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The phenotypic characterization of Rivet wheat accessions (Triticum turgidum L. ssp. turgidum) for grain quality and other agronomic properties

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Research Article

Keywords: Rivet wheat, grain vitreousness, gluten content in grain, dough rheology, yield components

Posted Date: October 13th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3426122/v1

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Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Genetic Resources and Crop Evolution on March 28th, 2024. See the published version at https://doi.org/10.1007/s10722-024-01909-4.

Abstract

Currently, there is an increased interest in obsolete accessions of cultivated species that do not meet modern agronomic requirements. A tetraploid Rivet wheat, which has a common genepool with durum wheat, is among them. Unlike the latter, Rivet wheat has so far been little involved in genetic and molecular studies. Technological properties of grain, which determine its end-use, are characterized especially weakly and fragmentarily. The purpose of this study was to evaluate a phenotypic diversity in six accessions of Rivet wheat from the collection of Gatersleben genebank (IPK, Germany) for these and other economically important traits. Milling parameters of grain, gluten content and quality, and physical properties of dough were studied. All characteristics, except flour particles diameter demonstrated a substantial variability. The difference for thousand grain weight was 12 g, for gluten content – 8%. The total grain vitreousness was in the range of 48–82%. Gluten, in general, had a low extensibility. One of the samples showed a high elasticity of dough and high flour strength according to alveograph. Two samples showed a darkening of gluten during washing, which indicates a high activity of polyphenol oxidases. The accessions also differed in yield components and in flowering date greater than 10 days. The variety in grain quality indicates the possibility of using Rivet wheat for a wider range of flour products compared to durum wheat. The obtained phenotypic data allow the purposeful use of Rivet wheat samples both in agricultural production and in scientific research.

Introduction

Tetraploid wheat (genomic formula AABB) is one of the oldest agricultural plants. According to the botanical classification of Van Slagereen (1994), its various forms are varieties of the species *Triticum turgidum* L. Among them, the durum subspecies (*Triticum turgidum* L. ssp. *durum* Desf.) is the most used for cultivation. Although the original cultivation area of the species is in the Mediterranean, it has been adapted for cultivation in various regions of the world through the efforts of breeders. Durum wheat grain is widely used in the production of pasta and specific local types of food. There is currently an increased interest in ancient, local as well as neglected forms of crop species, including tetraploid wheat (Mefleh et al. 2018), which have been adapted to low-input farming systems in past. This trend is associated with general deterioration of climatic conditions in the areas of cultivation, with the advancement of crops to the borders of natural areas and the introduction of organic farming, which avoids the use of chemical intensification of crop production. In addition, neglected forms have an unused reserve of genetic variability for breeding.

The closest relative of durum wheat is Rivet wheat or English wheat belonging to the subspecies *T. turgidum* ssp. *turgidum*. During the 20th century, this wheat was completely displaced from agricultural production, even in those areas where it was originally grown (the British Isles, the mountainous regions of Spain, Germany, Switzerland). At the same time, Rivet has such valuable properties as winter hardiness, flood resistance, disease resistance and high phenol content in grain (Ambrogio et al. 2019). Microsatellite markers were used to study the intraspecific diversity of the subspecies and its place in the Triticeae tribe (Li et al., 2006; Olivriea et al. 2012; Sicilia et al. 2022). Using nuclear and chloroplast SSRs,

Oliveira et al (2012) showed that Rivet and durum belong to the same genepool. The morphological and biochemical diversity of Rivet wheat has been studied to a certain extent (Zeven, 1990; Carmona et al., 2010; Alvarez et al. 2013). In particular, the diversity was studied in terms of alleles of grain storage proteins – glutenins and gliadins (Alvarez et al. 2013; Laidò et al. 2013), as well as the nutritional value of grain (Ambrogio et al. 2019). However, we were able to find only a few studies relating to grain technological properties of Rivet wheat, which determine grain end-use. N.I. Vavilov reported that "... cultivated from time immemorial in England and the mountainous regions of Germany, Italy and Spain, the most productive wheat of the Rivet type (*T. turgidum*) is characterized by low quality flour and is currently used for poultry feed and for the manufacture of biscuits" (Vavilov 1957). Historically cultivated in the mountainous areas of Sicily (800 to 1,200 m above sea level), the Bufala wheat group was used for food purposes - for the manufacture of certain types of local pastries (Sicilia et al. 2022). Involvement in breeding of this neglected genetic resource, now mostly stored in various genetic banks, requires the knowledge about certain grain characteristics. The purpose of this study was to assess the phenotypic diversity of grain and flour for a complex of technological properties, as well as other economically important traits in six randomly selected accessions of Rivet wheat. This will determine their possible use for agricultural production. In addition, the data obtained contribute to a more reasonable approach to the choice of ancient genotypes to improve modern varieties of tetraploid wheat.

