Final Report
K_20 NKFIH 135211 project
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Abstract

We compared the complex quality of spelt and common wheat in conventional and organic systems by examining globally significant sample population sizes. We demonstrated by 25K SNP array analysis that spelt is genetically distinct from wheat. We identified 406 spelt-specific SNP markers that are suitable for distinguishing the two species, as well as SNP markers related to fiber and fructan content. It was demonstrated that spelt varieties contained more protein and gluten, including more gliadin, which resulted in greater dough extensibility. Spelt starch had a higher pasting temperature, with more diverse starch viscosity properties, while the degree of starch damage was lower than in wheat. The arabinose/xylose ratio, which affects fiber solubility, was higher in spelt arabinoxylan. Several spelt genotypes had higher levels of arabinogalactan and short-chain carbohydrates than wheat varieties, but their average values were similar, and they contained similar amounts of FODMAPs than wheat. Spelt varieties were identified suitable for cultivation in low-input and organic farming systems, and gene bank accessions that could be promising genetic resources for improving the baking quality of spelt, and we produced three new spelt varieties. Our results help to find a professionally established place for spelt in modern nutrition, organic farming and baking industry.

1. Screening of the genetic diversity within and between spelt and wheat species

Twohundred spelt and onehundred common wheat genotypes were collected, sown, multiplied and analysed in our study. After selection**, genetic analysis** was carried out by using 25K array Illumina analysis on 282 lines, from which finally 268 (181 spelt and 87 common wheat) were appropriate for further analysis based on marker datas. Both the two species and the varieties could be well distinguished as shown by the principal component analysis and the hierarchical cluster analysis (Figure 1 and 2). The cluster analysis not only separated the two species, but it also separated a group of spelt genotypes (TSP2), which were genetically closer to the group of the common wheat than to the main group of spelt. This might be resulted by the presence of wheat in their genome.

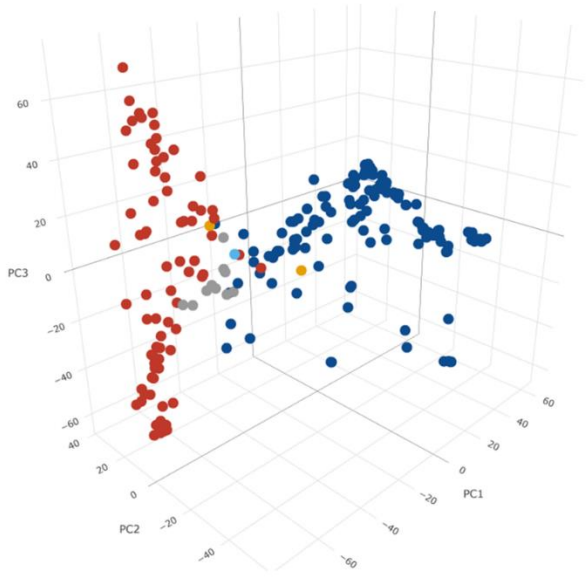

Figure 1. Comparison of wheat and spelt based on 25K SNP array analysis by using principal component analysis. Wheat (82) (red), Spelt (200) (blue), wild hexaploid species (32) (grey, light blue, yellow) (T. Compactum, T. Macha, T. Sphaerococcum).

