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The Effect of High Molecular Weight Glutenin Subunit Encoded by *Glu-B1k* Allele on Bread-Making Quality of Near-Isogenic Lines of Bread Wheat*

Glu-B1k Alleli Tarafından Kodlanan Yüksek Moleküler Ağırlıklı Glutenin Alt Biriminin Ekmeklik Buğday Yakın İzogenik Hatlarının Ekmek Yapım Kalitesine Etkisi

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Abstract: In this study, the effect of the 22 glutenin subunit encoded by the *Glu-B1k* allele on the chromosome B on the quality of wheat was investigated. Nevzatbey and a genotype of *Triticum aestivum* L. *subsp. sphaerococcum* were used as parents and produced the near-isogenic lines (NILs) in the generation of BC_4F_5. Plant morphological traits and protein content, sedimentation volume, lactic acid solvent retention capacity (SRC), and glutenin swelling index (GSI) of the NILs were determined. The mean protein content of the NILs carrying 22 glutenin subunit was higher than that of the NILs carrying the 7+9 glutenin subunit (20.4% and 16.2%, respectively). In contrast, the NILs with 22 glutenin subunit had a lower sedimentation volume than those of the NILs with 7+9 glutenin subunits. The 22 glutenin subunit decreased the sedimentation volume from 19.47 to 13.49 mL. The average GSI value of the NILs carrying 7+9 glutenin subunits was higher than that of the NILs carrying 22 glutenin subunit (3.05 and 2.92). In conclusion, in this study we were able to detect a quality difference between NILs with 22 and 7+9 glutenin subunits in a small amount of samples. These findings suggest that glutenin subunit 22 may be associated with low gluten strength.

Keywords: High molecular weight glutenin subunit, bread wheat technological quality, gluten quality, *Glu-B1* locus, *Glu-B1k* allele

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Öz: Bu çalışmada, B kromozomu üzerindeki *Glu-B1k* aleli tarafından kodlanan 22 glutenin alt biriminin buğday kalitesine etkisi araştırılmıştır. Nevzatbey ve *Triticum aestivum* L. *subsp. Sphaerococcum'a* ait bir genotip ebeveyn olarak kullanılmış ve BC4F3 generasyonunda yakın izogenik hatlar (NIL'ler) elde edilmiştir. NIL'lerin bitki morfolojik özellikleri, protein oranı, sedimantasyon hacmi, laktik asit solvent tutma kapasitesi (STK) ve glutenin şişme indeksi (GSI) değerleri belirlenmiştir. 22 glutenin alt birimini taşıyan NIL'lerin ortalama protein oranı, 7+9 glutenin alt birimini taşıyan NIL'lerden daha yüksek bulunmuştur (sırasıyla %20.4 ve %16.2). Buna karşılık, 22 glutenin alt birimine sahip NIL'ler, 7+9 glutenin alt birimine sahip NIL'lerden daha düşük bir sedimantasyon hacmine sahip olmuştur. 22 glutenin alt birimi, sedimantasyon hacmini 19.47'den 13.49 mL'ye düşürmüştür. 7+9 glutenin alt birimi taşıyan NIL'lerin ortalama GSI değeri, 22 glutenin alt birimi taşıyan NIL'lerden (3.05 ve 2.92) daha yüksek olmuştur. Sonuç olarak, bu çalışmada az miktarda numunede 22 ve 7+9 glutenin alt birimine sahip NIL'ler arasında bir kalite farkının olduğu belirlendi. Bu bulgular, 22 glutenin alt biriminin düşük gluten kücü ile ilişkili olabileceğini ortaya koymuştur.

Anahtar Kelimeler: Yüksek molekül ağırlıklı glutenin altbirimleri, ekmeklik buğday teknolojik kalitesi, gluten kalitesi, *Glu-B1* lokusu, *Glu-B1k* alleli

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INTRODUCTION

Gluten is the most important quality parameter of wheat flour (Perten et al., 1992) and the main component responsible for the viscoelastic properties of dough with its elasticity and extensibility (Pena, 2002). It is a storage protein of wheat endosperm, consists of monomeric gliadin and polymeric glutenin subunits (Alvarez and Guzmán, 2019; Li et al., 2020). Glutenin, which determines the quality of the wheat to turn into the final product, is a type of protein consisting of many polypeptide chains combined with disulfide bonds (Day, 2011). Reduction of interchain bonds with reducing agents results in the formation of high- and low-molecular-weight glutenin subunits (HMW-GS and LMW-GS) (Payne, 1987). A hundred genes encode gluten proteins in wheat (Asri et al., 2021). The genes encoding HMW-GS are located in the long arm of chromosome 1 (1AL, 1BL, and 1DL) of three different chromosome sets of bread wheat (Branlard et al., 2001). HMW-GS is encoded by the genes *Glu-A1, Glu-B1,* and *Glu-D1* at the Glu-1 locus located on the long arms of homologous chromosome 1 (Shewry and Halford, 2002). Significant allelic variations in HMW-GS combinations in wheat are responsible for 70% of genetic variation in dough characteristics (Payne et al., 1987). Among these genes, the cysteine codon in the 1Dx5 gene plays an important role in the formation of gluten's physicochemical properties. It has been shown that the 1Ax2 and 1Bx7 genes do not have additional cysteine codons and therefore have fewer disulfide bonds that increase the elasticity and viscosity of dough gluten (Wanous et al., 2003). Peng et al. (2015) stated that dough quality may vary depending on the molecular weight of different subunits and the number of cysteine groups. Many studies have been conducted to determine how high-molecular-weight subunits affect the quality of various product groups. One of the most important classifications revealed as a result of these studies is that wheat with the subunit combination of Dx5+Dy10, which is encoded by the genes on the D chromosome, is ideal for bread making with its strong gluten (Lei et al., 2006), whereas with the subunit combination of Dx2+Dy12, the ones with weak gluten properties are suitable for use in soft wheat products (Karaduman et al., 2022). However, the chromosome B has the largest genetic variation in HMW-GS-encoding genes and has an important place in the genotypic development of quality traits in wheat and the introduction of new gene sources into breeding programs. Several reports have shown that the allele at the *Glu-B1* is also important in determining dough strength (Lei et al., 2006). Nakamura, (2000) reported that the incidence of the *GluB1k* allele in 405 wheat cultivars used in a study investigating allelic diversity in HMW-GS in Chinese and Japanese bread wheat cultivars is very rare., alsothe cultivars with the allele were reported to be Chinese wheats. In the study, it was predicted that transferring the genes encoding this subunit to wheat cultivars lacking subunit 22 may lead to greater genetic diversity In another study by Filip (2018) bread quality was scored according to the combinations of HMW-GS in the A, B, and D chromosomes of 28 cultivated wheat cultivars. Mostly, the association of 7+9 subunits in the *Glu-B1* locus with subunits in other loci is included. Subunit 22 in the *Glu-B1* locus with 5+10 in the D chromosome and 1 subunit in the A chromosome is found in the Mydlniczanka and Udyczanka Czerwona varieties. These two wheats had 8 points according to the scoring system that says bread quality varies between 1 and 10, and in the evaluation made due to the variability of HMW-GS in the *Glu-B1* locus it was determined that 22 subunits negatively affected gluten quality.

