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**ENHANCEMENT OF THE ARABINOXYLAN CONTENT OF WHEAT AND
ANALYSIS OF ITS STABILITY AND MOLECULAR BACKGROUND**

Main points of the thesis

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Introduction

Over the last few decades the main aim of wheat breeding has been to improve the yielding ability, disease resistance and/or breadmaking quality. In other words, it has mainly considered the interests of farmers and the processing industry. Recently, however, increasing attention has been paid to the positive effects of foodstuffs on human health and on the enhancement of nutritional quality, so breeders have now shifted emphasis to increasing the content of chemical components known for their positive impact on health.

A number of components with health benefits are to be found in wheat. Among these the antioxidants are extremely sensitive to processing factors (temperature, pressure, light, etc.), while the microelement content is greatly influenced by factors such as the microelement content of the soil or the ability of the plant to absorb microelements. Fibres can be regarded as the least sensitive components. They can be relied upon to retain their biological effect even after processing, so their improvement in breeding programmes is a realistic aim.

It was demonstrated in earlier research that fibre content is basically a genetically determined trait, though environmental effects cannot be ignored, especially if no genetic markers are available and selection has to rely on biochemical measurements. Such quantitative traits are known to be substantially influenced by environmental factors. The aim was therefore to evaluate the effect of environmental factors, followed by the complex analysis of all the results.

In order to make the results less dependent on environmental factors and to achieve more efficient selection, a further aim was to identify molecular markers. For this purpose a segregating wheat population was examined to detect correlations between chemical traits or yield components and the genetic background.

The main aim of the present research was thus to breed for improvements in the health-related properties of wheat (more specifically the fibre content), to analyse factors influencing the fibre content and to identify markers linked to fibre content. To achieve this, the following investigations were performed:

1. Development and characterisation of wheat lines with high fibre (arabinoxylan) content
2. Analysis of the heritability, environmental sensitivity and stability of the arabinoxylan content
3. Analysis of the stability of arabinoxylan content in wheat composite cross populations and variety mixtures.
4. Identification of QTLs related to fibre content in the Mv Toborzó/Tommi population in order to develop molecular markers

Review of the literature

The most important fibre source for human nutrition is cereals, especially wheat. The major fibres in the wheat kernel are the cell wall polysaccharides, 70% of which are arabinoxylans (AX) and 20% (1–3)(1–4)- β -D-glucans (β -glucans) (Mares and Stone, 1973¹). Wheat and rye are rich in arabinoxylan, while barley and oats contain a larger proportion of β -glucan. AX has both water-extractable (WE) and water-unextractable fractions, the physiological effects of which differ. The water-unextractable fibres reduce the transition time in the intestinal tract and increase the quantity of stools and the frequency of defecation, while also playing a role in binding carcinogens. The water-extractable fibres, especially AX, influence the processing properties of wheat, including its breadmaking quality (Courtin and

¹ Mares, D. J., Stone, B. A. 1973. Studies on wheat endosperm. I. Chemical composition and ultra structure of the cell walls. Aust. J. Biol. Sci. 26:793-812.

Delcour, 2002²), the separability of gluten and starch (Frederix et al., 2004³), fodder quality (Bedford and Schulze, 1998⁴) and the manufacture of alcoholic drinks and biofuel (Shewry et al., 2010⁵).

The variability of bioactive components in cereals was investigated in the framework of the EU-FP6 Healthgrain Project, which aimed to develop healthy food products from wholegrain wheat or other cereals and to prove the health benefits of these products in clinical tests. A total of 150 wheat lines were examined in this project, chiefly to determine the fibre, starch, xylanase, alkylresorcin, lignan, sterol, tokol and folate contents of the genotypes, but also to record their protein and gluten contents, Zeleny sedimentation, starch viscosity, and agronomic and breadmaking properties. On the basis of the results genotypes were selected for use not only in breeding wheat with good breadmaking quality, but also for the manufacture of products rich in fibre and vitamins. Among other things, this process led to the identification of the wheat genotype Yumai-34, which has extremely high total and water-extractable AX content and was registered in China in 1988. This variety contains twice as much fibre as the approx. 150 European wheat cultivars included in the project. Thanks to its outstandingly high, genetically determined fibre content (Shewry et al. 2010⁵) and relatively good agronomic properties, Yumai-34 can be regarded as a good starting point for breeding (Ward et al., 2008⁶).

Analysing the chemical contents of wheat lines grown under diverse environmental and agronomic conditions makes it possible to separate environmental and genotypic effects. The high heritability of the TOT-AX and WE-AX contents of flour was proved by many authors including Hong et al. (1989⁷), who examined 18 wheat lines at two locations, Martinant et al. (1999⁸), who tested 19 genotypes at three locations, Dornez et al. (2008⁹), who studied 14 genotypes over three years, and Finnie et al. (2006¹⁰), who investigated seven spring and 20 winter wheat varieties in 10 and 12 environments, respectively. Li et al. (2009¹¹), however, reached the opposite conclusion for the wholemeal of 25 winter and 25 spring wheats grown at three locations. They demonstrated that the environment had a far greater effect than the genotype on the TOT-AX and WE-AX contents of winter wheats and on the WE-AX content

² Courtin, C.M., and Delcour, J. A. 2002. Arabinoxylans and endoxylanases in wheat flour bread-making. *J. Cereal Sci.* 35:225-243.

³ Frederix, S. A., Courtin, C. M., and Delcour, J. A. 2004. Influence of process parameters on yield and composition of gluten fractions obtained in a laboratory scale dough batter procedure. *J. Cereal Sci.* 39:29-36.