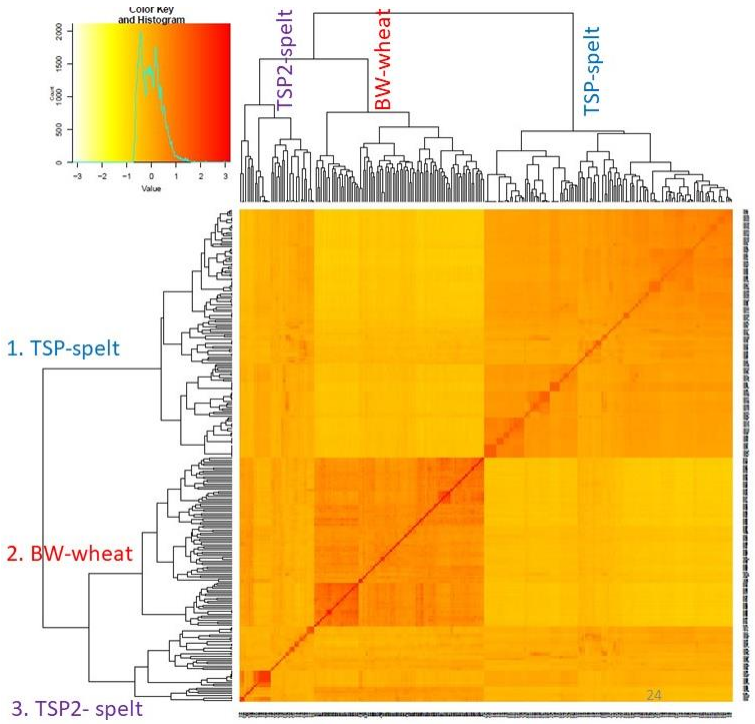

Figure 2. Hierarchical claster analysis by VanRaden kinship matrix based on genetic analysis of spelt and wheat with 25K SNP array. BW- bread wheat, TSP- Triticum spelta, TSP2- separate group of spelt geneticaly closer to bread wheat

Deatailed analysis of the agronomical, morphological traits of the plants and physical, compositional and processing quality of the seed was carried out and identification of the markers associated to the different traits was analysed (Figure 3.). Furthermore, 406 SNP markers specific to spelt wheat were identified.

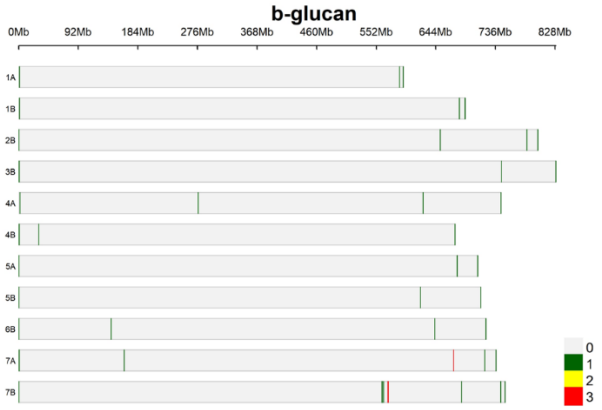

Figure 3. Determinant SNP markers associated to b-glucan content of the seed (an example)

The analysis was able to identified genotypes, which were taxonomically not belonged to the given species and those spelts which contained high ratio of common wheat in their genome.

Several papers are planned to be published from these results and is in progress. The reason of the small delay in publishing is due to the delay in the huge work of spelt treshing, cleaning and laboratory analysis during the Covid and energy saving period. However all the work is finished and ready for publishing.

2. Studies on association between genetic markers, composition and quality traits of spelt

The 268 genotypes (87 common wheat and 181 spelt) evaluated genetically was analysed in details for **physical, compositional and processing quality traits** as well. Hierarchical cluster analysis based on different combination of the traits (for example, compositional properties: protein, starch, arabinoxylan, b-glucan, fructan; breadmaking quality traits: gluten content, gluten index, gluten spread, Zeleny sedimentation; carbohydrate related traits: starch content, starch viscosity traits, starch damage, flour yield, fructan, arabinoxylan content) could well distinguish the group of common wheat from the group of spelts, similarly to the SNP marker results. However, spelt genotypes form two groups. One of the spelt group is more similar to common wheat in some trait combinations, but more similar to the main group of spelt in other combination of the quality traits used in the hierarchical claster analysis. Based on the breadmaking quality traits for example (Figure 4) all the spelts were similar to each other, and different from common wheat. Thus significant differences were observed for some traits **between the species**.
The results of these analysis are planned to be published together with the results of point 1.