In recent years, Sharma et al., (2020) a study conducted on how the protein composition of the wheat grain affects the quality of the final product. Among the cultivars tested in their study, six alleles at the *Glu-B1* locus were examined. They shown when alleles were evaluated separately; it was determined that subunit 22 is one of the four alleles that negatively affect gluten quality. However, in the same study by same researcher, the effect of different combinations of subunits in *Glu-A1, Glu-B1,* and *Glu-D1* on bread formation was scored according to the *Glu-1* scoring system, and when 22 subunits were carried together with the 5+10 subunit in the *Glu-D1* locus, it was scored as eight, also their study, the effect of the HMW-GS 22 encoded by the *Glu-B1k* allele on the B chromosome on the quality of wheat was investigated. Up to now, the limited number of studies on this subject in the literature has made the subject worth studying. Therefore, we here investigate subunit 22, encoded by the *Glu-B1k* allele, is found in the cultivated wild wheat species *Triticum aestivum* L. *subsp. sphaerococcum*. It was transferred to the Nevzatbey bread wheat cultivar and developed the NILs carrying 22 and 7+9 glutenin subunits in the $BC_4F_{2:3}$ generation.

MATERIAL AND METHOD

Materials

Triticum aestivum L. *subsp. sphaerococcum* species and the Nevzatbey bread wheat variety were used to develop the NILs. The wild wheat species *Triticum aestivum* L. *subsp. sphaerococcum* has N, 22, 2+12 HMW-GS, and the Nevzatbey cv. contains 2*, 7+9, 5+10 subunits. The NILs in the BC4F2:3 generation were obtained using the marker-assisted backcrossing method and the recurrent parent was Nevzatbey cv. As a biochemical marker-assisted selection, we used Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) (Figure 1) modifed from Payne et al. (1980). The gels contained 8.7% gradual sep-aration gel and 3% loading gel. Five wheat kernels randomly selected from the spike of each genotype were crushed in a mortar. The extraction solution was prepared to extract the glutenin proteins. For this purpose, 1 mL of distilled water, 0.40 mL of extract stock solution, and 0.07 mL of ME (mercapto-ethanol, 5%) were used. One mL of this solution was added to each sample. The samples were vortexed for 30 s with 15-min intervals for 2 h. At the end of this period, the samples were placed in a boiling water bath with 85 °C–90 °C for three to 4 min. The samples were centrifuged at 12.000 rpm for 6–7 min before loading to the gel. Glutenin proteins were loaded into each well as 5 µL. Special glasses of 16 × 18 cm dimensions were used for electrophoresis (Max ll and Bio-Rad Power PAC 3000). Electrophoresis was carried out for two gels at 60 mA and 15 °C for about 5 h. Gel imaging was performed using the "Kodak GL 200" gel documentation device. The plants were grown in the greenhouse under speed breeding conditions. Of the 72 plants used in the analysis, 25 of them carry the glutenin subunits 2*, 22, 5+10, and 47 of them carry 2*, 7+9, 5+10.

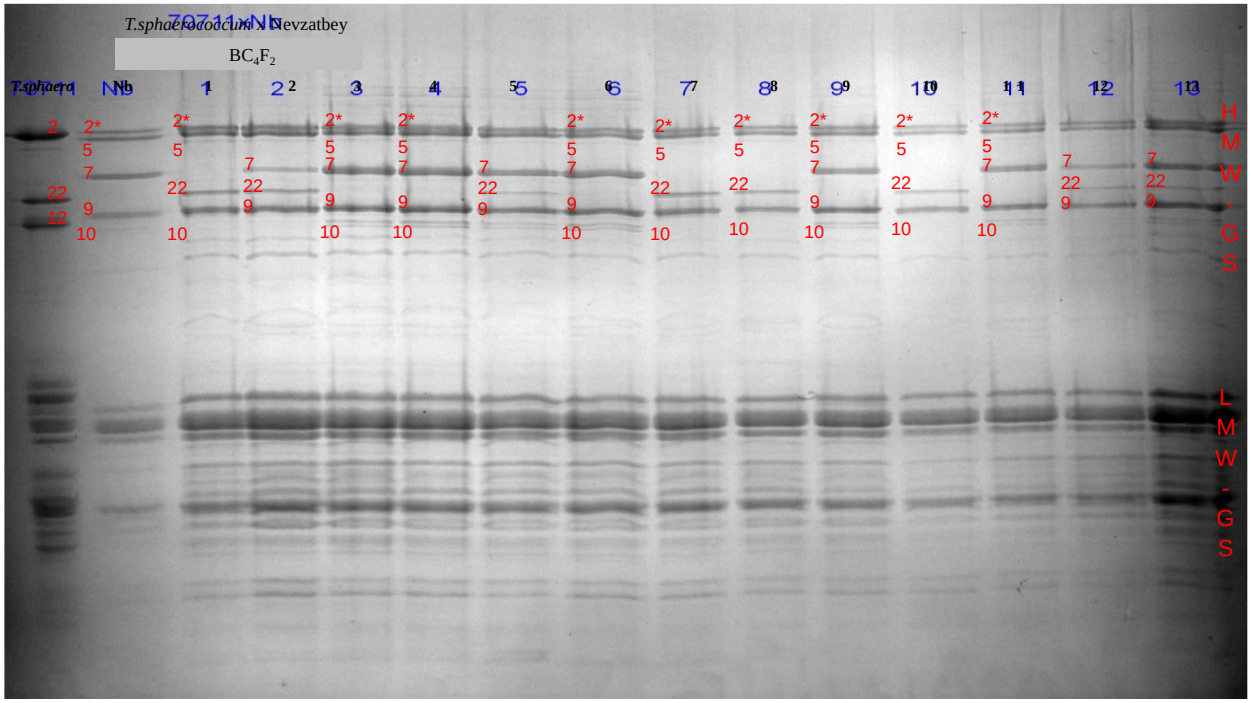

Figure 1. HMW-GS of the near-isogenic lines (NILs**)** in the BC4F2:3 generation obtained using the marker-assisted backcrossing method.

Şekil 1. Marköre dayalı geri melezleme metoddu ile elde edilen BC4F2:3 generasyonundaki yakın izojenik hatların (NIL'ler) YMA glutenin altbirimleri.

Obtaining Parents and Hybrid Seeds

The parent seeds used in the study and the seeds of *Triticum aestivum L. subsp. sphaerococcum,* Nevzatbey cv., and the NILs carrying glutenin subunits 7+9 and 22 were germinated in a few steps. The seeds were kept in an incubator for two days with the addition of an appropriate amount of water in glass petri dishes with filter paper. After germination, it was vernalized in a refrigerator at 4 °C for a month in order

to meet the vernalization need. The seeds that completed the vernalization period were planted in 3- to 3.5-liter pots. From the $BC_4F_{2:3}$ plants obtained before the study, fourty-seven plants with 7+9 subunits, twenty five plants with 22 subunits, and their parents were planted. These plants were grown up in the greenhouse conditions and agronomical practices (irrigation, fertilization, spraying, etc.) were applied. Klassman TS1 peat (Germany) was used to grow the plants. The leaves of the plants were sprayed with 1% of calcium nitrate once a week. We harvested and threshed the material by hand. The temperature of the greenhouse was kept between 25 and 28 °C during the day and between 15 and 18 °C at night. Halogen lamps with 400 watts of power remained on from 17:00 p.m. to 2:00 a.m. Agronomic characteristics such as plant height, spike length, number of grains per spike, and grain weight were measured (Figure 2).

Figure 2. Producing of the near-isogenic lines (NILs) in the generation of BC_4F_3 in the speed breeding condition in the greenhouse.
Şekil 2. Serada hızlı ıslah koşullarında BC4F3 generasyonunda yakın izojenik çizgilerin (NIL'ler) eldesi.

