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Increased grain protein and gluten contents of bread wheat caused by introgression of a *T. timopheevii* segment into chromosome 2A

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Abstract The end use and value of bread wheat grain is determined by its protein and gluten content. Therefore, stable manifestation of this trait in a cultivar is an important breeding goal. According to some reports, the tetraploid species *T. timopheevii* is characterized by high grain protein and gluten contents. However, there is no example of the transfer of genes for these traits from this species to common wheat. The aim of this study was to transfer chromosome 2A with an introgression from *T. timopheevii* from an experimental line (Line 821) to the bread wheat cultivar Saratovskaya 29 (S29) and to show its association with increased protein and gluten contents in grain. Chromosome 2A from Line 821 was transferred to cv. S29 by means of chromosome substitution, and its integrity was checked using 14 molecular markers. Substitution line S29(821 2A) was grown at 5 different locations: Novosibirsk, Omsk, Barnaul, Ussuriysk and Gatersleben, and in a greenhouse. The protein and gluten contents in the grain of S29(821 2A) produced under all conditions were significantly higher by 1–2% and 3–4%, respectively, than in the parental cultivar. In most cases, this introgression did not reduce grain yield and retained thousand grain weight. Substitution line S29(821 2A) had high rheological properties of dough comparable to the high-quality cv. S29. The new genetic factor might belong to the homoeoallelic series *Gpc-2* for high grain protein content.

Keywords Common wheat · Protein content · Chromosome substitution · Yield components

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Introduction

Vegetable protein makes up an average 57% of the human diet (Ghanem 2010). Wheat grain accounts for 20% of the world's calories and protein consumption (Erenstein et al. 2022; https://www.wheatinitiative.

org/ last accessed 04.08.2022). Plant-based protein consumption is predicted to rise in the future along with growth of the world population, which could reach 10 billion people in 2060 (United Nations 2021, https://news.un.org/en/story/2022/07/1122272, last accessed 27.09.2022). The protein content in wheat grain characterizes its nutritional and dietary value. The percentage of protein and gluten in the grain, as well as gluten quality, determine its use for food, feed or industrial purposes. The domestic and international grain trade is based on this indicator. Grain with high protein content has a higher market value and generates more income for producers. Therefore, this trait and its stable manifestation in cultivars is one of the most important selection targets in wheat breeding. Genotypes with a high protein content can be found among bread wheat cultivars; for example, the winter cultivar Atlas 66 (Shewry 2009). The donors of high protein content can be related species, such as *Thinopyrum ponticum* (Labuschagne et al. 2002) and *Aegilops speltoides* (Shchukina et al. 2020). The cultivar Plainsman V has a high protein content due to an introgression from *Ae. ovata* (Sharma and Gill 1983). The gene *Gpc-B1*, located on chromosome 6B was transferred to durum and common wheat from the wild tetraploid wheat species *Triticum dicoccoides* (Tabbita et al. 2017). The grain of the tetraploid species *T. timopheevii*, which is closer to bread wheat, also contains a large amount of protein and gluten (Mitrofanova and Khakimova 2016; Relina et al. 2018). The protein content in this species correlates with the level of expression of the gene *Gpc-G1*, an orthologue of *Gpc-B1* (Hu et al. 2013). *T. timopheevii* has contributed disease resistance genes to bread wheat (McIntosh et al. 2013), but we are not aware of examples of the transfer of genes for high protein content. Line 821 obtained from a cross of the Russian spring cultivar Saratovskaya 29 (S29) and *T. timopheevii* ssp. *viticulosum* had resistance to leaf rust (Leonova et al. 2008) and 43% wet gluten content in the grain (Obukhova et al. 2008). An analysis using molecular markers showed that the line carries introgressions on chromosomes 2A, 2B, and 5A. Chromosome 5A carried a small distal fragment on the long arm, whereas chromosomes 2A and 2B carried introgressions of extended length involving both chromosome arms. Since the latter chromosome introgressions were large we considered them to be the more likely carriers of genes contributing to high protein