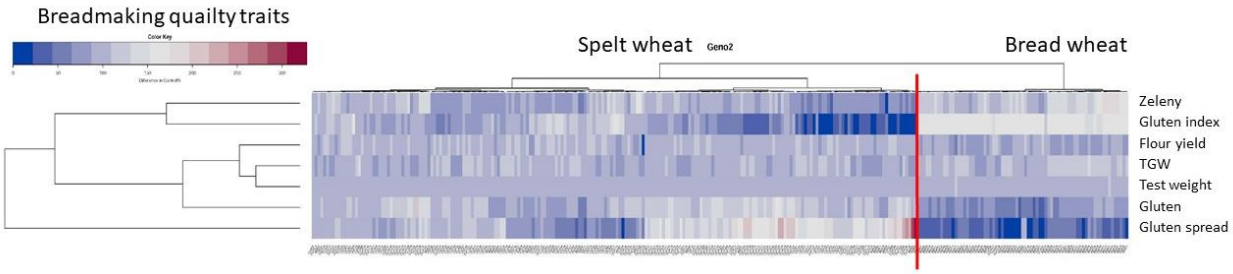

Figure 4. Hierarchical Cluster Analysis based on processing quality traits (example)

A high variation of the compositional and quality traits was found **within the species** as well. Thus, the TOTAX content of common wheat white flour was between 0.99 and 3.37%, with 1.9% mean value, while they were 1.07-2.8% and 1.72%, respectively for spelt. The WEAX content varied between 0.37 and 1.54% for common wheat and 0.3-0.88% in spelt. The arabinogalactan content ranged from 0.22 to-0.57% in wheat and form 0.21 to 0.86% in spelt The variation of the ß-glucan content in common wheat was slightly higher than in spelt. The antioxidant capacity of common wheat (0.66-1.19 mg GAE/g (dm)) and spelt wheat (0.80-1.17 mg GAE/g (dm)) fell in a similar range, but the variation within spelt wheat was lower. The content of FODMAP (fermentable oligo-, di-, and mono-saccharides, and polyols) is important for those patientes suffering from irritable bowel syndrome (IBS). Thus, short chain carbohydrate content was determined. The excess fructose content in wheat ranged from 0.004 to 0.16% with 0.09% mean and 0.01%. median value. The range was 0.01-0.14%, in spelt with the mean value of 0.08, and the median value of 0.07%. Less common wheat samples were identified with calculated excess fructose content than spelt, but the non-outlier and interquartile range was wider in wheat samples. (Schall et al., 2024). The falling number and viscosity properties of spelt wheat was lower than that of common wheat, but its variability was greater (Figure 5.) (Jaksics et al., 2024). Relation of these traits and their genetic variation will be analysed in more details.

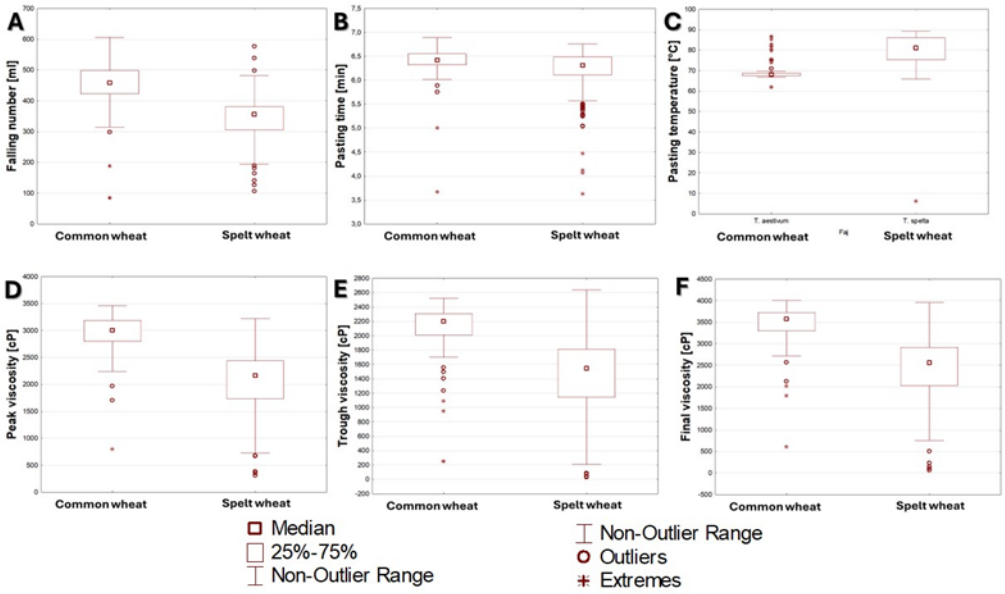

Figure 5. The figure shows the parameters of viscous properties A – Falling number [s], B – Pasting time [min], C – Pasting temperature [°C], D – Peak viscosity [cP], F – Trough viscosity [cP], E – Final viscosity [cP]