Materials and methods

We used six accessions of the species *T. turgidum* ssp. *turgidum*. from the IPK genebank, Gatersleben, Germany (Table 1). They were selected for the presence of the conventional wheat name Rivet in the designation. Four accessions had a botanical identification and belonged to varieties *falsejodurum* (TRI 1256 and TRI 28439) and *dinurum* (TRI 4810 and TRI 28415). Two accessions (TRI 28386 and TRI 24752), although they were identified only to the level of the genus, were assigned to the species *T. turgidum* ssp. *turgidum* by the name Rivet. All accessions are of winter habit.

Six accessions were grown in a greenhouse at the Institute of Cytology and Genetics during three vegetative seasons. Before sowing, the seedlings were vernalized for two months under temperature + 2°C and 12-hour day length. Plants were grown under hydroponic conditions 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. The grain obtained under these conditions was studied for milling properties and the quantity and quality of gluten. 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

Table 1
List of Rivet accessions used in the study

#	Accession name in the collection of IPK	Botanical name	Accession number
1	“England Rivet Wheat”	*Triticum* sp.	TRI 28386
2	“Rivets Grannen”	*T. turgidum* convar. *turgidum* var. *falsejodurum* Flaksb.	TRI 1256
3	“Heines Rivetts Bearded”	*T. turgidum* convar. *turgidum* var. *dinurum* (Alef.)	TRI 28415
4	“Heines Rivetts Bearded”	*T. turgidum* convar. *turgidum* var. *dinurum* (Alef.)	TRI 4810
5	“RIVETTS GRANNEN”	*Triticum* sp.	TRI 24752
6	“RIVET”	*T. turgidum* convar. *turgidum* var. *falsejodurum* Flaksb.	TRI 28439

was the sum of fully vitreous grains and half the number of partially vitreous grains according State Standards 10987–76 (1977). 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 State Standard R 54478–2011 (2013) modified for research. Gluten was washed manually in tap water from 1 g of whole meal. The amount of wet gluten was expressed as a percentage of the weight. The properties of this washed gluten were also assessed for stickiness and extensibility manually. In addition, plants from these greenhouse seasons were studied for flowering date, ear morphology, stem length, and ear yield components. Three samples from this set, which showed contrasting grain quality, were grown in the field in Gatersleben. Plants were grown in plots 1.5 m × 2.5 m in size with sowing density of 60 g of grains per plot. The grains from the field were studied according to the above scheme and additionally for dough physical properties with the use of a Chopin alveograph equipped with a fifty-gram kneader (ISO-5530-4-91 1-2013) modified for research. The average values of grain quality parameters and yield components were calculated for each accession from the data of three greenhouse seasons. Two-way analysis of variance was used to assess the significance of the genotype and environmental factors. Least significant differences (LSD_{05}) were calculated to determine genotype differences. Genotypes were fixed effects, and environments (greenhouse seasons) and their interactions were random effects.

Results

Grain quality parameters

Figure 1 shows photographs of grain sections of tetraploid accessions and hexaploid common wheat. *T. durum* had a vitreous grain (Fig. 1, cv. Langdon) which was characterized by a transparent cut. This type of endosperm is also found in common wheat varieties that are classified as "hard" (Fig. 1, cv. Alpine). In contrast is the class of "soft-grain" wheat (Fig. 1, cv. Golubka) which have a mealy, opaque grain cut. A

