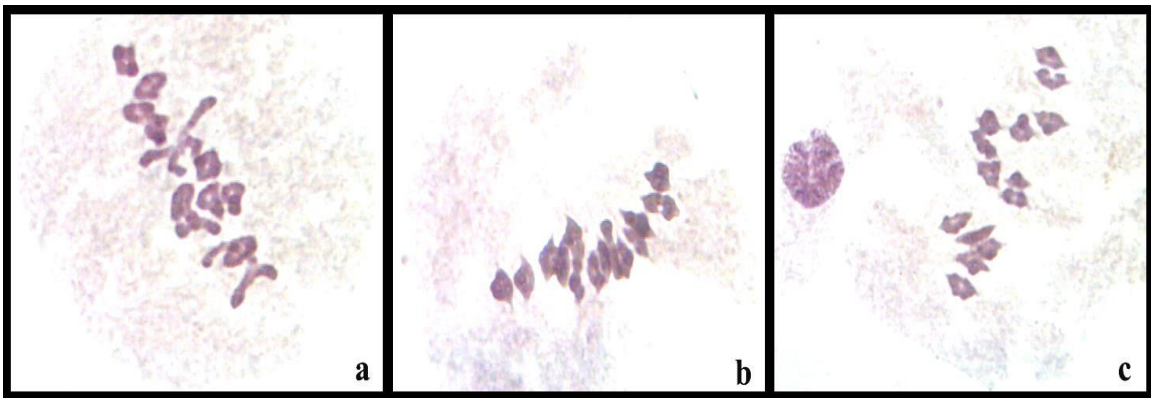

Fig 2 Chromosome pairing at metaphase-I of meiosis of disomic substitution lines. a-1D3597 (1D(1A)); b-1D3601 (1D(1A)); c-1D3598 ((1D(1B))

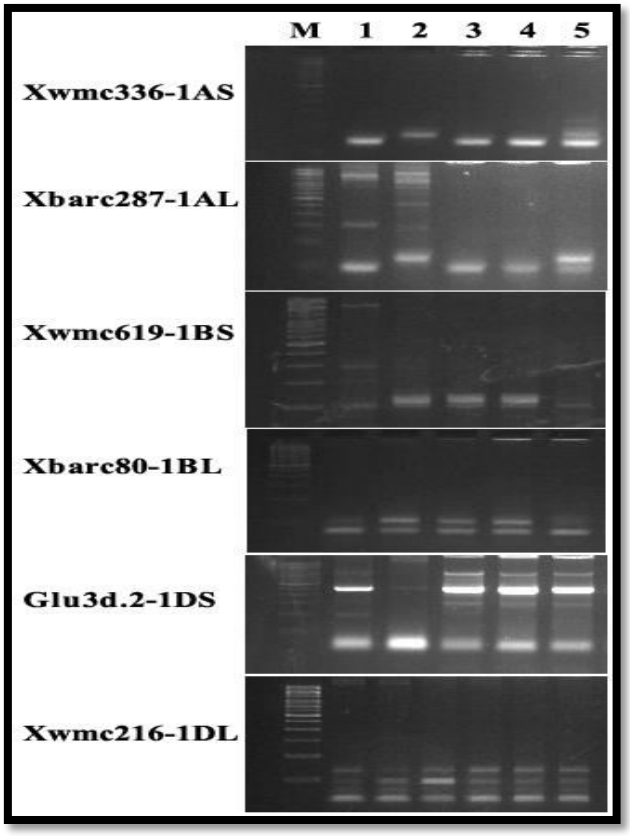

Fig 3 Molecular markers analysis for determination of chromosome constitution of substitution lines for chromosome 1D(1A) and 1D(1B)1. Ladder, 2. *Aegilops tauschii*, 3. PBW114, 4.1D3597, 5.1D3601, 6.1D3598

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Analysis of seed storage protein

The SDS-PAGE analysis showed, the parent durum cultivar 'PBW114' possesses 6+8 subunits of high molecular weight gluten subunit, belonging to the long arm of 1B chromosome (Fig 4). Line 1D3597 and 1D3601 carried two extra subunits which belong to the long arm of the 1D chromosome. The cytological and molecular analysis describes above suggest that the extra subunit was encoded by the genes present on the long arm of chromosome 1D. The low molecular weight glutenin profile of line 1D3597 and 1D3601 possesses the *Glu-B3* allele and the red arrow indicate the absence of the *Glu-A3* allele. Line 1D3598 carried only the extra glutenin subunit (2t+T2) which was encoded by genes of the long arm of chromosome 1D, however, it did not carry the parent subunit (6+8) which belongs to the long arm of 1B chromosome and the low molecular weight glutenin subunit of line 1D3598 possesses of *Glu-A3* allele and the black arrow indicates the absence of *Glu-B3* allele. This analysis suggests that lines 1D3597 and 1D3601 had a substitution of chromosome 1A (1D) and line 1D3598 had a substitution of chromosome 1B(1D).