3. Screening of the agronomical, compositional and processing traits of spelt and the effect of the environment on them

Speltwheat (Triticum aestivum subsp. spelta L.) is an underexploited hexaploid wheat species that has become an increasingly fashionable raw material of bakery products in the last decades, partly because of its ability to grow under organic agricultural conditions and partly because of the growing number of people following the trend of having a healthy diet. However, due to its difficult threshing, most research on spelt seed is based on a very limited number of genotypes. Therefore, we determined the physical, compositional, and breadmaking quality traits of 90 spelt genotypes in order to highlight the variation of these properties and to **identify possible genetic resources** for spelt improvement. The thousand kernel weight of the spelt genotypes ranged between 23.2 and 49.7 g, the protein content between 12.1% and 22.2%, the gluten index between 0.7 and 98.8, the dough stability between 0.0 and 19.6 min, and the starch damage between 6.3 and 19.4 UCD value. The average values showed that spelt has higher protein and gluten contents (Rakszegi et al., 2023), but weaker dough strength and stability than common bread wheat. The starch pasting temperature was also higher in spelt, but the starch damage was lower, resulting in lower water absorption. Some genebank accessions (MVGB142, 145, 353, and 525) and internationally available cultivars (Bohemia, Bodensonne, Black-Bearded, and White-Beardless) were identified as good genetic resources for improving the breadmaking-quality traits of spelt (Tóth et al., 2022).

Recently, interest in the production and expansion of spelt wheat has been also boosted due to its significance in the production of healthy food, mostly originated from **organic production**. Thus, we examined and compared the quality parameters (gluten content, Zeleny sedimentation, farinograph dough properties), protein content and composition (by the Dumas method, Size Exclusion (SE) and Reversed Phase (RP) High Performance Liquid Chromatography (HPLC) analyses) of bread and spelt wheat varieties grown under conventional and organic production in Hungary and under conventional production in Serbia, thanks to a cooperation in a COST Action. According to our results most of the analyzed traits showed significant differences between varieties, wheat species and growing sites. Total protein content was significantly higher in spelt than in bread wheat and under conventional than under organic production. In comparison to spelt, bread wheat showed better breadmaking

quality, characterized by a higher amount of glutenins (in particular high molecular weight glutenin subunits) and unextractable polymeric proteins. The proportion of the gliadins was also found to be different under conventional and organic systems. Spelt Ostro and Oberkulmer-Rotkorn and bread wheat varieties Balkan, Estevan and Pobeda proved suitable for low input and organic systems (Takac et al., 2021).

The effect of the **organic vs. conventional growing conditions** was studied by Jaksics et al., (2024) on some spelt wheat varieties and breeding lines. No significant differences was found in the total dietary fiber content of spelt samples between different harvest years and cultivation methods. However. there were significant differences between the soluble fiber content of the varieties and lines, as well as between harvest years and cultivation methods. The effect of cultivation method also depended on the genotype and the harvest year. The ß-glucan content varied significantly from year to year and also depended on the cultivation method. However, the difference between years and varieties was lower at organic site, than at conventional site. The antioxidant capacity varied significantly from year to year. At the same time, it can be stated that the antioxidant capacity of conventionally grown samples was higher than that of organically grown samples. The quantity of the short chain carbohydrates was influenced mostly by the effect of harvest year and the genotype, while the effect of the cultivation conditions was lower and depended on the genotype. Comparing the data of the four harvest years, only minor differences were found in the falling number and RVA starch viscosity parameters of the individual varieties and lines. The 2021 data was significantly different from the other years for most samples, especially at conventional site, due to the different environmental conditions. In this year the degree of deviation between conventionally grown varieties and lines was higher, than at organic site (Figure 6) (Jaksics et al., 2024).