variety for grain vitreousness was found among the tetraploid accessions of Rivet wheat. TRI 28386 had a vitreous endosperm similar to Langdon and Alpine (Fig. 1). TRI 28439 had mealy endosperm similar to Golubka. Samples TRI 28415 and TRI 4810 were found to be a mixture of hard and soft grains. TRI 1256 and TRI 24752 consisted of mealy and semi-vitreous grains (Fig. 1). The total vitreousness (%) ranged from 44 to 82% (Table 2). It was lowest in TRI 28439 with phenotypically mealy endosperm. The value 44.8% was comparable to the vitreousness of the soft-grain wheat variety Golubka (49.4%). The highest total vitreousness, 80.0%, was found in TRI 28386 with vitreous endosperm (Fig. 1). This value is close to the value in the durum wheat variety Langdon (92.0%). Sufficiently high vitreousness over 70% was shown by TRI 28415 and TRI 1256 exhibited a mixture of phenotypically hard and mealy grains. Two other accessions, TRI 4810 and TRI 24752, had a low trait value. The differences among accessions 1–3 and 4–6 in Table 2 were significant. TRI 28386, TRI 1256 and TRI 24752 maintained similar values of total vitreousness under field conditions of Gatersleben (Table S1).

Table 2
ANOVA analysis of technological parameters of the grain among the Rivet accessions from IPK collection together with cultivars Langdon, Golubka and Alpine, grown in the greenhouse (autumn 2020 and spring 2021)

Number in order	Accession number in IPK	Technological parameters of the grain				
		Vitreousness, %	Flour particle size, µm	TGWt*, g	Gluten content, %	Gluten quality, manually
1	TRI 28386	80.0	28.7	45.0	37.0	Gray, sticky
2	TRI 1256	73.5	26.2	39.1	30.0	Light, non-sticky
3	TRI 28415	72.0	27.8	41.7	36.3	Light, elastic
4	TRI 4810	58.8	27.3	38.0	28.3	Light, elastic
5	TRI 24752	51.8	27.7	39.8	35.5	Gray, very sticky
6	TRI 28439	44.8	28.5	38.7	32.8	Light, elastic
-	cv. Langdon§	92.0	30.1	39.2	28.5	—
-	cv. Golubka§	49.7	9.9	32.0	35.8	—
-	cv. Alpine§	100.0	19.1	37.6	38.5	—
F		6.4	0.8	13.4	6.5	
F_{05}	5.1					
LSD		11.0	-	2.9	1.4	

* - thousand grain weight determined after sample cleaning; § - reference data

Flour particles size after milling did not differ much among the accessions. The diameter was between 26 and 28 microns which is quite close to that of Langdon durum wheat (30.1 microns) (Table 2). Under field conditions, the particle diameter decreased (Table S1). Significant differences in TGW were found. The smallest grain had TRI 4810 with a value of 38.0 g. The largest grain was in TRI 28386 with TGW 45.0 g in greenhouse conditions and 54.5 g in the field.

The accessions differed significantly in gluten content and quality both under greenhouse and field conditions (Table 2). TRI 28386 showed the highest content – 37.0% in greenhouse and 43.0% in the field (Table S1). TRI 24752 and TRI 1256 showed the lowest gluten content in the greenhouse – 28.3% and 30.0%, respectively. According to this feature, they corresponded to the Langdon variety of durum wheat. The remaining accessions were comparable to common wheat varieties (Table 2). In the field, TRI 1256 showed a value close to greenhouse (32%), but TRI 24752 accumulated 8% more gluten in grain (Table S1).

Figure 2 shows gluten balls placed on graph paper. Their sizes also indicate the different gluten content in the grain of the accessions. In addition, the gluten balls varied in color. The darkest, gray shade was gluten of TRI 24752 both in greenhouse and field conditions (Fig. 2, sample 5). The gluten of TRI 28386 was also darkish. The remaining samples showed a light gluten. The shaped balls were tested manually for stickiness and rheological properties. All of them were sticky to varying degrees (Fig. 3). TRI 24752 gluten was the most sticky and difficult to shape, while TRI 1256 and TRI 28386 were more resilient. Extensibility was assessed by stretching a gluten ball. The most extensible at a length of 7 cm was the gluten of TRI 28439. The remaining accessions were significantly inferior and stretched only up to 3.5–4.5 cm. The most elastic and short-tearing gluten was in TRI 1256 (Fig. 3).

The three most rheologically contrasting Rivet accessions were grown in the field to determine the dough physical properties using alveograph. The results are presented in Table 3. They generally reflect the rheological properties of the samples identified manually. TRI 1256 which showed the most elastic gluten in manual tests showed the greatest flour strength – 303 J * 10 – 4. The value of elasticity on the device was also the highest – 151 mm. These values of flour strength and elasticity are characteristic of “strong” varieties of common wheat (Shchukina et al. 2022). The other two samples had a very low flour strength and 2.5 times lower dough elasticity. TRI 24752 was characterized by all the lowest values (Table 3). The ratio of elasticity to extensibility (P/L), which characterizes dough rheological properties as a whole, ranged from 2.7 to 4.1. TRI 1256 with high flour strength showed the highest P/L value.