Technological Quality Traits

Since it is an early-generation material, developed under greenhouse conditions and providing preliminary information before going to the field, only small-scale quality tests could be performed on the material with a smaller sample amount. A laboratory type mill (Perten Lab Mill 3100, PerkinElmer Inc., USA), equipped with a 0.5 mm sieve, was used to mill whole wheat flours. Grain protein, ash, moisture, and L value were determined using Perten Inframatic 9500 (Perten Instruments). The instrument was calibrated according to AACC methods 46-19.01, 08-21.01, and 44-01.01 (AACC, 2010) for protein content, ash content, and moisture content, and Hunterlab colorimeter results for L value. The modified macro SDS sedimentation (MSDS) test (AACC Method 56-70) was performed according to Sayaslan et al. (2006). According to this method, two grams of the grinded samples were weighed and

transferred to sedimentation tubes with a volume of 100 mL. After adding 20.0 mL of water containing 0.0004% (w/v) bromophenol blue to each of the tubes, the tubes were shaken manually for 15 s and in a sedimentation device for 225 s. Then, 20.0 mL of a 2.5% (w/v) SDS solution was added to the tubes and shaken in the sedimentation device for 6 min. At the last stage, 10.0 mL of a 0.47% (v/v) lactic acid solution was added to the tubes and shaken for 6 min in the sedimentation device. It was kept on a flat surface for 20 min, and the sedimentation volumes were read. The glutenin swelling index was determined according to the method developed by Wang and Kovacs (2002). First, the isopropanol-lactic acid solution was prepared (AACC 56–60). 45 mL of the 25% lactic acid solution prepared for this was taken, and the volume was completed to 250 mL after adding 50 mL of isopropanol. Approximately 40 mg of flour was weighed into a 2 mL centrifuge tube, and 0.8 mL of distilled water was added and vortexed for 5 s. It was kept in a thermomixer at 1400 rpm for 10 min at 25 °C. 0.4 mL of isopropanol-lactic acid solution was added and vortexed again for 5 s. It was kept in the thermomixer at 1400 rpm for 10 min at 25 °C and centrifuged at 100 g for 5 min. Then, after the solution was poured and the top of the tube was wiped with a paper towel, it was weighed. Lactic acid solvent retention capacity (SRC) test was made according to Guzman et al. (2015). 5% lactic acid (v/v) was used in the study. Accordingly, 0.3 g flour was weighed into a 2 mL centrifuge tube, and 1.5 mL of lactic acid was added to it. After homogeneous mixing in the vortex, it was quickly placed in the thermomixer and kept at 1400 rpm at 25 °C for 5 min. The tube contents were then centrifuged at 400 g for 2 min. After the solvent content was poured, it was kept at room temperature at a 45 degree angle for 10 min, the upper part of the tubes was wiped with a paper towel, and the tube and residue were weighed.

Statistical Analysis

The basic statistics such as mean, standard error of the mean, coefficient of variation (CV), minimum and maximum were first determined for all parameters. Then, analysis of variance (ANOVA) was performed according to the Augmented Experimental Design (Patterson and Hunter, 1983). A student t-test (LSD) was used to compare the means when the ANOVA F-test indicated a significant effect of the treatments ($p < 0.05$). For all statistical analyses, the JMP 12.0.1 statistical program were used (JMP, 2013).

RESULTS AND DISCUSSION

Plant, Spike, and Grain Traits of the NILs

Some morphological traits of the NILs were given in Table 1. It was determined that the difference between plant height, spike length, number of grains per spike, and grain weight per spike between the lines in the $BC_4F_{2:3}$ generation was statistically significant ($p < 0.01$). According to the data obtained, the plant height values of the genotypes in the population varied between 53.93 and 83.40 cm in the greenhouse. The 39 of these genotypes in the population have higher plant heights above the average plant height of the NILs included in the trial. In this study, the plant height of 46 genotypes and Nevzatbey cv. was between 70 and 83 cm (Supplementary Table 1). Short-bread wheat varieties are more resistant to lodging. The fact that the wild wheat species used as a parent in the study is a dwarf wheat may be effective in shortening the stature of some NILs. It was observed that the difference between the average plant heights of the lines carrying the 22 and 7+9 subunits encoded by the *Glu-B1* locus in the study was statistically insignificant. Longer spike length in cereals may cause more grain and, therefore, an increase in grain yield during the grain filling period. In the study, the maximum spike length was 9.23 cm and the minimum was 4.57 cm (Table 1). Approximately 50% of the genotypes in the population and the Nevzatbey cv. had spike lengths above the average (Supplementary Table 2). The short and compact structure of the wild-type spikes used in the development of NILs may be effective in observing a wide variation in spike length. No statistically significant difference was observed between the spike length values of the NILs carrying different HMW-GS. In the $BC_4F_{2:3}$ plants included in the study, the average number of grains in the NILs carrying the 7+9 glutenin subunit was higher than that of the NILs carrying the 22 subunits (30.5 and 22.5, respectively), and this difference was statistically significant ($p < 0.01$) (Supplementary Table 3). The fact that a wide phenotypic variation was observed in the number of grains per spike of the NILs. According to the average values of the lines, the grain weight in the spike

varied between 0.3 and 2.04 g. The compact grain structure of the wild-type parent could cause a significant phenotypic variation in terms of grain weight (Supplementary Table 4). As a result of the comparison of the NILs used in the study with the 22 and 7+9 gluten subunits for the grain weight in the spike, we determined the grain weights of the NILs carrying the 7+9 subunit were statistically higher than those of the NILs carrying the 22 glutenin subunit.

Table 1. Some plant morphological characteristics of the NILs.
Çizelge 1. NIL'lerin bazı bitki morfolojik özellikleri.

Some plant morphological characteristics of the NILs										
	Average			Maximum			Minimum			CV (%)
Plant height (cm)	71.57	±	3.54	83.40	±	5.25	53.93	±	2.47	9.58 **
Spike length (cm)	7.42	±	0.35	9.23	±	0.53	4.57	±	0.25	9.37 **
Number of grains per spike	27.83	±	3.30	49.72	±	4,89	8.08	±	2,30	23.97 **
Grain weight per spike (g)	1.05	±	0.31	2.04	±	0.19	0.31	±	0.09	24.13 **
Means of some plant morphological characteristics of the NILs carrying different HMW-GS										
				Subunit 22			Subunit 7+9			
Plant height (cm)				72.82	±	0.75	72.14	±	0.61	0.74 n.s.
Spike length (cm)				7.35	±	0.07	7.52	±	0.05	12.67 n.s.
Number of grains per spike				22.50	±	0.90b	30.50	±	0.70a	41.2 **
Grain weight per spike (g)				0.88	±	0.03b	1.14	±	0.02a	36.50 **

Means with the same the letters are statistically not significant; **: Significant at $p < 0.01$ level; *: Significant at $p < 0.05$ level; n.s: not significant.

Technological Quality Properties

Findings about quality parameters of the $BC_4F_{2:3}$ generation of Nevzatbey x *Triticum aestivum* L. *subsp. sphaerococcum* and the NILs carrying different HMW-GS are given in Table 2.

Protein content

Protein content is an important quality criterion in determining the baking quality and bread volume of bread as well as its nutritional value (Guzman et al., 2022). The difference between the protein contents of the NILs was significant ($p < 0.01$). The values for the average protein content of the NILs in the $BC_4F_{2:3}$ generation are given in Supplementary Table 5. The average protein content of the NILs and parents used in the study was 17.67% (d.m.). The protein content of 68% of the NILs carrying the 22 subunits was above average. When the parents were examined, *Triticum aestivum* L. *subsp. sphaerococcum* had a protein content of 23.15% (d.m.), and Nevzatbey cv. had 11.85% (d.m.). There were 17 lines with a protein content above 23.15 %. The fact that *Triticum aestivum* L. *subsp. sphaerococcum* had a high protein content was due to its compact structure and small size. In many wild species, the small and compact structure of the grains could cause a relative increase in the protein content. The material in the NILs had the potential to be used as a source population to increase the protein ratio in breeding programs. The difference between the protein contents of the NILs carrying different HMW-GS was significant ($p < 0.01$). The average protein content of the NILs carrying 22 subunits was higher than that of the NILs carrying the 7+9 subunit (20.4% and 16.2%, respectively), and this result was found to be statistically significant ($p < 0.01$).