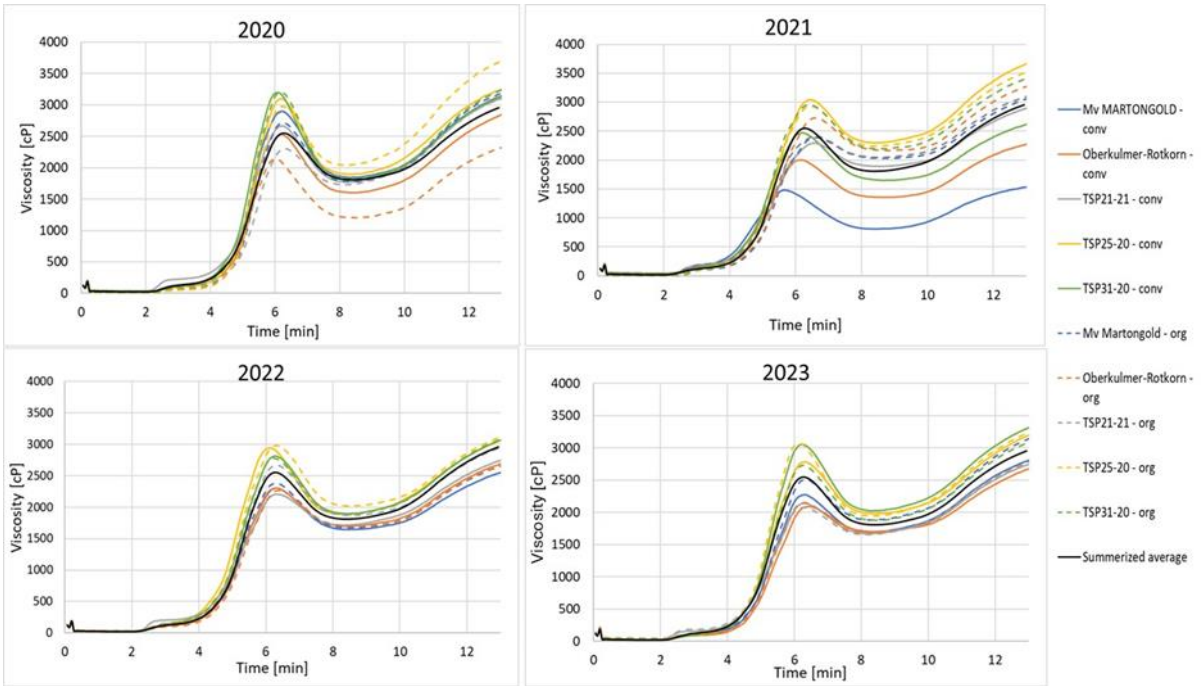

Figure 6. The RVA curves of the two varieties and three breeding lines from the two cultivation methods and from four years

4. Identification of the methods most appropriate for studying the techno-functional properties of spelt and its relation to compositional properties of the flour

Analysis of spelt flour and wholemeal was carried out by the methods applied for common wheat samples and most of those were appropriate for screening spelt properties. The **total and water-extractable arabinoxylan** (TOTAX and WEAX) and **arabinogalactan** (AG) content of the white flour were measured by modified GC method. The determination of AG is a new element either in our analítical tools and worldwide in the complex caracterisation of dietary fiber compositin of spelt wheat lines and varieties. The WEAX and AG were extracted in cold water and the supernatant was used in further steps. The calculation was made taking into account that the average arabinose/galactose ratio in arabinogalactans is 0.7.

Two HPLC analitical methods were adapted and further developed for the analysis of **short chain carbohydrate** profile (including FODMAPs) (Szentmiklóssy et al., 2023). Monomers and sugar alcohols were separated by a ligand exchange column, while the DP2-DP4 oligomers (sucrose, maltose, trehalose, kestose, raffinose, nystose, and stachyose), were measured using HILIC column.

Our formerly developed method for **reduced-scale baking tests** was adapted and further developed for the determination and comparison of baking quality of spelt and normal wheat lines and varieties. The modification was necessary due to the often significantly different technological behaviour of spelt wheat (i.e. weaker dough-forming tendency, lower gluten strength, etc.). The dough is mixed with a farinograph until the optimal dough consistency (until development time) is reached, then after 60 minutes of rising, the loaves were baked for 15 minutes. After cooling, the loaves were stored in a sealable HDPE bag until qualification tests.