and gluten contents in the grain of Line 821. Moreover, QTLs for these traits were found on these two chromosomes in previous studies (Groos et al. 2003; Li et al. 2009; Bogard et al. 2011; Sun et al. 2016; Liu et al. 2019). The purpose of this study was to transfer chromosome 2A from Line 821 to cultivar S29; to show the presence of a responsible genetic factor; and to study the effect of the introgression on grain quality and yield components in different environments.
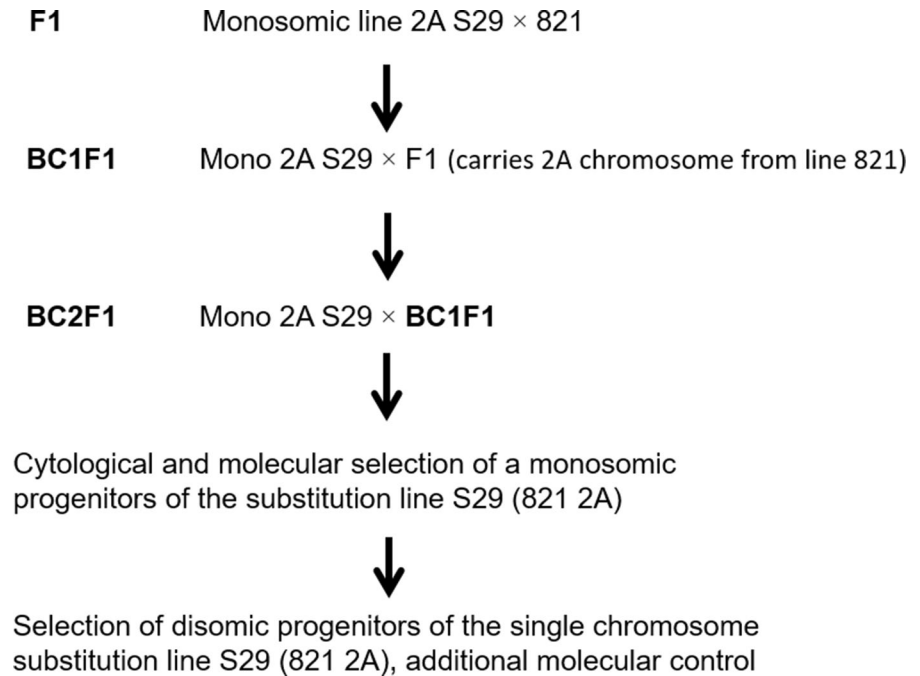
Materials and methods

Initial genotypes and development of a single chromosome substitution line

Line 821 belongs to a collection of stable bread wheat lines with introgressions from *T. timopheevii* produced by E. Budashkina at the Institute of Cytology and Genetics SB RAS. The line was the donor of increased grain protein and wet gluten content for this study. The line is in the genetic background of spring cultivar Saratovskaya 29 (S29) and carries introgressions into chromosomes 2A, 2B and the long arm of chromosome 5A (Leonova et al. 2008). S29 has excellent grain and flour properties but relatively low grain protein and wet gluten contents.

Transfer of chromosome 2A from Line 821 was carried out using a 2A monosomic line of S29 (mono 2A S29, Fig. 1). A monosomic line has 41 chromosomes, and MI of meiosis forms a configuration of twenty bivalents and one univalent 2A chromosome $(20'' + 1')$. The maternal gametophyte of a monosomic plant mostly forms 20-chromosome gametes lacking the 2A chromosome. In the first cross, Line 821 was used as a male and crossed to a selected monosomic plant. Monosomic F1 plants were selected and backcrossed as male to selected S29 2A monosomic plants over two generations. This procedure enabled an unchanged chromosome 2A to be passed from one generation to the next. After two backcrosses two monosomic plants $(20'' + 1')$ were selected and selfed before identification of disomic progeny as chromosome substitution lines named S29(821 2A) D1 and S29(821 2A) D2. Absence of the 2B and 5A introgressions in these lines was verified using molecular markers.

Fig. 1 Transfer the chromosome 2A from Line 821 into cv. S29

F1   Monosomic line 2A S29 × 821

↓

BC1F1   Mono 2A S29 × F1 (carries 2A chromosome from line 821)

↓

BC2F1   Mono 2A S29 × **BC1F1**

↓

Cytological and molecular selection of a monosomic progenitors of the substitution line S29 (821 2A)

↓

Selection of disomic progenitors of the single chromosome substitution line S29 (821 2A), additional molecular control

Chromosome configurations were determined by acetocarmine staining at the metaphase I stage of meiosis in pollen mother cells.

Genotyping of the S29(821 2A) lines

The correctness of the chromosome substitution with introgression from *T. timopheevii* in the SILs S29(821 2A) D1 and D2 was verified by identifying the alleles of microsatellite markers of the 2A chromosome in parental forms. The original set of molecular markers (Leonova et al. 2008) was expanded using the genetic map (Röder 1998). A total of 27 markers were tested, of which 14 were informative and were used to validate the substituted chromosome. The absence of the introgression in chromosome 5A was confirmed using morphological markers (presence of awns, leaf pubescence, spike shape) and microsatellite markers *Xgwm179, Xgwm126, Xgwm6* and *Xgwm291*. Absence of the 2B chromosome introgression was determined using microsatellite markers *Xgwm257, Xgwm148, Xgwm630, Xgwm319, Xgwm120, Xgwm047* and *Xgwm526*.

DNA isolation was performed according to the protocol from Plaschke et al. (1995). Primer sequences and conditions for PCRs for most were as described in Röder et al. (1998). Information for

markers *Xgwm817* and *1053* was obtained from M. Röder (IPK, Gatersleben, Germany), and information for markers *Xgwm1508, 1539, 4276, 4601, 4849* was obtained from M. Ganal (TraitGenetics, GmbH). The resulting PCR products were separated in 3% agarose gels and photographed under UV light using the Molecular Imager® Gel DocTM XR + System (Bio-Rad Laboratories, Inc., Hercules, CA, USA).