Ash Content and L (Brightness) Value

The values for the average ash content and L value of the NILs were given in Supplementary Tables 6 and 7. The difference both in the ash content and L values of the NILs was statistically significant ($p < 0.01$). The ash content of all genotypes analyzed in the study was above 1.00%. Grinding the samples to be analyzed as whole wheat flour caused high ash contents. The difference between the ash contents and L values of the NILs carrying different HMW-GS was significant ($p < 0.01$). Accordingly, the average ash content of the NILs carrying the 22 glutenin subunit was higher than that of the NILs carrying the 7+9 subunit. The average L color value of the NILs carrying 7+9 subunits was higher than the average L color

value of the NILs carrying 22 subunits. Due to the higher ash content of the lines carrying the 22 subunits, the L color value was also low in these lines.

Table 2. Some quality analysis results of the NILs.
Çizelge 2. NIL'lerin bazı kalite analiz sonuçları.

Quality characteristics of the NILs													
	Average			Maximum			Minimum			CV	Significance		
Protein content (%)	17.67	±	0.32	25.40	±	0.32	10.10	±	0.32	2.55	**		
Moisture content (%)	10.50	±	0.02	11.20	±	0.02	9.95	±	0.02	0.26	**		
Ash content (%)	1.35	±	0.02	1.65	±	0.02	1.20	±	0,02	1.62	**		
L (brightness)	84.97	±	0.09	86.15	±	0.09	82.75	±	0.09	0.15	**		
Means of quality characteristics of the NILs carrying different HMW-GS													
				Subunit 22			Subunit 7+9						
Protein content (%)				20.4	±	0.07a	16.2	±	0.05b	28.15	**		
Moisture content (%)				10.50	±	0.03	10.49	±	0.02	6.76	n.s.		
Ash content (%)				1.40	±	0.01a	1.32	±	0.09b	6.76	**		
L (brightness)				84.44	±	0.03b	85.23	±	0.02a	36.50	**		

Means with the same the letters are statistically not significant; **: Significant at $p < 0.01$ level; *: Significant at $p < 0.05$ level; n.s.: not significant.

Gluten Quality Properties

Findings about gluten quality parameters of the $BC_4F_{2:3}$ generation of Nevzatbey x *Triticum aestivum* L. *subsp. sphaerococcum* and the NILs carrying different HMW-GS are given in Table 3.

Sedimantation Volume

It was determined that the difference between the sedimentation volumes of the wheat genotypes grown under fully controlled greenhouse conditions was statistically significant ($p < 0.01$). The sedimentation volumes of the NILs used in the study ranged from 8.5 to 24.5 mL. Nevzatbey cv. had the highest sedimentation value, and the wild-type parent had the lowest value. The NILs 34 and 30 with high sedimentation volumes followed Nevzatbey cv. (Supplementary Table 8). The NILs showed variation between the sedimentation values of the parents. This result shows the effect of target genes encoding gluten subunits, 22 and 7+9. Determining the differences between the sedimentation values of the NILs grown in the greenhouse is important in terms of obtaining preliminary information about the quality characteristics of the NILs before the field tests. Sedimentation volume plays an important role in determining gluten quality. A high value indicates that gluten holds water well and that breads made from flours with a high sedimentation value have a high volume (Guzman et al., 2016a,b). We determined that the difference between the sedimentation volume values of the NILs carrying 22 and 7+9 glutenin subunits was significant ($p < 0.01$). The NILs carrying 22 glutenin subunits had a lower sedimentation volume than those of the NILS carrying 7+9 glutenin subunits. We found that 22 glutenin subunits decreased the sedimentation volume. Considering that the genotypic effects on protein quality are higher, it is important to obtain these results in the form of a preliminary screening under greenhouse conditions.

Glutenin Swelling Index, GSI

The glutenin swelling test is an important test for rapid assessment of end product quality in wheat (Labuschagne et al., 2021). The glutenin swelling index (GSI) method determines the swelling power of gluten to predict dough quality characteristics and end-use quality, especially those related to dough strength and baking properties. As in the sedimentation volume, the differences between the GSI values of the NILs were found to be statistically significant ($p < 0.01$). The GSI values of the NILs ranged from 2.68 to 3.83. Nevzatbey cv. has a GSI value of 3.18, while the wild-type parent used as a parent has a GSI value of 2.95. A wide variation was observed between the values (Supplementary Table 9). The difference

between the GSI values of the NILs and those carrying different HMW-GS was found to be significant at the level of 1%, and the average GSI value of the NILs carrying 7+9 glutenin subunits was higher than that of the NILs carrying 22 glutenin subunits (3.05 and 2.92, respectively). The GSI test, in which very small amounts of samples are used, is especially important for the pre-evaluation of materials grown in greenhouses.

Lactic Acid Solvent Retention Capacity (SRC)

The solvent retention capacity (SRC) test, which is used to predict the commercial bakery performance of soft wheat (*Triticum aestivum* L.) flours, provides information about whether the viscosity is due to protein properties (Kweon et al., 2011; Karaduman, 2020). The difference in lactic acid SRC values between the NILs was found to be statistically significant ($p < 0.01$). The lactic acid SRC values of the genotypes varied between 83.26% and 118.28%. SRC values of the parents, Nevzatbey cv. and the genotype from *Triticum aestivum* L. *subsp. sphaerococcum*, were 104.76 and 95.33%, respectively. SRC values below 85% are defined as "weak" and those above 105% or 110% as "strong" gluten. Accordingly, two of the NILs (lines 8 and 37) were lower than 85% (weak), and six of them had a value greater than 105% (strong) (Supplementary Table 10). It was determined that the difference between the lactic acid SRC values of the NILs carrying 22 and 7+9 glutenin subunits was not statistically significant.

Table 3. Gluten quality properties of the NILs.
Çizelge 3. NIL'lerin gluten kalite özellikleri.

Gluten quality characteristics of the NILs											
	Average			**Maximum**			**Minimum**			**CV**	**Significance**
Sedimentation volume (mL)	17.43	±	0.28	24.50	±	0.28	8.50	±	0.28	2.30	**
Glutenin swelling index (GSI)	3.01	±	0.04	3.83	±	0.04	2.68	±	0.04	0.26	**
Lactic acid SRC (%)	97.20	±	0.74	118.28	±	0.74	83.26	±	0,74	1.08	**
Means of gluten quality characteristics of the NILs carrying different HMW-GS											
				Glu-B1, Subunit 22			*Glu-B1*, Subunit 7+9				
Sedimentation volume (mL)				13.49	±	0.43b	19.47	±	0.31a	17.76	**
Glutenin swelling index (GSI)				2.92	±	0.03b	3.05	±	0.02a	7.71	**
Lactic acid SRC (%)				95.95	±	0.8	97.84	±	0.6	6.08	n.s.

Means with the same the letters are statistically not significant; **: Significant at $p < 0.01$ level; *: Significant at $p < 0.05$ level; n.s.: not significant.

CONCLUSION

The fact that the quality analyses were carried out in small sample amounts on the plant samples grown in the greenhouse is important in terms of the rapid evaluation of the breeding material before it is released into field conditions and the evaluation of the results of the backcross breeding programs. According to results of this study, it was found that the 22 glutenin subunit may be associated with weak gluten strength. With this study, its effectiveness on gluten quality was investigated under greenhouse conditions using a small sample amount. In addition, the results obtained showed that some NILs showed superior characteristics to parents and would be useful for developing new varieties in breeding studies. *Triticum aestivum L. subsp. sphaerococcum* could be used as genetic source material in wheat breeding programs for quality and plant morphological traits.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DECLARATION OF AUTHOR CONTRIBUTION

Betül Altınsoy, Nevzat Aydın and Yaşar Karaduman designed the study, participated in experiment, and drafted themanuscript

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REFERENCES

AACC (2010). *Approved Methods of Analysis, eleventh ed*. AACC,St. Paul, MN.