Table 3
Physical properties of dough of the three Rivet accessions from IPK collection

Accession number in IPK	Accession name	Physical properties of dough			
		Dough strength, $J*10^{-4}$	Tenacity, P, mm	Extensibility, L, mm	P/L ratio
TRI 28386	England Rivet Wheat	65	66	35	3.2
TRI 1256	Rivets Grannen	303	151	37	4.1
TRI 24752	“RIVETTS GRANNEN”	51	66	25	2.7

Other agronomic traits

Rivet wheat accessions were also evaluated for a number of morphological and agronomically important traits. They differed in the number of days before flowering ranging from 54 to 68 days. TRI 24752 was the earliest. TRI 28415 and TRI 4810 of the botanical variety *T. turgidum* convar. *turgidum* var. *dinurum* (Alef.) did not differ in the date of flowering. In contrast, accessions TRI 1256 and TRI 28439 of *T. turgidum* convar. *turgidum* var. *falsejodurum* Flaksb. differed by 8 days. The samples showed large differences in plant height. The shortest stem length was 77.6 cm in TRI 24752. The maximum length of about 99 cm was in TRI 28386 and TRI 28415. Stem length was significantly different between the two accessions of *T. turgidum* convar. *turgidum* var. *dinurum* (Alef.) (TRI 28415 and TRI 4810) (Table 4).There were also significant differences in the spike length of the accessions of the same variety. The accessions with the longest stem had the longest spike (8-9 cm). The shortest spike (6.6 cm) was found in the accession TRI 24752 with the shortest stem. However, it should be noted that accession TRI 1256 with a stem length of more than 90 cm also had a short spike (6.9 cm). These ratios were maintained when grown under Gatersleben field conditions (Table S1).

Table 4
ANOVA analysis of yield components and number of days until flowering among the Rivet accessions from IPK collection grown in the greenhouse (autumn 2020 and spring 2021)

Number in order	Accession number in IPK	Number of days till flowering and yield components						
		NDF	SL (cm)	SpL	SpN	GN	GW	M1000
1	TRI 28386	54.1	99.9	9.0	19.1	32.3	1.34	43.0
2	TRI 1256	55.1	92.1	6.9	15.1	28.9	1.13	38.1
3	TRI 28415	61.5	98.6	8.4	17.6	46.9	1.84	40.3
4	TRI 4810	62.7	89.3	7.7	18.9	51.2	2.11	37.5
5	TRI 24752	68.5	77.6	6.6	18.3	45.2	1.58	34.8
6	TRI 28439	63.4	96.3	8.2	18.0	47.9	1.94	37.2
F		122.1	13.0	20.4	3.5	4.8	6.5	6.8
F_{05}		2.3			2.4			
LSD		2.0	6.4	0.6	1.9	11.5	0.39	2.8

Abbreviations: NDF – number days till flowering; SL – stem length; SpL – spike length; SpN – spikelet number; SN – grain number from the spike; GW - grain weight from the spike; M1000 – calculated thousand grains weight

Figure S1 shows the spikes of the studied accessions. Spikelet glumes of all of them were pubescent. At the same time, they differed in the color. Accessions of the botanical variety *T. turgidum* convar. *turgidum* var. *falsejodurum* Flaksb., as well as TRI 28386 of *Triticum* sp. had a colored ear and colored awns.

Accessions of the variety *T. turgidum* convar. *turgidum* var. *dinurum* (Alef.), as well as TRI 24752, had an uncolored spike and uncolored awns.

Discussion

In this work, a complex of technological characteristics of grain and flour the totality of which is usually defined by the general term “grain quality” was studied in Rivet wheat accessions. These characteristics include milling performance, gluten content and quality, and the physical properties of flour and dough. Flour milling characteristics include TGW, vitreousness and flour particles diameter. The average flour particle diameter of all Rivet accessions was about 27 microns. Although this value was slightly lower than that of the durum Langdon variety (Table 2), it was generally in line with the hard wheat class. This indicator did not show significant variation. It is well known that this property of tetraploid wheats is associated with the absence of D genome, which introduced *Ha* gene into the hexaploid common wheat genome. The dominant allele of the gene determines grain softness and introduces the diversity in flour particle size and vitreousness (Morris 2002). In an artificially synthesized tetraploid wheat with AADD genomic formula, the endosperm was super soft with a flour particle size of 8.7 μk and a vitreousness of 50% (Kurkiev et al. 2019).