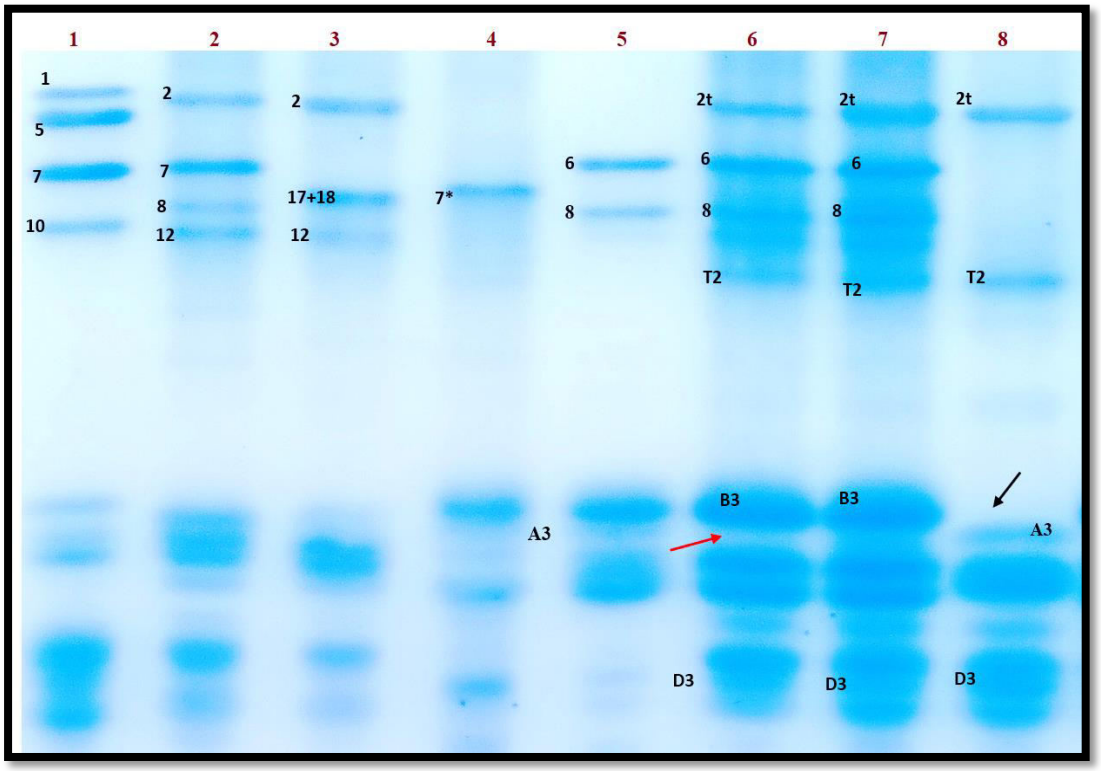

Fig 4 SDS-PAGE analysis of substitution lines for chromosome 1D(1A) and 1D(1B) along the control durum cultivars. 1. PBW343 (*Lr24+Yr57*), 2. Chinese Spring 3. WL711, 4. PDW233, 5. PBW114, 6. 1D3597, 7. 1D3601, 8. 1D3598

Analysis of protein content, carotenoid content, gluten content, water absorption capacity and micro-sedimentation analysis of bread-making quality

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To examine the effect of the addition of the 1D chromosome and the substitution of chromosome 1A (1D3597, and 1D3601) and substitution of chromosome 1B (1D3598) on bread-making quality concerning grain protein content, SDS-sedimentation value, loaf volume, and water absorption capacity (Table 2). Line 3598 has high grain protein content than its parent durum cultivar. Line 3597 and its parent durum cultivar had similar grain protein content and line 3601 had slightly higher grain protein content than their parent durum cultivar. Water absorption capacity/specific sedimentation volume range in descending order 3598>PBW114>1D3597>1D3601. The water absorption capacity suggests that line 3598 and the parent durum cultivar have higher starch content, and lines 1D3597 and 1D3601 have lower starch values. The SDS-sedimentation volume range in descending order is 3597>3601>PBW114>3598.

Line 3597 and 3601 with disomic substitution of chromosome 1D(1A) was significantly higher SDS-sedimentation volume than the parent durum cultivar (Fig. 5). The increment suggests that the addition has a positive effect on the 1D chromosome on dough strength and bread-making quality. Line 3598 with the substitution of chromosome 1D for 1B, showed SDS-sedimentation volume slightly decreased than to the parent durum cultivar 'PBW114'.Loaf volume directly impacts on bread-making quality of the wheat flour. The loaf volume of these lines ranges in the following descending order 1D3601>1D3597>PBW114>1D3598. The level of carotenoid content is nearly the same as the parent durum cultivar PBW114.