Alvarez, J. B., & Guzmán, C. (2019). Recovery of wheat heritage for traditional food: Genetic variation for high molecular weight glutenin subunits in neglected/underutilized wheat. *Agronomy*, *9*(11), 755. https://doi.org/10.3390/agronomy9110755

Asri, N., Rostami-Nejad, M., Anderson, R. P., & Rostami, K. (2021). The gluten gene: unlocking the understanding of gluten sensitivity and intolerance. *The Application Of Clinical Genetics*, *14*, 37. DOI: 10.2147/TACG.S276596

Branlard, G., Dardevet, M., Saccomano, R., Lagoutte, F., & Gourdon, J. (2001). Genetic diversity of wheat storage proteins and bread wheat quality. *Euphytica*, *119*(1), 59-67. https://doi.org/10.1023/A:1017586220359

Day, L. (2011). *Wheat gluten: production, properties and application*. In Handbook of food proteins (pp. 267-288). Woodhead Publishing

Filip, E. (2018). Composition of high molecular weight glutenin subunits in polish common wheat cultivars (*Triticum aestivum L.). Journal of Food Quality*, Article ID 2473420. https://doi.org/10.1155/2018/2473420

Guzman, C., Posadas-Romano, G., Hernandez-Espinosa, A., Morales- Dorantes, A., & Peña, R. J. (2015). A new standard water absorption criteria based on solvent retention capacity (SRC) to determine dough mixing properties, viscoelasticity, and bread-making quality. *Journal of Cereal Science*, *66*, 59-65. https://doi.org/10.1016/j.jcs.2015.10.009

Guzman, C., Peña, R. J., Singh, R., Autrique, E., Dreisigacker, S., Crossa, J., Rutkoski, J., Poland, J., & Battenfield, S. (2016a). Wheat quality improvement at CIMMYT and the use of genomic selection on it. *Applied & Translational Genomics*, *11*, 3-8. https://doi.org/10.1016/j.atg.2016.10.004

Guzman, C., Mondal, S., Govindan, V., Autrique, J. E., Posadas-Romano, G., Cervantes, F., Crossa, J., Vargas, M., Singh, R. V., & Peña, R. J. (2016b). Use of rapid tests to predict quality traits of CIMMYT bread wheat genotypes grown under different environments. *LWT-Food Science and Technology*, *69*, 327–333. https://doi.org/10.1016/j.lwt.2016.01.068

Guzman, C., Crossa, J., Mondal, S., Govindan, V., Huerta, J., Crespo-Herrera, L., Vargas, M. P., Singh, R., & Ibba, M. I. (2022). Effects of glutenins *(Glu-1* and *Glu-3*) allelic variation on dough properties and bread-making quality of CIMMYT bread wheat breeding lines, *Field Crops Research*, *284*, 108585. https://doi.org/10.1016/j.fcr.2022.108585

JMP. 2013. *13.0.0.* Scintilla - Copyright (C) 1998-2014 by Neil Hodgson;neilh@scintilla. Org SAS Institute. JMP *13.0 users guide*. Carry, NC: Release SAS Institute Inc.

Karaduman, Y. (2020). Assessing gluten strength with a new small-scale LASRC method useful for soft wheat breeding programs. *Cereal Chemistry, 97* (2), 196-204. https://doi.org/10.1002/cche.10235

Karaduman, Y., Yeşildağ, Z. S., & Akın, A. (2022). Evaluating selection efficacy of high molecular weight glutenin subunits (HMWGs) by relating gluten quality parameters. *LWT-Food Science and Technology*, *155*, 112949. https://doi.org/10.1016/j.lwt.2021.112949

Kweon, M., Slade, L., & Levine, H. (2011). Solvent retention capacity (SRC) testing of wheat flour: Principles and value in predicting flour functionality in different wheat-based food processes and in wheat breeding – a Review., *Cereal Chemistry*, *88*, 537–552. https:// doi.org/10.1094/CCHEM-07-11-0092

Labuschagne, M., Guzmán, C., Phakela, K., Wentzel, B., & van Biljon, A. (2021). Solvent Retention Capacity and Gluten Protein Composition of Durum Wheat Flour as Influenced by Drought and Heat Stress. *Plants, 10,* 1000. https://doi.org/10.3390/plants10051000

Lei, Z. S., Gale, K. R., He, Z. H., Gianibelli, C., Larroque, O., Xia, X. C., Butow, B. J., & Ma, W. (2006). Y-type gene specific markers for enhanced discrimination of high-molecular weight glutenin alleles at the *Glu-B1* locus in hexaploid wheat. *Journal of Cereal Science*, *43*(1), 94-101. https://doi.org/10.1016/j.jcs.2005.08.003

Li, Y., Fu, J., Shen, Q., & Yang, D. (2020). High-molecular-weight glutenin subunits: Genetics, structures, and relation to end use qualities. *International Journal of Molecular Sciences*, *22*(1), 184. https://doi.org/10.3390/ijms22010184

Nakamura, H. (2000). Allelic variation at high-molecular-weight glutenin subunit Loci, Glu-A1, *Glu-B1* and *Glu-D1*, in Japanese and Chinese hexaploid wheats. *Euphytica*, *112*(2), 187-193. https://doi.org/10.1023/A:1003888116674

Patterson, H. D., & Hunter, E. A. (1983). The efficiency of incomplete block designs in National List and Recommended List cereal variety trials. *The Journal of Agricultural Science*, *101*(2), 427-433. https://doi.org/10.1017/S002185960003776X

Payne, P. I., Law, C. N., & Mudd, E. E. (1980). Control by homoeologous group 1 chromosomes of the high-molecular-weight subunits of glutenin, a major protein of wheat endosperm. *Theoretical and Applied Genetics, 58*, 113–120. https://doi.org/ 10.1007/BF00263101

Payne, P. I., Nightingale, M. A., Krattiger, A. F., & Holt, L. M. (1987). The relationship between HMW glutenin subunit composition and the bread-making quality of British-grown wheat varieties. *Journal of the Science of Food and Agriculture*, *40*(1), 51-65. https://doi.org/10.1002/jsfa.2740400108

Pena, R. J. (2002). *Wheat for bread and other foods. Bread wheat improvement and production*. Food and Agriculture Organization of the United Nations. Rome, 483-542

Peng, Y., Yu, K., Zhang, Y., Islam, S., Sun, D., & Ma, W. (2015). Two novel y-type high molecular weight glutenin genes in Chinese wheat landraces of the Yangtze-River region. *PLoS One*, *10*(11), e0142348. https://doi.org/10.1371/journal.pone.0142348

Perten, H., Bondesson, K., & Mjorndal, A. (1992). Gluten index variations in commercial Swedish wheat samples. *Cereal Foods World*. 37, 655-660.

Sayaslan, A., Seib, P. A., & Chung, O. K. (2006). Wet-milling properties of waxy wheat flours by two laboratory methods. *Journal of Food Engineering*, *72*(2), 167-178. https://doi.org/10.1016/j.jfoodeng.2004.11.033

Sharma, A., Garg, S., Sheikh, I., Vyas, P., & Dhaliwal, H. S. (2020). Effect of wheat grain protein composition on end-use quality. *Journal of Food Science and Technology*, *57*(8), 2771-2785. 10.1007/s13197-019-04222-6

Shewry, P. R., & Halford, N. G. (2002). Cereal seed storage proteins: structures, properties and role in grain utilization. *Journal of Experimental Botany*, *53*(370), 947-958. https://doi.org/10.1093/jexbot/53.370.947

Wang, C., Kovacs, & M. I. P. (2002). Swelling index of glutenin test. II. Application in prediction of dough properties and end-use quality. *Cereal Chemistry*, *79*(2), 190-196. https://doi.org/10.1094/CCHEM.2002.79.2.190

Wanous, M., Munkvold, J., Kruse, J., Brachman, E., Klawiter, M., & Fuehrer, K. (2003). Identification of chromosome arms influencing expression of the HMW glutenins in wheat. *Theoretical and Applied Genetics*, *106*(2), 213-220. https://doi.org/10.1007/s00122-002-1098-7

Supplementary Table 1. Plant heights (cm) of the NILs.
Ek Tablo 1. NIL'lerin bitki boyları (cm).