Growing conditions

Development of S29(821 2A) D1 and D2 was carried out in a hydroponic greenhouse at the Institute of Cytology and Genetics at Novosibirsk. Plants were grown in tubs (dimension: 4,0 × 1,0 × 0.35 m) filled with artificial soil (expanded clay); Knop's solution was used for nutrition. A 14/10-h photoperiod with day/night air temperatures of 18/20 °C before and 20/22 °C after tillering was applied. Ten plants were grown per row.

One of the lines was included in the experiment to study the effect of contrasting irrigation conditions on the manifestation of agronomically important traits. The experiment was carried out during three growing seasons in a hydroponic greenhouse. Seven plants of each line and recipient variety S29 were sown in two independent replicates. From germination to the

tillering stage all plants in the baths were watered twice a day. One of the tubs was disconnected from the regular water supply at the beginning of tillering to create artificial drought conditions. The second tub (control) continued to be watered twice daily until the end of the growing season. The moisture level was measured once a week at a depth of 6 cm using a moisture metre (AMF038, Amtast USA Inc, China). Soil moisture in the control variant averaged 28–30% per season. In the drought variant, the soil moisture gradually decreased and after a month of drought reached an average of 10–12%.

Field trials using the S29(821 2A) line were carried out at five locations: Novosibirsk (Western Siberia, 55°01′N; 82°55′E), Omsk (Western Siberia, 54°58′N; 73°23′E), Barnaul (Western Siberia, 53°42′N; 83°51′E), Ussuriysk (Russian Far East, 43°01′N; 131°57′E) and Gatersleben (Germany, 51°49′N; 11°17′E) (Online Resource 1). The lines were grown at Novosibirsk in 2016–2020, at Ussuriysk in 2018–2020, at Gatersleben in 2019 and 2020, at Omsk in 2017–2021 and at Barnaul in 2021. The experiments at each site were organized according to accepted experimental designs. A detailed description of agronomic practices and experimental conditions in different regions is given in Online Resource 2.

Phenotyping of technological properties of grain and flour

The technological properties included thousand grain weight, grain vitreousness, flour particle diameter after milling, and grain protein and wet gluten contents. For grain harvested in the field, the physical properties of flour and dough, water absorption and flour mixing characteristics were determined. Thousand-grain weight (TGW) was determined by weighing 100 grains. Total grain vitreousness was determined visually after cutting 100 grains in halve. The indicator of total vitreousness was the sum of fully vitreous grains and half the number of partially vitreous grains (GOST 10987-76). The average diameter of flour particles was determined using a PSH-4 device (Pshenichnikova et al. 2010). The wet gluten content was determined according to GOST R 54,478–2011 modified for research. Gluten was washed manually in water from 1 g of whole meal. The amount of wet gluten was expressed as a percentage of the weight. Grain protein in samples from

Novosibirsk and Ussuriysk was determined using an infrared analyser (OmegAnalizer G, Germany). Data on protein and gluten contents in grain produced in the field at Omsk and Barnaul were obtained using an Infralum FT-10 infrared analyser (Lumex, S.-Petersburg, Russia). Wet gluten content in grain and physical properties of dough were determined at the Section of Grain Quality Genetics in the Institute of Cytology and Genetics. Dough physical properties were determined on a Chopin alveograph equipped with a fifty-gram kneader (ISO-5530-4-91 1-2013 modified for research). Flour mixing characteristics were determined on a Brabender farinograph with a fifty-gram kneader (ISO 5530–1-2013 modified for research).

Analysis of yield components

To study the relationship between protein and gluten contents and productivity the yield components were studied along with the target traits from all locations except Gatersleben and in greenhouse conditions with normal and restricted water supply. The following traits were assessed at Novosibirsk (greenhouse and field): number of tillers per plant, stem length, number and weight of grains on the main and secondary tillers, number and weight of grains from the whole plant, and TGW. At other locations the number of tillers per plant, stem length, number and weight of grains in the main spike, yield from the whole plant and TGW were determined.

Statistical analysis

Two-way analysis of variance was used to assess the significance of the genotype and environmental factors in all cases where possible. Least significant differences (LSD_{05}) were calculated to determine differences. Genotypes were fixed effects, and environments (geographical locations and water regimes in the greenhouse), replications and their interactions were random effects. This statistical approach was applied for the analysis of protein and gluten content in grain grown at Novosibirsk and Ussuriysk and for other technological properties of grain grown at Novosibirsk. Two-way analysis of variance without replicates was used in analyses of protein and wet gluten content in grain grown at Gatersleben, Barnaul and Omsk. In these cases, genotypes were fixed

effects, and the environments (geographical locations) and their interactions were random effects. Student's *t*-tests were used to evaluate significant differences of the S29(821 2A) lines from the recipient cultivar S29 for yield components at Novosibirsk under field and greenhouse conditions. The relationships among wet gluten content, plant grain weight and TGW were studied using Pearson's correlation analysis. Statistical data processing was carried out using the EXCEL 2013 analysis package.