Grain vitreousness is determined by the nature of the binding of starch granules to protein matrix in endosperm and is in close positive correlation with flour particles size during milling (Pshenichnikova et al. 2008). Despite the lack of diversity in particle size, we found significant variation among Rivet accessions for vitreousness. This important indicator of durum wheat grain quality is associated with the production of semolina. The presence of grains with a soft texture can impair pasta quality. In some studies, the variation in vitreousness in durum wheat was in the range of 70-90% (Korkut et al. 2007; Alsaleh et al. 2019). The percentage of starchy (soft) grains in various Sicilian Rivet accessions ranged from 6 to 72 percent (Spina et al. 2023). It has been shown that this trait is highly dependent on growing conditions, in particular, on air humidity during grain ripening (Sandhu et al. 2009). In our work, the vitreousness in Rivet showed the variability in the range of 45-82%. The accessions retained the level of the trait under different growing conditions. For example, TRI 24752 was soft-grained and TRI 1256 was hard-grained both in the greenhouse and in the field. This variability suggests that Rivet accessions can be used for different bread products.

The maximal difference in TGW among the accessions was 13 g (Table 2) which indicates that Rivet can be a donor of a high value of the trait. TRI 28386 and TRI 1256 from Table 2 showed high values in different greenhouse seasons and even exceeded them under the field conditions of Gatersleben. This may indicate a genetically based control of grain size. The samples differed in gluten content by 9% between the minimum and maximum average values. At the same time, TRI 28386 with the largest grains showed the highest gluten content. The persistence of this property in the field (Table S1) may indicate a genetic control for the trait level. Ancient Sicilian Rivet accessions contained between 20 and 30 percent of raw gluten content (Spina et al. 2023).

Gluten quality studied manually was rather different. The quality was the lowest in TRI 24752; the gluten disintegrated very quickly when washed. Extensibility was small in five accessions and varied within 1 cm (from 3.5 to 4.5 cm). This is generally the case for durum wheat (Ammar et al. 2000; Marchylo et al. 2001; Trad et al. 2014). Interestingly, the artificial tetraploid with AADD genomic formula that we studied earlier also had a high gluten elasticity (Kurkiev et al. 2019). Only one accession, TRI 28439 with the lowest vitreousness of 44.8% had an elastic gluten and exhibited an extensibility 1.8 times higher than the rest of the accessions (Figure 3).

Upon washing, the gluten acquired a different color. In two accessions, TRI 28386 and TRI 24752, it was dark. It is known that this occurs due to the activation of polyphenol oxidase enzymes in the aquatic environment (Fuerst et al. 2006). This activity promotes the synthesis of melanins and is associated with certain alleles of the genes located on the chromosomes of the second homoeologous group (Demeke and Morris 2002; Zhai et al. 2020). For grain used for food this feature is undesirable. However, the grain of such accessions may be used for the technical purposes of deep processing or feed, since the synthesized phenolic compounds have protective, antioxidant, and adsorbing properties.

The physical properties of flour and dough in the three Rivet wheat accessions with contrasting properties of gluten quality were studied using alveograph. They corresponded to the quality of gluten, determined by hand. TRI 1256, which had slightly extensible, lightest and non-sticky gluten, showed the highest flour strength (303 J*10-4) and high dough elasticity. These properties are comparable to the best quality bread wheats and allow this sample to be classified as extra strong (Marchylo et al. 2001). Such wheat can be an improver for weak bread wheat. Spina et al. (2023) found no high flour strength genotypes among 20 Sicilian Rivet wheats that were used in ancient times for local traditional bread. A large variability in flour strength is also characteristic of durum wheat. Thus, among 54 Canadian varieties, the differences were 140-150 $J*10^{-4}$ (Marchylo et al. 2001), and among 27 varieties of different geographical origin, the difference in flour strength reached 300 J*10-4 (Ammar et al. 2000). The other two accessions we studied in the field of Gatersleben (Table S1) had very low flour strength and elasticity and are unlikely to be suitable for food use. However, the high gluten content of the TRI 28386 sample makes it possible to recommend it, for example, for animal feeding.