GN	ADAVE	AVE	SE (±)	GN	ADAVE	AVE	SE (±)
1	73.60	72.00	4.42	41	74.74	73.14	3.20
2	69.26	67.67	4.42	42	72.60	71.00	3.37
3	69.80	68.20	3.61	43	79.60	78.00	3.61
5	76.88	75.29	3.20	44	72.02	70.43	3.20
6	55.93	54.33	4.42	45	74.20	72.60	3.61
7	53.93	52.33	4.42	46	67.40	65.80	3.61
8	70.20	68.60	3.61	47	76.76	75.17	3.37
9	70.80	69.20	2.85	48	64.26	62.67	4.42
10	81.41	79.82	2.77	49	73.65	75.25	3.06
11	68.17	66.57	3.20	50	69.40	71.00	5.25
12	68.60	67.00	3.61	51	69.40	71.00	3.93
13	65.88	64.29	3.20	52	59.07	60.67	4.42
14	69.69	71.29	3.20	53	67.20	68.80	3.61
15	70.40	72.00	3.93	54	70.20	71.80	3.61
16	64.65	66.25	3.06	55	81.07	82.67	3.37
17	74.69	76.29	2.60	56	73.55	75.14	3.20
18	83.40	85.00	2.85	57	79.15	80.75	3.93
19	73.15	74.75	3.93	58	76.40	78.00	3.20
20	77.74	79.33	2.95	59	78.07	79.67	3.37
21	72.57	74.17	3.37	60	81.74	83.33	3.37
22	77.59	79.18	2.77	61	77.40	79.00	3.20
23	78.07	79.67	2.71	62	76.49	78.08	2.71
24	77.40	79.00	3.37	63	78.15	79.75	3.93
25	66.20	67.80	3.61	64	76.29	77.89	2.95
26	57.10	55.50	3.93	65	69.65	71.25	3.93
27	58.60	57.00	3.20	66	76.24	77.83	3.37
28	68.43	66.83	3.37	67	79.40	81.00	3.93
29	66.00	64.40	3.61	68	75.90	77.50	3.93
30	71.60	70.00	4.42	69	76.40	78.00	2.95
31	71.60	70.00	3.61	70	78.00	79.60	3.61
32	83.40	81.80	3.61	71	72.90	74.50	3.93
33	70.85	69.25	3.93	72	69.65	71.25	3.93
34	69.35	67.75	3.93	TA	60.06	60.29	2.65
35	59.85	58.25	3.93	NB	74.75	74.75	2.47
36	65.10	63.50	3.93	General Average	**71.57**	**71.59**	**3.54**
37	64.80	63.20	3.61	Maximum	**83.40**	**85.00**	**5.25**
38	66.85	65.25	3.93	Minimum	**53.93**	**52.33**	**2.47**
39	74.93	73.33	3.37	CV%		**9.58**	
40	64.10	62.50	3.93				

GN: Genotype, ADAVE: Adjusted mean AVE: arithmetic mean. SE: Standart error, NB: Nevzatbey, TA: *Triticum aestivum* L. *subsp. Sphaerococcum*, CV: Coefficient of Variation.

Supplementary Table 2. Spike lengths (cm) of the NILs.
Ek Çizelge 2. NIL'lerin başak uzunlukları (cm).

GN	ADAVE	AVE	SE (±)	GN	ADAVE	AVE	SE (±)
1	8.06	8.00	0.44	41	7.63	7.57	0.32
2	7.90	7.83	0.44	42	7.31	7.25	0.34
3	8.26	8.20	0.36	43	7.76	7.70	0.36
5	8.49	8.43	0.32	44	7.21	7.14	0.32
6	9.23	9.17	0.44	45	7.26	7.20	0.36
7	7.40	7.33	0.44	46	7.26	7.20	0.36
8	7.36	7.30	0.36	47	7.40	7.33	0.34
9	7.56	7.50	0.29	48	6.40	6.33	0.44
10	8.70	8.64	0.28	49	7.06	7.13	0.31
11	6.63	6.57	0.32	50	7.19	7.25	0.53
12	5.86	5.80	0.36	51	7.31	7.38	0.39
13	7.28	7.21	0.32	52	7.10	7.17	0.44
14	6.11	6.17	0.32	53	7.14	7.20	0.36
15	6.94	7.00	0.39	54	7.34	7.40	0.36
16	6.69	6.75	0.31	55	7.69	7.75	0.34
17	7.26	7.32	0.26	56	7.94	8.00	0.32
18	7.89	7.95	0.29	57	8.44	8.50	0.39
19	8.19	8.25	0.39	58	7.58	7.64	0.32
20	7.22	7.28	0.30	59	7.19	7.25	0.34
21	7.10	7.17	0.34	60	7.60	7.67	0.34
22	7.44	7.50	0.28	61	7.87	7.93	0.32
23	7.90	7.96	0.27	62	8.19	8.25	0.27
24	7.44	7.50	0.34	63	7.69	7.75	0.39
25	6.84	6.90	0.36	64	8.38	8.44	0.30
26	7.44	7.38	0.39	65	6.81	6.88	0.39
27	8.78	8.71	0.32	66	7.27	7.33	0.34
28	7.73	7.67	0.34	67	7.44	7.50	0.39
29	7.46	7.40	0.36	68	6.81	6.88	0.39
30	7.73	7.67	0.44	69	7.38	7.44	0.30
31	7.26	7.20	0.36	70	7.54	7.60	0.36
32	7.26	7.20	0.36	71	7.31	7.38	0.39
33	7.19	7.13	0.39	72	7.44	7.50	0.39
34	8.06	8.00	0.39	TA	4.56	4.57	0.27
35	7.44	7.38	0.39	NB	7.56	7.56	0.25
36	7.31	7.25	0.39	General Average	**7.42**	**7.42**	**0.35**
37	6.66	6.60	0.36	Maximum	**9.23**	**9.17**	**0.53**
38	7.19	7.13	0.39	Minimum	**4.56**	**4.57**	**0.25**
39	7.81	7.75	0.34	CV (%)		**9.37**	
40	6.44	6.38	0.39				

GN: Genotype, ADAVE: Adjusted mean AVE: arithmetic mean. SE: Standart error, NB: Nevzatbey, TA: *Triticum aestivum* L. *subsp. Sphaerococcum*, CV: Coefficient of Variation.

Supplementary Table 3. Number of grains per spike of the NILs.
Ek Çizelge 3. NIL'lerin başak başına tane sayısı.