Results

Line 821 developed in the genetic background of cv. S29 carries introgressions from *T. timopheevii* into chromosomes 2A, 2B, and the long arm of chromosome 5A. A 3-year study of wet gluten content in the line grown in the greenhouse and field seasons showed that the average value of the trait was significantly higher than that of the recipient S29 (Table 1). The increase in the introgression line was 5.8% and 8.6% under the conditions of hydroponic growth and in the field, respectively.

Development of S29(2A) chromosome substitution lines and their phenotypic and molecular characterization

Backcrossing was used to transfer chromosome 2A to S29 (Fig. 1). With such a crossing scheme, the 2A chromosome is transferred into the genotype of the recipient variety in an unchanged state, while introgressions in other chromosomes are eliminated. Since Line 821 was already in S29 genetic background, two backcrosses were sufficient to eliminate the *T.*

Table 1 Wet gluten contents in grain of Line 821 grown under greenhouse and field conditions

Genotype	Conditions	
	Greenhouse	Field
S29	40.3	31.3
Line 821	46.1**	39.9***
F_G	24.72	51.44
LSD_{05}	3.13	3.24
F_{05}	5.98	

$**P < 0.01$; $***P < 0.001$

timopheevii chromatin in chromosomes 5A and 2B. Line 821, unlike the original cultivar, has an awned spike as the introgression involved the subtelomeric region of the 5A chromosome carrying the awn inhibitor allele *B1*. Chromosome elimination was monitored visually by the absence of awns on the spike in offspring at each stage of crossing. At the stage of isolating disomic plants, its absence was also checked using the *Xgwm291* microsatellite marker linked to the *B1* gene (McIntosh et al. 2013) (Fig. 2). In addition, leaf pubescence, determined by the Hl^{tt} gene of *T. timopheevii* (Simonov et al. 2021), was used as a morphological marker of the 5A introgression. Elimination of the 2B chromosome was checked using 7 chromosome-specific microsatellite markers located across both arms at the stage of the second backcross and at the stage of isolation of the disomic plants (Fig. 2). Twenty-seven markers were used to check the integrity of the introgression fragment in chromosome 2A and its identity to the original fragment in Line 821. Of these, 14 were polymorphic and confirmed the 2A substitution. The size of the introgression from *T. timopheevii* into the chromosome 2A and distribution of the molecular markers are shown in Fig. 3. The development of the substitution lines was accompanied by tests of wet gluten content. At the stage of the first backcross, the value was 49% in the lines versus 43% in S29. At the stage of isolation of disomic plants, these values were 43% and 36%, respectively. This indicated the preservation of the target trait in the process of development. The sister substitution lines S29(821 2A) D1 and S29(821 2A) D2 were further studied in Novosibirsk and one of the lines was studied at other geographical conditions.

Protein and wet gluten contents in the grain of the substitution lines grown under field conditions
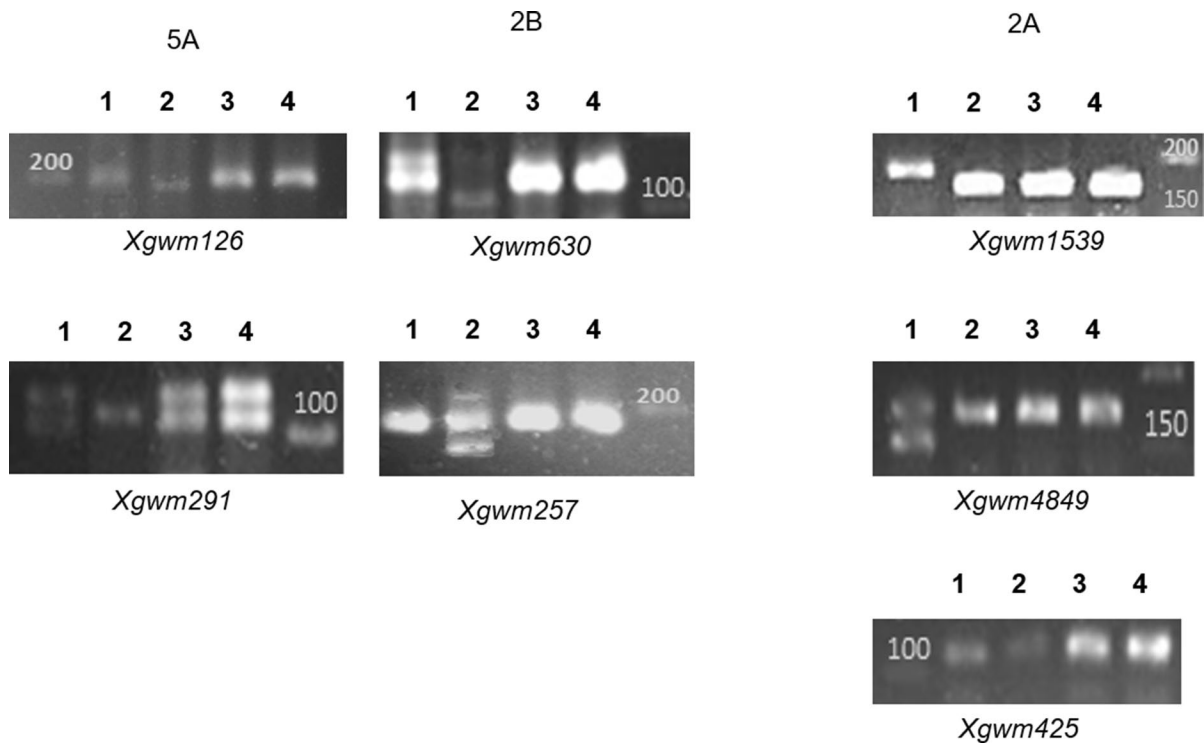
The average contents for these traits are presented in Table 2. The wet gluten contents of the substitution lines were 36.5 and 36.9%, respectively, and were significantly different from that of S29 (32.3%). The protein contents in lines D1 and D2 were 18.0 and 18.04%, respectively; an increase of at least 1.5% compared to S29. Under water deficit conditions the wet gluten contents of the lines exceeded S29 by 3–4% (Table 3).