In our work, we did not find any relationship between belonging to a particular variety and the quality of gluten. Two accessions belonged to *dinurum* variety. They were very similar in spike morphology; both had a white head of similar size, number of days to flowering, stem length and yield (Table 4, Fig. 4). They were also close in the technological properties of grain except contrasting vitreousness. TRI 1256 and TRI 28439 assigned to *falsejodurum* variety, not only differed in the same trait, but also had contrasting dough physical properties - flour strength and elasticity. They had a close M1000 value, but significantly differed in yield components (Table 4).

Rivet accessions also showed a great diversity in other morphological and agronomic traits. In particular, they differed significantly in the date of flowering. Two accessions (1 and 2, Table 4) were the earliest in both spring and autumn greenhouse growing seasons. Their difference with other accessions was

determined by the date of flowering in the autumn season. Samples 3-6 (Table 4) delayed flowering in autumn by 7-14 days. This may indicate the genetic diversity in terms of photoperiod response among Rivet wheats.

Morphometric parameters were also different. The maximum differences in stem length reached 20 cm, in ear length- more than 3 cm. It is interesting that these two characters did not always correlate with each other. Thus, TRI 1256 with a stem length of 92 cm had an ear 6.9 cm long, which is not significantly different from the length of the ear in TRI 24752 with the smallest stem length (Table 4). The two earliest accessions did not have the highest productivity per plant, but they showed a high TGW value, an important milling trait. It should be noted that the same two samples retained a high value of this trait in the field.

Conclusion

Many accessions of cultivated plants that are kept in the genebanks of the world can be involved in the breeding process. However, only a small part of them has been characterized by agronomically important traits. This is especially true for underutilized species as *T. turgidum* var. *turgidum*. This tetraploid wheat subspecies called Rivet is the closest relative of the *durum* subspecies, and shares a genepool with it. Although it was excluded from intensive farming systems in the 20th century, it can now be re-used as a source of genetic diversity for newly emerging crop production and breeding needs. Rivet wheat accessions stored in the IPK genebank in Gatersleben and studied in our work, demonstrated a great diversity in the technological properties of grain and flour. This diversity can serve the purposes of both food and feed, or technical end-use. On the other hand, the individual properties, such as diversity in grain vitreousness and dough physical properties can be used in genetic experiments to map the responsible loci in tetraploid wheats. The use of characterized accessions of Rivet wheat will help researchers or breeders to avoid mistakes in the choice of initial parents for crossing and can make the results more predictable.

Declarations

Funding

This work was supported by the Russian Science Foundation (project #23-26-10046) and the Ministry of Science and Innovation policy of Novosibirsk Region (project №p-59). Plants were grown in the Center for Shared Use “Laboratory of Artificial Plant Cultivation” (Institute of Cytology and Genetics SB RAS, FWNR-2022-0017)

Competing Interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Genetic material preparation and field experiment were performed by Andreas Börner; data collection and analysis were performed by Ludmila Shchukina and Tatyana Pshenichnikova. The first draft of the manuscript was written by Tatyana Pshenichnikova and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

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Figures



Figure 1

Grain cross-sections of Rivet wheat and bread wheat accessions demonstrating grain vitreousness

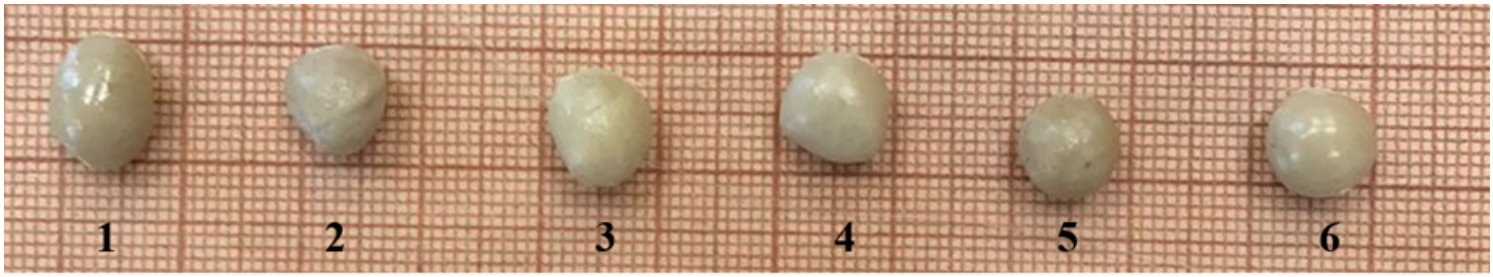


Figure 2

Gluten balls of Rivet accessions. 1. TRI 28386. 2. TRI 1236. 3. TRI 28415. 4. TRI 4810. 5. TRI 24752. 6. TRI 28439