GN	ADAVE	AVE	SE (±)	GN	ADAVE	AVE	SE (±)
1	48.72	46.00	4.11	41	34.72	32.00	2.98
2	44.39	41.67	4.11	42	42.72	40.00	3.14
3	46.32	43.60	3.36	43	43.92	41.20	3.36
5	36.29	33.57	2.98	44	27.29	24.57	2.98
6	44.05	41.33	4.11	45	24.52	21.80	3.36
7	40.05	37.33	4.11	46	36.52	33.80	3.36
8	26.12	23.40	3.36	47	30.72	28.00	3.14
9	12.62	9.90	2.66	48	35.05	32.33	4.11
10	34.45	31.73	2.58	49	37.15	39.88	2.85
11	18.86	16.14	2.98	50	34.28	37.00	4.89
12	12.72	10.00	3.36	51	28.78	31.50	3.66
13	13.58	10.86	2.98	52	27.61	30.33	4.11
14	10.14	12.86	2.98	53	27.08	29.80	3.36
15	12.28	15.00	3.66	54	30.08	32.80	3.36
16	8.78	11.50	2.85	55	20.28	23.00	3.14
17	22.35	25.07	2.42	56	24.14	26.86	2.98
18	24.78	27.50	2.66	57	15.03	17.75	3.66
19	12.78	15.50	3.66	58	19.85	22.57	2.98
20	14.72	17.44	2.74	59	19.95	22.67	3.14
21	11.28	14.00	3.14	60	24.45	27.17	3.14
22	22.92	25.64	2.58	61	21.71	24.43	2.98
23	21.36	24.08	2.52	62	29.61	32.33	2.52
24	18.28	21.00	3.14	63	14.03	16.75	3.66
25	8.08	10.80	3.36	64	23.72	26.44	2.74
26	36.72	34.00	3.66	65	21.78	24.50	3.66
27	49.72	47.00	2.98	66	10.95	13.67	3.14
28	42.05	39.33	3.14	67	16.53	19.25	3.66
29	41.92	39.20	3.36	68	11.28	14.00	3.66
30	44.72	42.00	4.11	69	15.83	18.56	2.74
31	39.32	36.60	3.36	70	17.48	20.20	3.36
32	39.92	37.20	3.36	71	13.28	16.00	3.66
33	40.72	38.00	3.66	72	10.03	12.75	3.66
34	43.47	40.75	3.66	TA	28.18	28.57	2.47
35	38.72	36.00	3.66	NB	48.63	48.63	2.30
36	37.47	34.75	3.66	General Average	**27.83**	**27.88**	**3.30**
37	32.72	30.00	3.36	Maximum	**49.72**	**48.63**	**4.89**
38	38.22	35.50	3.66	Minimum	**8.08**	**9.90**	**2.30**
39	45.05	42.33	3.14	CV (%)		**23.97**	
40	27.97	25.25	3.66				

GN: Genotype, ADAVE: Adjusted mean, AVE: arithmetic mean, SE: Standart error, NB: Nevzatbey, TA: *Triticum aestivum* L. *subsp. Sphaerococcum*, CV: Coefficient of Variation.

Supplementary Table 4. Grain weight in the spike of the NILs.
Ek Çizelge 4. NIL'lerin başaktaki tane ağırlığı.

GN	ADAVE	AVE	SE (±)	GN	ADAVE	AVE	SE (±)
1	2.04	1.89	0.16	41	1.38	1.22	0.11
2	1.15	1.00	0.16	42	1.53	1.38	0.12
3	1.53	1.38	0.13	43	1.57	1.41	0.13
5	1.34	1.18	0.11	44	1.33	1.17	0.11
6	1.53	1.38	0.16	45	1.01	0.85	0.13
7	1.57	1.41	0.16	46	1.25	1.09	0.13
8	1.18	1.02	0.13	47	1.54	1.38	0.12
9	0.61	0.45	0.10	48	1.33	1.18	0.16
10	1.32	1.17	0.10	49	1.22	1.38	0.11
11	0.87	0.71	0.11	50	1.06	1.22	0.19
12	0.61	0.45	0.13	51	1.05	1.21	0.14
13	0.61	0.45	0.11	52	0.96	1.12	0.16
14	0.40	0.56	0.11	53	1.06	1.22	0.13
15	0.43	0.59	0.14	54	1.14	1.30	0.13
16	0.48	0.64	0.11	55	0.73	0.89	0.12
17	0.83	0.99	0.09	56	0.97	1.13	0.11
18	1.00	1.15	0.10	57	0.62	0.78	0.14
19	0.50	0.66	0.14	58	0.88	1.04	0.11
20	0.62	0.78	0.11	59	0.52	0.68	0.12
21	0.48	0.63	0.12	60	0.62	0.77	0.12
22	0.85	1.01	0.10	61	0.66	0.82	0.11
23	0.89	1.05	0.10	62	0.94	1.10	0.10
24	0.74	0.90	0.12	63	0.56	0.72	0.14
25	0.31	0.47	0.13	64	0.88	1.03	0.11
26	1.17	1.01	0.14	65	0.77	0.93	0.14
27	1.77	1.61	0.11	66	0.49	0.65	0.12
28	1.55	1.39	0.12	67	0.66	0.82	0.14
29	1.44	1.29	0.13	68	0.46	0.62	0.14
30	1.90	1.74	0.16	69	0.69	0.85	0.11
31	1.70	1.55	0.13	70	0.69	0.85	0.13
32	1.53	1.37	0.13	71	0.56	0.72	0.14
33	1.43	1.27	0.14	72	0.36	0.52	0.14
34	1.62	1.46	0.14	TA	0.90	0.92	0.10
35	1.51	1.35	0.14	NB	1.94	1.94	0.09
36	1.43	1.28	0.14	General Average	**1.05**	**1.06**	**0.13**
37	1.22	1.06	0.13	Maximum	**2.04**	**1.94**	**0.19**
38	1.52	1.37	0.14	Minimum	**0.31**	**0.45**	**0.09**
39	1.73	1.57	0.12	CV (%)		**24.13**	
40	1.24	1.08	0.14				

GN: Genotype, ADAVE: Adjusted mean, AVE: arithmetic mean, SE: Standart error, NB: Nevzatbey, TA: *Triticum aestivum* L. *subsp. Sphaerococcum*, CV: Coefficient of Variation.

Supplementary Table 5. Grain protein contents (d.m.) of the NILs.
Ek Çizelge 5. NIL'lerin tane protein içerikleri (k.m.).

GN	ADAVE	SE (±)		GN	SE (±)
1	13.35		41	14.75	
2	12.40		42	12.00	
3	10.35		43	12.25	
5	13.90		44	15.10	
6	14.65		45	14.50	
7	14.60		46	11.25	±0.32
8	23.45		47	14.05	
9	24.75		48	11.05	
10	19.15		49	10.80	
11	23.15		50	10.10	
12	23.10		51	13.50	
13	24.40		52	13.00	
14	24.00		53	12.45	
15	22.65		54	13.40	
16	22.70		55	21.95	
17	20.50		56	21.90	
18	19.80		57	25.40	
19	24.80		58	17.05	
20	22.95		59	24.95	
21	24.50		60	22.00	
22	20.65		61	23.20	
23	19.65		62	20.30	
24	23.00		63	23.25	
25	24.40		64	21.30	
26	12.85		65	23.20	
27	13.75		66	24.05	
28	11.85		67	21.85	
29	11.65		68	22.80	
30	13.90	±0.32	69	23.45	
31	12.55		70	22.65	
32	11.40		71	23.50	
33	11.50		72	24.90	
34	12.65		TA	23.15	
35	12.60		NB	11.85	
36	11.05		General Average	17.67	0.32
37	11.00		Maximum	25.40	0.32
38	11.90		Minimum	10.10	0.32
39	11.05		CV (%)	2.55	
40	12.20				

GN: Genotype, ADAVE: Adjusted mean, SE: Standart error, NB: Nevzatbey, TA: *Triticum aestivum* L. *subsp. Sphaerococcum*, CV: Coefficient of Variation. d.m.: dry matter base.

Supplementary Table 6. Ash contents (d.m.) of the NILs.
Ek Tablo 6. NIL'lerin kül içerikleri (k.m.).