Target traits were studied in geographic areas located thousands of kilometres apart (Table 4).

Fig. 2 Allele-specific PCR products of primers of some microsatellite markers used for detecting presence/absence of introgressions from *T. timopheevii* on chromosomes 5A, 2B and 2A. 1, S29; 2, Line 821; 3, S29(821 2A) D1; 4, S29(821 2A) D2; *Xgwm*—microsatellite marker; 100—150—200 – molecular weight ruler (bp)

Under Gatersleben conditions, the wet gluten content in the grain of the substitution line was 35.2%, or 3.9% higher than that of S29. The protein content under the same conditions was 16.5% and 15.1% in S29. Similar results were obtained at Omsk. The wet gluten content in the grain of the substitution line was 34.3%, and in the original variety 31.4%, the protein content was 17.6% and 15.7%, respectively. Less gluten and protein in the grain accumulated at Ussuriysk and Barnaul compared to the other locations. Nevertheless, the values for the substitution line were significantly higher than those for S29. The protein content in the line grown at Ussuriysk was 15.5%, whereas it was 13.4% for cv. S29, and the wet gluten contents were 26.5% and 23.5%, respectively. At Barnaul, the protein content was 14.8% for the substitution line and 13.8% for S29, and the wet gluten contents were 28.0% and 24.6%, respectively. Therefore, S29(821 2A) consistently showed a 1–2% increase over S29 in protein content and a 3–4% increase in wet gluten content at each location.

Other technological and physical properties of the grain, flour and dough in the S29(821 2A) lines and S29 at various locations were consistent with the strong wheat class (suitable for blending). To determine whether the introgression affects other technological parameters one line was grown at all locations. Average values obtained over four years at Novosibirsk are listed Online Resource 3. Grain vitreousness and flour particle size were maintained at the level of S29. According to the alveograph data, the sister substitution lines showed a significant increase in dough extensibility by approximately 16 mm compared to S29. Line D2 had lower dough elasticity than D1 and S29. This resulted in lower flour strength (315.0 J*10–4) and P/L ratio (Online Resource 3). Lines D1 and D2 also had slight differences in farinograph parameters; dough development time for D1 was significantly higher (275 s compared to 250 and 260 s for S29 and line D2, respectively). Both lines showed significantly reduced dough thinning during mixing by 13 and 17

2A
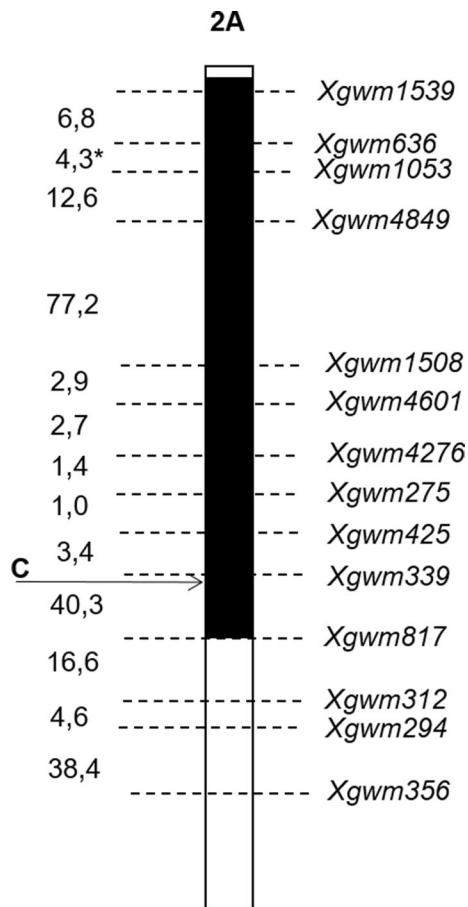
Fig. 3 Molecular map of chromosome 2A in S29(821 2A). *Xgwm* – microsatellite marker. Black color shows the region of introgression from *T. timopheevii*. Distances between markers are calculated from the map in Röder et al. (1998); *, distance between *Xgwm636* and *Xgwm1053* is according to Iqbal et al. (2007)