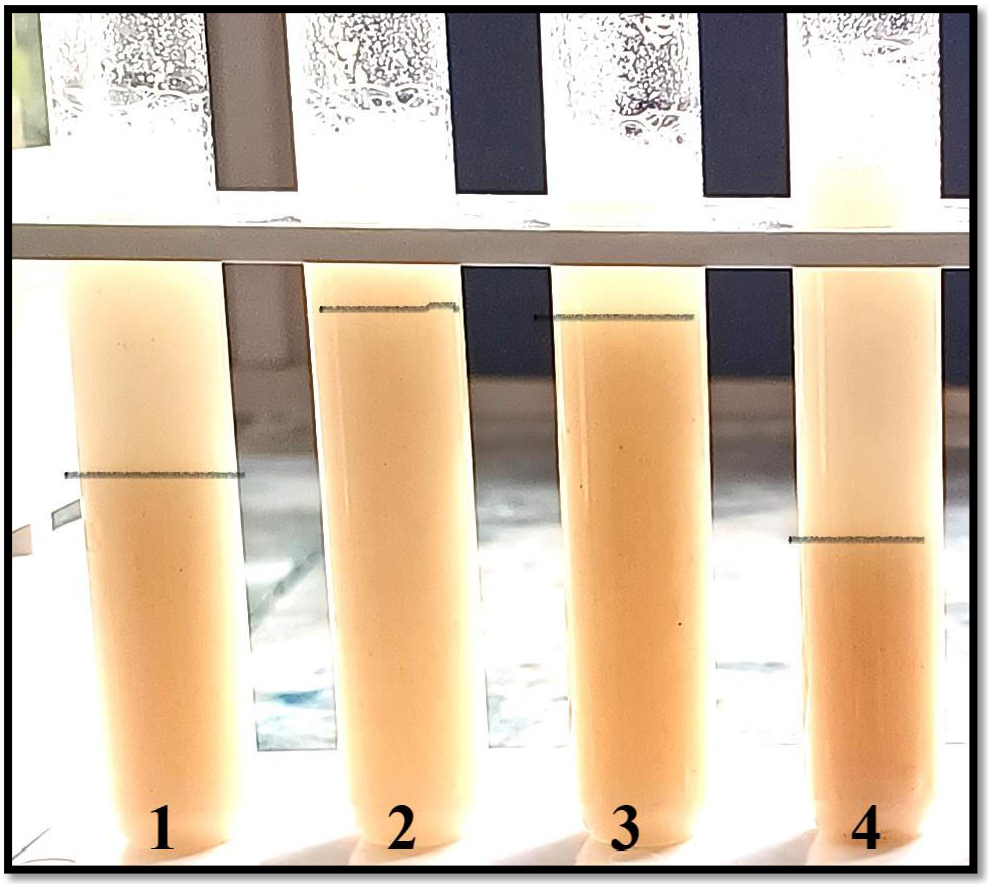

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Fig-5 SDS-sedimentation volume of substitution lines for chromosome 1D(1A) and 1D(1B) along with their parent durum cultivar PBW114. 1. PBW114, 2. 1D3597, 3. 1D3601, 4. 1D3601

Table 2 Nutritional characters and end-use baking quality test of substitution lines for chromosome 1D(1A) and 1D(1B) along with its parent durum cultivar PBW114

Sample ID	**Moisture content (%) (Mean±SD)**	**Specific sedimentation volume (ml) (Mean±SD)**	**SDS-micro-sedimentation volume (mm) (Mean±SD)**	**Grain protein content (%) (Mean±SD)**	**Loaf volume (ml)**	**Carotenoid content (L* a* b*) (Mean±SD)**		
PBW114	11.8±0.25	2.6±0.45	38±1.5	12.99±1.55	340±2.5	83.22±2.5	1.59±0.22	13.69±0.36
1D3597	10.8±0.19	1.8±0.26	64±4.5	12.32±0.55	450±3.9	83.78±1.9	1.73±0.19	13.09±0.25
1D3598	10.3±0.54	2.7±0.33	34±1.5	13.01±0.95	310±2.1	13.01±0.65	1.62±0.02	14.5±0.39
1D3601	11.3±0.26	1.6±0.39	62±3.4	12.32±0.83	455±4.2	81.98±4.5	1.61±0.05	13.21±0.21

Discussion

The main objective of durum wheat breeding is to produce varieties with efficient elasticity and extensibility to produce dual-purpose wheat for pasta and bread products. The 1D chromosome of bread wheat carries genes for seed storage protein and it is an efficient approach for enhancing the bread-making quality of durum wheat (Coriton et al. 2019). In the previous year's substitution line for chromosome 1D(1A) had been developed in durum cultivar 'Langdon' had increased two-fold in SDS-sedimentation value including rheological properties, mixing time (Blanco et al. 2002; Joppa et al. 1998). However, because of reduced yield and meiotic instability, the use of these lines is not recommended for release variety purposes [35]. Disomic substitution line for chromosome 1D (1B) and 1D(1A) has arisen spontaneously by the selfing of the monosomic 1D addition line for several years (Dhaliwal et al; 1990).