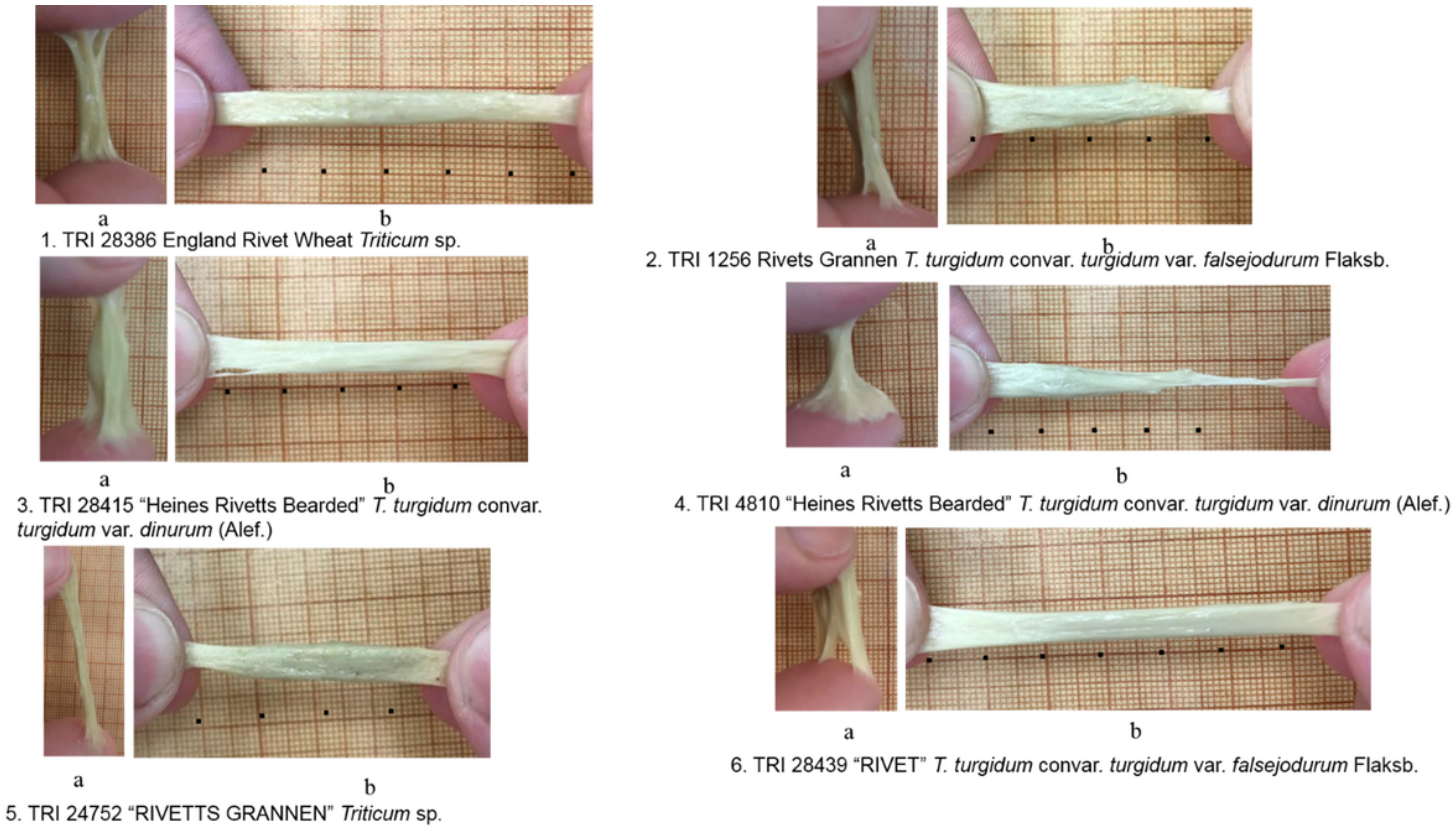


Figure 3

Gluten quality of Rivet accessions determined manually. Stickiness (a) and extensibility (b) are demonstrated. The black marks denote 1 cm

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