With the use of this baking method, we have found that, good or acceptable quality bread could be made from all spelt varieties and lines, regardless of the harvest year and cultivation method (Figure 7).

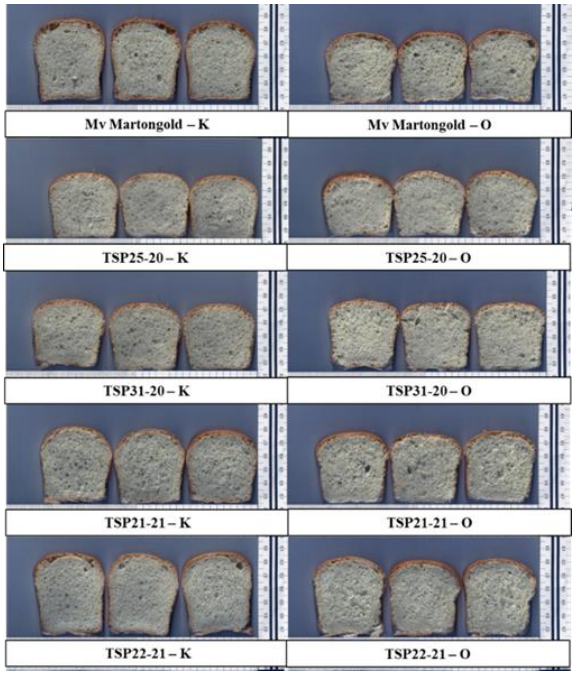

Figure 7. The bread crumb from spelt Crumb of breads made from conventional and organic spelt flour (K-conventional, O-organic)

The specific volume and baking loss of spelt wheat bread showed little fluctuation regardless of variety, harvest year and cultivation method. Examining the properties of the crumb, there was little difference between the harvest years. The cultivation method only had significant effect on the hardness of the crumb, but it depended on the variety and the harvest year as well.

The changes of the measurable total and soluble arabinoxylan and short chain carbohydrate content during bread processing were also investigated. The WEAX content decreased during processing. The glucose, fructose and disaccharide contents increased, while the amount of oligosaccharides decreased most likely due to the enzymatic effects during dough fermentation (leavening) process. Processing had a much greater influence on the composition than the plant cultivation method.

5. Development of new spelt lines

In order to broaden the genetic variability, we created **spelt x spelt** and **spelt x wheat** combinations. Then, a line-by-line **pedigree selection** was carried out of spike offsprings in both wheat and spelt directions. In the early generations (F3-F5), the selection was mainly based on agronomic properties (earliness, plant height, fitness and resistance properties), while for more developed strains (F6-F12), the grain yield was the determining factor. This was determined in a comparative multi-site field experiments sown in several replications. The aim of the selection in the direction of wheat was to transfer certain resistance and compositional traits, but this trial was not successful, as in the F7-F8 generation the lines were not able to exceed the yield average of the control varieties. In the direction of spelt, we aimed to improve endurance, early maturity and to achieve better baking properties. The selection in this direction was successful.

In the period between 2020-2024, we created 120 new lines, from which, in the end, seven strains were selected and was sent for the NÉBIH variety registration trials. These lines not only have outstanding yield, but also have good field disease resistance and excellent endurance. We currently have four candidate varieties and three new spelt varieties, of which Mv Pangolin received state breed recognition in 2023, while Mv Marduk and Mv Armadillo got it in 2024. Based on our results, we recommend our varieties primarily for eating purposes, but due to their high protein content, they can also be used excellently in animal feed. Due to their favorable pathological and resistant properties, they are also suitable for cultivation under ecological conditions.

Mv Pangolin is a high-yielding pure spelt variety with excellent endurance and frost tolerance. Its awnless ears are red in color, and its heading date is May 28 on average. With an average plant height of 123 cm, it belongs to the medium-height spelts. It is moderately resistant to powdery mildew and yellow rust, while moderately susceptible to leaf rust. Its average thousand grain weight is 42.8 g, hectoliter weight is 75 kg/hL. It has a high crude protein content, which is 16% on average, with high gluten content (32%), and an optimal gluten spread (4.7 mm/hour) and B2 Farinographic group, as a result of which it has a favorable quality for the baking industry (Tóth et al., 2023).