GN	ADAVE	SE	GN	ADAVE	SE
1	1.30	±0.02	41	1.32	
2	1.34		42	1.27	
3	1.36		43	1.26	
5	1.32		44	1.27	
6	1.38		45	1.27	
7	1.29		46	1.28	
8	1.48		47	1.21	
9	1.55		48	1.22	
10	1.34		49	1.20	
11	1.37		50	1.22	
12	1.41		51	1.25	±0.02
13	1.59		52	1.24	
14	1.50		53	1.26	
15	1.40		54	1.22	
16	1.33		55	1.42	
17	1.38		56	1.34	
18	1.32		57	1.49	
19	1.50		58	1.35	
20	1.38		59	1.51	
21	1.65		60	1.51	
22	1.33		61	1.48	
23	1.35		62	1.40	
24	1.36		63	1.44	
25	1.44		64	1.40	
26	1.46		65	1.42	
27	1.44		66	1.38	
28	1.34		67	1.35	
29	1.34		68	1.43	
30	1.30		69	1.39	
31	1.27		70	1.34	
32	1.24		71	1.38	
33	1.29		72	1.47	
34	1.27		TA	1.43	
35	1.32		NB	1.28	
36	1.26		General Average	1.35	0.02
37	1.25		Maximum	1.65	0.02
38	1.27		Minimum	1.20	0.02
39	1.26		CV%	1.62	
40	1.21				

GN: Genotype, ADAVE: Adjusted mean, SE: Standart error, NB: Nevzatbey, TA: *Triticum aestivum* L. *subsp. Sphaerococcum,* CV: Coefficient of Variation, d.m.: dry matter bases.

Supplementary Table 7. L value of the NILs.
Ek Çizelge 7. NIL'lerin L değeri.

GN	ADAVE	(±) SE	GN	ADAVE	(±) SE
1	85.55		41	85.30	
2	85.45		42	85.75	
3	85.20		43	85.65	
5	85.35		44	85.50	
6	85.10		45	85.35	
7	85.40		46	85.60	
8	83.90		47	85.85	
9	83.70		48	85.95	
10	84.90		49	86.15	
11	84.45		50	86.10	
12	84.40		51	85.65	
13	83.25		52	85.70	
14	83.70		53	85.65	
15	84.50		54	85.65	
16	84.50		55	84.60	
17	84.30		56	84.60	
18	84.30		57	84.05	
19	84.15		58	84.80	
20	84.40		59	84.30	
21	82.75		60	84.25	
22	84.45		61	84.45	
23	84.20		62	84.55	
24	84.35		63	84.40	
25	84.15	±0.09	64	84.70	±0.09
26	84.90		65	84.55	
27	85.05		66	84.50	
28	85.45		67	84.95	
29	85.55		68	84.65	
30	85.60		69	84.65	
31	85.70		70	84.85	
32	85.80		71	84.75	
33	85.65		72	84.35	
34	85.75		TA	84.80	
35	85.40	±0.09	NB	85.45	
36	85.85		General Average	**84.97**	**0.09**
37	85.85		Maximum	**86.15**	**0.09**
38	85.75		Minimum	**82.75**	**0.09**
39	85.80		CV (%)	**0.15**	
40	85.90				

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Supplementary Table 8. Sedimentation volume of the NILs (ml).
Ek Çizelge 8. NIL'lerin sedimantasyon hacmi (ml).

GN	ADAVE	(±) SE	GN	ADAVE	(±) SE
1	17.25	±0.28	41	23.25	
2	18.25		42	23.25	
3	16.75		43	20.25	
5	18.25		44	20.50	
6	22.25		45	20.50	
7	19.75		46	19.75	
8	10.25		47	19.25	
9	10.25		48	19.25	
10	19.25		49	20.00	
11	10.25		50	17.25	
12	10.25		51	19.25	±0.28
13	14.00		52	19.25	
14	12.00		53	19.00	
15	12.75		54	22.00	
16	12.25		55	17.50	
17	17.25		56	18.75	
18	12.00		57	14.25	
19	9.25		58	16.75	
20	14.25		59	18.00	
21	9.50		60	18.25	
22	12.25		61	17.75	
23	10.50		62	20.00	
24	11.50		63	16.50	
25	8.50		64	18.75	
26	23.25		65	17.25	
27	22.25		66	15.75	
28	22.25		67	15.25	
29	22.25		68	14.00	
30	23.50		69	17.50	
31	22.00		70	16.25	
32	20.25		71	17.00	
33	21.00		72	18.75	
34	24.00		TA	8.50	
35	20.75		NB	24.50	
36	20.00		General Average	17.43	0.28
37	18.50		Maximum	24.50	0.28
38	19.50		Minimum	8.50	0.28
39	20.00		CV (%)	2.30	
40	20.00				

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Supplementary Table 9. GSI value of the NILs.
Ek Çizelge 9. NIL'lerin GSI değeri.

GN	ADAVE	(±) SE	GN	ADAVE	(±) SE
1	2.98		41	3.83	
2	3.04		42	3.50	
3	3.22		43	3.46	
5	3.10		44	3.20	
6	3.51		45	3.27	
7	3.45		46	3.21	
8	2.74		47	2.88	
9	2.79		48	2.93	
10	3.01		49	2.89	
11	2.68		50	2.93	
12	2.70		51	3.05	
13	3.04		52	3.39	
14	2.82		53	2.95	
15	2.79		54	3.32	
16	2.71		55	2.89	
17	2.92		56	2.88	
18	2.78		57	2.94	
19	2.80		58	2.78	
20	2.72		59	2.98	
21	2.93		60	2.87	
22	3.41		61	3.07	
23	2.76		62	3.16	
24	2.76	±0.04	63	2.91	±0.04
25	2.70		64	2.98	
26	3.33		65	2.94	
27	3.32		66	2.95	
28	3.16		67	2.75	
29	3.10		68	2.83	
30	3.14		69	2.88	
31	2.95		70	2.78	
32	2.95		71	2.82	
33	3.17		72	3.11	
34	3.20		TA	2.95	
35	3.25		NB	3.18	
36	2.76	±0.04	General Average	**3.01**	**0.04**
37	2.80		Maximum	**3.83**	**0.04**
38	3.04		Minimum	**2.68**	**0.04**
39	2.95		CV (%)	**1.73**	
40	2.95				

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Supplementary Table 10. Lactic acid SRC value of the NILs.
Ek Çizelge 10. NIL'lerin laktik asit SRC değeri.

GN	ADAVE	SE	GN	ADMEAN	SE
1	97.27	±0.74	41	108.33	
2	100.89		42	100.89	
3	104.62		43	95.57	
5	98.14		44	98.13	
6	118.28		45	99.24	
7	106.37		46	98.68	
8	83.26		47	97.70	
9	90.76		48	92.86	
10	92.83		49	90.44	
11	89.64		50	88.52	
12	93.15		51	97.73	
13	97.98		52	103.46	
14	92.93		53	95.41	±0.74
15	92.57		54	103.04	
16	92.50		55	100.95	
17	102.25		56	105.97	
18	95.61		57	101.02	
19	89.72		58	96.11	
20	93.61		59	110.39	
21	88.44		60	92.71	
22	97.02		61	100.52	
23	94.90		62	97.52	
24	98.00		63	93.06	
25	92.93		64	95.81	
26	105.27		65	96.03	
27	99.48		66	97.08	
28	98.53		67	93.16	
29	98.12		68	93.21	
30	99.21		69	103.88	
31	96.60		70	104.74	
32	95.11		71	101.13	
33	99.22		72	98.79	
34	100.58		TA	95.33	
35	100.41		NB	104.76	
36	91.31		General Average	**97.20**	**0.74**
37	84.58		Maximum	**118.28**	**0.74**
38	87.99		Minimum	**83.26**	**0.74**
39	91.40		CV (%)	**1.08**	
40	92.22				

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