Table 2 Protein and wet gluten contents in grain of S29(821 2A) and parental cultivar S29 grown under field conditions at Novosibirsk. D1, D2 – sister disomic substitution lines

Genotype	[a]Wet gluten, %	[b]Protein, %
S29	32.3	16.5
S29(821 2A) D1	36.5**	18.0*
S29(821 2A) D2	36.9**	18.4*
F_G	8.53	13.33
LSD_{05}	2.58	0.86
F_{05}	3.89	5.14

**P<0.01; *, P<0.05

[a]Mean, 2016—2020

[b]Mean, 2018 and 2020

Table 3 Wet gluten contents (%) in S29(821 2A) grown in the greenhouse under contrasting water supply over three growing seasons

Growing conditions	Genotype	
	S29	S29(821 2A)
Normal watering	32.7	35.3
Drought	34.6	38.2*
Mean	33.7	36.7*
F_G	4.84	
LSD_{05}	2.8	
F_{05}	4.35	
F_F	ns	

*P<0.05

farinograph units, respectively, along with improved valorimeter scores (Online Resource 3).

Under other growing conditions the substitution line slightly exceeded S29 in vitreousness. This trait was highly dependent on location (Online Resource 4); the lowest values were from Omsk and then in ascending order from Ussuriysk, Gatersleben and Barnaul. There was no difference in grain hardness. Flour strength in samples from Ussuriysk and Barnaul was lower than those from Gatersleben and Omsk. Tenacity was decreased in samples from three of the four locations. Dough extensibility was higher in samples from all locations (Online Resource 4). Therefore, the physical properties of flour and dough from the substitution line were at least equal to the high-quality cv. S29.

The mixing characteristics of flour from the substitution line and S29 were studied in samples from Gatersleben and Barnaul in addition to Novosibirsk. As seen from the data, the Water absorption capacity of the flour was higher in samples from Gatersleben (Online Resource 5). The line also had increased resistance to mixing and improved valorimetric value and dough thinning consistent with the data from samples at Novosibirsk. Differences between the substitution line and S29 in samples from Barnaul were minimal.

Table 4 Protein and wet gluten contents in grain of S29(821 2A) and cultivar S29 grown under field conditions at different geographical locations

Genotype	Wet gluten, %	Protein, %
Gatersleben, Germany, 51°49′ N; 11°17′ E		
S29	31.3	15.1
S29 (821 2A)	35.2*	16.5**
F_G	43.0	208.0
LSD_{05}	1.9	0.29
F_{05}	18.5	18.5
Omsk, Western Siberia, 54°58′N; 73°23′E		
S29	31.4	15.7
S29 (821 2A)	34.3*	17.6***
F_G	21.3	224.8
LSD_{05}	2.10	0.43
F_{05}	10.1	10.1
Ussuriisk, Far East, 43°01′N; 131°57′E		
S29	23.5	13.4
S29 (821 2A)	26.5*	15.5*
F_G	6.67	11.9
LSD_{05}	2.53	1.4
F_{05}	6.0	7.7
Barnaul, Western Siberia, 53°20′N; 83°46′E		
S29	24.6	13.8
S29 (821 2A)	28.0***	14.8*
F_G	38.1	12.5
LSD_{05}	1.2	0.63
F_{05}	5.0	5.0

**$P < 0.01$; *$P < 0.05$

Yield components in S29(821 2A) grown at different locations

Increased protein content in wheat grain is often associated with a decrease in yield, particularly with decreased TGW (Simmonds 1996). Therefore, yield components were also measured at each location (Table 5). The sister substitution lines behaved similarly. Stem length was increased by 5.8 cm compared to S29. The spike length of the main tiller was 0.5 cm longer, but the number of spikelets remained the same. The grain number in the main and secondary spikes increased by 3.3 and 19.8, and grain weight increased by 0.12 g and 0.68 g, respectively. The overall yield per plant was increased by 0.5 g, but TGW was unchanged relative to S29. In contrast to the field conditions, the substitution line was significantly inferior to S29 in almost all yield indicators under greenhouse conditions (Table 6). Although the stem of the substitution line was

5.6 cm longer, the grain weight per plant was 1.4 g lower, and TGW decreased by 13.7 g.