Mv Marduk is an extra early spelt variety with good endurance and frost tolerance originating from a spelt x wheat combination. Its heading date is on May 11 on average, which is two weeks earlier than typical for spelt. Its ear type is awnless and white in color. The height of the plant is low, with an average plant height of 96 cm. It is moderately resistant to powdery mildew and yellow rust, while moderately susceptible to leaf rust. The average thousand grains weight is 47.1 g, while the hectoliter weight is 78 kg/hL. Its compositional and baking quality is good, with 14.5 % crude protein content, 29.9 % wet gluten content, low gluten spread (5.5 mm/hour), and B2 Farinographic group, which is extremely favourable on the market (Tóth et al., 2024a).

Mv-Armadilló is a spelt variety with excellent endurance and frost tolerance originating from a cross combination of spelt and wheat. Its average heading date is May 21. The type of ear is awnless with white color. Its plant height is low, which is 101 cm on average. It is moderately resistant to powdery mildew and yellow rust, while moderately susceptible to leaf rust. The average thousand grain weight is 46.5 g, while the test weight is 79 kg/hL. Its crude protein content is high (15.1%), which is close to the value typical of "pure" spelt. In addition, its baking quality is also good, with an average 35.5% wet gluten content, 11.3 mm/hour gluten spread and B2 Farinograph group (Tóth et al., 2024b).

Main results and conclusions

1. We showed with 25K SNP array analysis that spelt wheat can be genetically distinguished from common wheat and that spelt containing the wheat genome could be separated from the main group of spelt.
2. We identified 406 spelt-specific SNP markers, which may be suitable for distinguishing the two species.
3. We identified SNP markers related to fiber and fructan content, which could help in the selection of genotypes and achieving a healthier diet.
4. We showed that spelt varieties contained more protein and gluten, including more gliadin, which resulted in greater extensibility of the dough.
5. Among the properties of the starch, the pasting temperature was higher, while the degree of starch damage was lower in spelt.
6. Spelt has a demonstrably narrower falling number range and more varied starch viscosity properties than wheat.
7. The ratio of the arabinose to xylose in arabinoxylan, determining the solubility of the fibers, was higher in spelt than in wheat, that also affect the technological properties
8. Several spelt genotypes had lower arabinogalactan content than wheat, but on average they were similar. This property was not studied before in splet.
9. Several short chain carbohydrates were studied in spelt, and in some cases we identified differences between the two species, but we did not find differences in the average amount of FODMAPs, which is important information for those patientes suffering from irritable bowel syndrome (IBS).
10. The organic site resulted a larger grain size, a lower HMW/LMW glutenin and alpha+beta gliadin ratio, but a higher gamma-gliadin content compared to the conventional growing site, which resulted in lower traditional breadmaking quality.
11. Ostro and Oberkulmer-Rotkorn spelt varieties were found to be most suitable for cultivation in low-input and organic farming systems, which were more tolerant to limited nitrogen application.
12. We have identified some gene bank accessions (MVGB142, 145, 353, and 525) and internationally available spelt varieties (Bohemia, Bodensonne, Black-Bearded, and White-Beardless), which can be promising gene sources for improving the baking quality of spelt.
13. Three spelt varieties were developed (Mv Pangolin, Mv Armadilló, Mv Marduk) and registered by NÉBIH

Utilization of the results

Spelt wheat is becoming increasingly popular among farmers and consumers alike. Since modern spelt varieties contain wheat genomes, the results of genetic studies contribute to more reliable identification of spelt wheat and to the identification and development of markers that make selection more efficient for breeders. The identification and production of spelt varieties that can be grown efficiently in an ecological environment contributes to the spread and development of environmentally friendly technologies and cultivation and to the promotion of a healthier diet. Our results are also intended to support the activities of the processing industry, providing processing technology and value-added raw materials for agriculture and the food industry, and can help to find a place for spelt in modern, health-promoting and sustainable food production.

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