In wheat end-use quality depends on the amount of grain protein content and the amount of water absorption capacity of gluten content. Wheat with low grain protein content with low SDS-sedimentation value is used to make cakes, biscuits, pastries, etc. On the other side, wheat having high grain protein contents with high SDS-sedimentation value is used to make bread products. While durum wheat has high grain protein content, less SDS-sedimentation value, and high carotenoid content used to make pasta and macaroni, etc. Apart from SDS-sedimentation water absorption capacity of gluten content also determined end-use

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quality. High water absorption capacity is described as weak flour in terms of bread-making quality while low water absorption capacity is used to make bread products. Durum wheat is not used for bread-making purposes because of its low SDS-sedimentation volume. In the present analysis line, 3598 showed low SDS-sedimentation value, high-water absorption capacity, and high grain protein content suggesting that in durum wheat there has been no correlation between grain protein content and SDS-sedimentation value.

In hexaploid wheat (*Triticum aestivum*) higher grain protein content, low water absorption capacity, and low moisture content increase SDS-sedimentation values. The analysis also proved on the parent durum cultivar possesses high grain protein content, high water absorption capacity, and lower moisture content showing less SDS-sedimentation volume. Line 3597 and 3601 possess protein content approximately equal to parent durum with high SDS-sedimentation volume and low water absorption capacity showing that chromosome 1D had a positive impact on the dough strength in durum wheat. All analyses suggested that chromosomes 1D and 1B are both important alleles to enhance the bread-making quality of durum wheat. The long arm of chromosome 1B of Indian durum cultivar PBW114 carries 6+8 high molecular weight gluten subunit which was associated with improved bread-making quality.

The gliadin subunit of parent durum cultivar PBW114 possesses the γ-45 type of gliadin which was associated with good pasta and bread-making quality in contrast to a standard durum cultivar 'Langdon' widely used in durum wheat breeding possesses the γ-42 type of gliadin subunit (Carrilo et al. 1990). The removal of chromosome 1B with 6+8 gluten subunit from line 3598 and decrease in SDS-sedimentation value from parent durum cultivar 'PBW114' suggest that chromosome 1B is associated with good bread-making quality along with γ-45 type of gliadin subunit associated with good pasta making quality and can be used in future breeding purpose with high confidence towards better end-use quality. The key determinant allele for bread-making quality in wheat is the *Glu-D1* allele with some alleles in descending order 5+10 > 2+12 > t2+T2 are the most important allele associated with improved bread-making quality with increased SDS-sedimentation volume and loaf volume. In the present study by addition of the HMW gluten subunit, in this case, is 2t+T2, with the approximate increase in two-fold SDS-sedimentation value in lines 3597 and 3601.

Parallel wise disomic substitution line for chromosome 1B for 1D has SDS-sedimentation volume approximately equal or slightly less to the parent durum cultivar indicating that chromosome 1B with 6+8 subunit is a slightly more important allele than the t2+T2. The Increment of 2-fold SDS-sedimentation volume in lines 3597 and 3601 by the addition of 1D allele and slightly decreased SDS-sedimentation value by removal of *Glu-B1* allele from line 3598 also suggest that chromosome 1B with (6+8) gluten subunit has slightly more important allele than to the chromosome 1D with (2t+T2) gluten subunit. But both subunits play a major role to enhance bread making the quality of tetraploid durum wheat.

Conclusions

Durum wheat is widely used for pasta preparation because the ratio of carotenoid content and elasticity is much higher than other wheat. Due to the absence of the *Glu-D1* allele durum wheat is not used for bread-making purposes. The long arm of the 1D chromosome with (t2+T2) subunit in the presence of chromosome 1B with (6+8) HMW-GS which is proved a beneficial allele for bread-making purposes. Durum wheat variety 'PBW114' with substitution line for chromosome 1D(1A) and chromosome 1D(1B) concludes that chromosome 1D plays a major role to improve bread-making quality. The

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substitution line for chromosome 1D(1A) can be used to induce 1AS.1DL translocation and the substitution line for chromosome 1D(1B) can be used to induce 1BL.1DL translocation.

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