In other growing regions, the absolute values of the yield components differed. The stem length of the substitution line was 4–6 cm longer on average compared to S29 at three of the four locations; however, there were no significant differences between Line S29(821 2A) and S29 in yield components at Novosibirsk, Barnaul and Ussuriysk. The grain yield per plant was lower only at Omsk. The highest number of productive tillers, largest number of spikelets per spike, and highest plant productivity were at Ussuriysk, the region with highest humidity. The grain yield per plant for both genotypes was approximately 5 g compared to approximately 3 g at other locations (Online Resource 6). Yield components at Gatersleben were not determined, but no difference in TGW was found between the substitution line and cv. S29 (Online Resource 4). Correlation analysis including the data from all field conditions and the greenhouse indicated no significant dependences among

Table 5 Yield components of S29 (821 2A) lines and parental cultivar S29 grown under field conditions[#] at Novosibirsk

Genotype	StL (cm)	NT	Main spike			Secondary spikes		Total yield/plant		
			SL (cm)	GN	GW (g)	GN	GW (g)	GN	GW (g)	TGW (g)
S29	76.6±10.3	4.8±1.3	6.6±0.9	25.0±6.0	0.85±0.3	56.7±24.3	1.6±0.8	81.7±28.0	2.5±1.0	27.5±7.3
S29(821 2A) D1	81.0±7.1*	4.9±1.1	7.0±0.7**	28.1±5.7*	0.94±0.3	74.3±26.0***	2.1±0.9**	102.4±29.1***	3.0±1.1**	27.9±6.6
S29(821 2A) D2	83.8±8.5***	5.1±1.3	7.2±0.9***	28.6±6.2*	1.0±0.3**	78.6±27.9***	2.3±1.0***	107.2±31.0***	3.3±1.2***	29.0±6.7

StL stem length; *NT* number of tillers per plant; *SL* spike length; *GN* grain number; *GW* grain weight; *TGW* thousand grain weight

#, Seasons 2017, 2018, 2020

wet gluten content, grain weight per plant and TGW (Online Resource 7).

Discussion

Grain protein and gluten contents are sensitive to climatic factors and agronomic practices. For example, it was estimated that less than 30% of the variation in protein content among UK winter wheat varieties is determined by genetic factors (Worland and Snape 2001). To maintain an acceptable level of protein in the future, it is necessary to expend resources to maintain water and nitrogen balance in the soil (Guttieri et al. 2005). Therefore, obtaining wheat cultivars with stable, genetically controlled protein levels is an important breeding goal. Despite significant variation in protein and gluten contents, related species are also considered a potential source of genes that could contribute to maintaining acceptable levels.

Line 821 showed higher wet gluten contents that S29 in grain produced in both the field and greenhouse (Table 1). However, it contained no introgressed chromatin from chromosomes of homoeologous group 6, which possibly carry genes from the orthologous *Gpc-1* series. To determine which of the introgressions in chromosomes 2A, 2B, and 5A carried the gene(s) for high protein and wet gluten contents, we decided to separately 'reintrogress' them into S29. We chose 2A preferentially because QTLs associated with increased protein content had already been mapped on that chromosome. Using a population of recombinant inbred lines (RILs) from cross Renan×Récital, Groos et al. (2003) localized a QTL associated with increased protein content in the region of molecular marker *Xgwm636* located in the short arm of chromosome 2A included in Line 821 (Fig. 3). In another set of RILs involving a cross of Chinese varieties Neixiang 188 and Yanzhan 1, Li et al. (2009) found that the *QGpc.caas-2A.1* was associated with a high protein content in the region of marker *Xwmc455*. According to the chromosome 2A consensus map (https://wheat.pw.usda.gov/GG3/), this marker was located about 7.6 cM from *Xgwm339* located in the centromere region of the introgressed fragment (Fig. 3). Bogard et al. (2011) showed an association between the marker *wPt-9104* from the telomeric region of the short arm of chromosome 2A and protein content in double haploid

Table 6 Yield components of S29(821 2A) and parent S29 grown under contrasting watering conditions in a greenhouse

Growing conditions	Normal watering		Drought	
	S29	S29(821 2A)	S29	S29(821 2A)
StL, cm	96.7	100.2	88.4	93.6*
NT	4.8	4.1**	3.1	2.9
GN per main spike	26.1	24.4*	26.1	22.9**
GW per main spike, g	1.13	0.99	0.99	0.68***
GN per secondary tiller	80.8	57.5***	38.2	39.3
GW per secondary tiller, g	2.9	1.8***	1.23	0.92*
GN per plant	105.7	78.4***	64.1	59.9
GW per plant	4.3	2.2***	2.9	1.5*
TGW	38.9	34.1	39.4	25.7***

$*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

StL stem length; *NT* number of tillers per plant; *GN* grain number; *GW* grain weight; *TGW* thousand grain weight

lines derived from cross Toisondor × CF9107. Finally, *QGpc.sdau-2A.1* was mapped near marker *Xgwm47* in RILs of cross Chuan 35,050 × Shannong 483 (Sun et al. 2016). This marker was located 6.8 cM from the marker *Xwmc455* mentioned above (https://wheat.pw. usda.gov/GG3/), and near *Xgwm339* used in the present work.

Genotyping with molecular markers confirmed that S29(821 2A) carries an introgressed chromosome 2A that was identical in structure to the original line (Fig. 2). The two substitution lines studied independently for agronomically important traits under field conditions at Novosibirsk showed approximately equal superiority in grain protein and gluten contents compared to the S29 parent (Table 2). Studies at various geographic locations (Online Resource 1) showed that one of the substitution lines retained a superiority of 1–2% for protein and 3–4% for wet gluten across sites (Table 4). Date of sowing and the duration of growing season did not affect the ranking of these values (Online Resource 2).

Our overall results indicated that the introgression from *T. timopheevii* in S29(821 2A) carried a genetic factor(s) determining higher grain protein and wet gluten contents. However, the difference in wet gluten content between S29 and the substitution line was considerably less than that between S29 and Line 821 (Table 1). This indicated that Line 821 also carries an additional gene(s) for this trait on chromosome 2B or 5A. Separate substitution lines for these chromosomes have been obtained and are being studied.

The recipient cultivar S29 has excellent physical properties of flour and dough and belongs to the 'strong' class. Although the introgression could have affected the unique physical properties S29(821 2A)

maintained flour strength at the level of S29 and remained in the 'strong' class (Online Resource 3, Online resource 4) while having a significant increase in dough extensibility and improved mixing characteristics expressed as increased dough formation time, decreased dilution and increased valorimeter score (Online Resource 3). These results were consistent with the those of Kerfal et al. (2010) who identified genes associated with physical dough properties measured by alveograph and mixing tolerance and dough extensibility measured by mixograph. These were located on the short arm of chromosome 2A in the region of molecular markers *Xwmc177* and *Xwmc522*, close to the marker *Xgwm4849*, which was monitored in the present introgression (Fig. 3).

Previous studies reported negative links between protein content and yield (Simmonds 1996; Bogard et al. 2010). Other work showed that this connection can be broken (Blanco et al. 2012, Liu et al. 2019). Here we document a consistent increase in protein and wet gluten content in substitution line S29(821 2A) compared to its recurrent parent S29 without affecting yield components (Table 6). However, under the field conditions at Novosibirsk, where both substitution lines were studied over four seasons there were opposite results. They outperformed S29 in terms of stem and ear length and weight and number of grains per plant (Table 5) but TGW was equal to S29. In other areas of Western Siberia (Barnaul and Omsk), the substitution line also had a longer stem (Online Resource 6). In terms of grain yield per plant, the substitution line did not differ from the parent cultivar at Barnaul but had reduced yield at Omsk. Under conditions of high moisture at Ussuriysk, the substitution line had the highest yield per plant and was not

different from S29. It can be assumed that introgression to the 2A chromosome reduces the drought tolerance of S29. It is known that *T. timopheevii* is adapted to the ecological conditions of a humid cool climate (Dorofeev et al., 1987). Previously, it was shown that chromosome 2A of S29 contained a cluster of QTLs associated with physiological response to drought. These genes were associated with molecular marker *Xgwm339* located in the centromere region (Pshenichnikova et al. 2021). The yield of the substitution line was not evaluated at Gatersleben, but the TGW was similar to S29 (Online Resource 4).

As already noted, the only well documented gene that increases grain protein content is *Gpc-B1*. Its functional role as a transcription factor is to initiate plant senescence (Uauy et al. 2006). In the same work, a paralogous copy of the gene was found on chromosome 2B, but not on chromosome 2A of *T. durum*. However, another gene was found in *T. urartu* (Uauy et al. 2006), the likely donor of the A genome for wheats of the *timopheevii* group (Dvorák et al. 1993; Goncharov 2011). Thus, the genetic factor that we detected in chromosome 2A from *T. timopheevii* could be related to *Gpc-1*. To date, we have not observed physiological differences between the S29 and the substitution line except for stem length, drought response and some physical parameters of dough. For the genetic position of this gene on chromosome 2A and elucidation of its functional role, it will be necessary to dissect the introgression into smaller fragments by recombination. Considering its stability under various growing conditions this gene could be useful in breeding for stable grain protein and gluten contents.

Conclusion

Genetically controlled high grain protein and gluten contents is a valuable breeding target. Various wheat relatives are potential sources of such genes. In this work, we extracted a genetic factor(s) from *T. timopheevii* capable of increasing grain protein content by up to 2%, and wet gluten content by up to 4%. For this, we substituted chromosome 2A in *T. timopheevii* derived Line 821 into spring cultivar S29. The target traits of the substitution line grown in widely different geographic regions were preserved across seasons. Comparison of yield components of the substitution line with the recipient S29 showed that the introgression in most cases did not affect agronomic traits while retaining the same TGW. A decrease in yield per plant was observed only in the arid climate of Western Siberia and during artificial drought in a greenhouse. The substitution line not only retained excellent flour physical and mixing properties of S29, but also improved some of them. In particular, the dough of the substitution line liquefied less during mixing. It is suggested that the new genetic factor could be related to the *Gpc-1* gene series for high protein content.

Author contributions All authors contributed to the study conception and design. The lines were developed by LV Shchukina and TAP. Field investigations, data collection and analysis were performed by AGK, VPS, VEP, SBL, MVC, VAP, AB; molecular work was done by AVS and MAD. LVS and TAP wrote the first draft of the manuscript, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Declarations

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