⁴ Bedford, M.R. and Schulze, H. (1998) Exogenous Enzymes for Pigs and Poultry. *Nutrition Research Reviews*, 11, 91-114. <https://doi.org/10.1079/NRR19980007>

⁵ Shewry, P. R., Piironen, V., Lampi, A.-M., Edelman, M., Karluoto, S., Nurmi, T., Fernandez-Orozco, R., Ravel, C., Charnet, G., Andersson, A. A., Aman, P., Boros, D., Gebruers, K., Dornez, E., Courtin, J. A., Rakszegi, M., Bedő, Z., Ward, J. L. 2010a. The HEALTHGRAIN wheat diversity screen: effects of genotype and environment on phytochemicals and dietary fiber components. *J Agric Food Chem.* 58:9291-8.

⁶ Ward, J. L., Poutanen, K., Gebruers, K., Piironen, V., Lampi, A. M., Nyström, L., Andersson, A. A. M., Aman, P., Boros, D., Rakszegi, M., Bedő, Z., Shewry, P. R. 2008. The HEALTHGRAIN cereal diversity screen: concept, results, and prospects. *J. Agric. Food Chem.* 56:9699-9709.

⁷ Hong, B. H., Rubenthaler, G. L., and Allan, R. E. 1989. Wheat pentosans. II. Estimating kernel hardness and pentosans in water extracts by near-infrared reflectance. *Cereal Chem.* 66:374-377.

⁸ Martinant, J. P., Billot, A., Bouguennec, A., Charnet, G., Saulnier, L. and Branlard, G. 1999. Genetic and environmental variations in water-extractable arabinoxylans content and flour extract viscosity. *J Cereal Sci.* 30:45-8.

⁹ Dornez, E., Gebruers, K., Joye, I. J., De Ketelaere, B., Lenartz, J., Massaux, C., Bodson, B., Delcour, J. A., Courtin, C. M. 2008a. Effects of genotype, harvest year and genotype-by-harvest year interactions on arabinoxylan, endoxylanase activity and endoxylanase inhibitor levels in wheat kernels. *J Cereal Sci.* 47:180-9.

¹⁰ Finnie, S. M., Bettge, A. D. and Morris, C. F. 2006. Influence of cultivar and environment on water-soluble and water-insoluble arabinoxylans in soft wheat. *Cereal Chem.* 83:617-23.

¹¹ Li, S., Morris, C. F. and Bettge, A. D. 2009. Genotype and environment variation for arabinoxylans in hard winter and spring wheats of the US Pacific Northwest. *Cereal Chem.* 86:88-95.

of spring wheats. The analysis published by Gebruers et al. (2010¹²) indicated that the AX content of flour exhibited a high degree of heritability, with the genotype determining around 60% of the total variance for WE-AX and 70% for TOT-AX. A heritability of around 50% was found for β -glucan in grist and for WE-AX in bran. Török et al. (2019¹³) detected a very significant year effect and G×E effect for TOT-AX, while 21% of the total variance in WE-AX was determined by the genotype in tests on 41 genotypes in three years. The results for the TOT-AX content of the flour thus appear to be contradictory, but selection for the WE-AX content could be promising for plant breeding programmes.

Organic farming is steadily gaining ground and is now an accepted method in over 160 countries throughout the world, taking up approx. 0.9% of the total sowing area. This technique was first applied in the early years of the 20th century and by 2009 organic products made up 5% of the market. Conventional agriculture uses a wider range of inputs to compensate for environmental effects (plant protection, mineral fertiliser, etc.) than organic farming, which therefore requires more flexible, robust cultivars capable of adjusting to the given environmental conditions and giving stable yields with satisfactory quality. Organic farmers are increasingly interested in cultivar diversity, and more and more papers are published on genetic variability at the population level. One reason for this is the temporal and spatial change in the environment (Ceccarelli et al., 2007¹⁴, Ostergard et al., 2009¹⁵, Wolfe et al., 2008¹⁶). In such an environment only the genetic diversity of the crops and the use of technologies that preserve biodiversity will be able to protect cultivar yields and quality from biotic and abiotic stressors (Finckh et al. 2000¹⁷). Based on this knowledge seven composite cross populations and three variety mixtures were developed in three breeding institutes (UK, A, HU) in the framework of the SOLIBAM EU-FP7 project and were sown in three years (2011–2013) under various climatic and technological conditions (organic and low-input) in Europe. These genetic stocks could be excellent tools for examining the role played by biodiversity and the cultivation system in preserving the stability of the chemical content and quality, and thus that of the fibre content.

Improvements in molecular genetic methods, especially QTL analysis, brought a breakthrough in the genetic analysis of quantitative traits. Many results have been published on fibre content. Martinant et al. (1998¹⁸) used two mapping population to map the genes involved in arabinoxylan synthesis. One of these originated from a cross between Courtot and Chinese Spring and contains 91 doubled haploid (DH) lines, while the other consists of 115 SSD (single seed descent) lines in the F₇ generation obtained from the synthetic W7984 × Oparta cross. The

¹² Gebruers, K., Dornez, E., Bedo, Z., Rakszegi, M., Fras, A., Boros, D., Courtin, C. M., Delcour, J. A. 2010. Environment and genotype effect on the content of dietary fibre and its components in wheat in the HEALTHGRAIN diversity screen. *J. of Agric. and Food Chem.* 58:9353–9361.

¹³ Török, K., Szentmiklossy, M., Tremmel-Bede, K., Rakszegi, M., Tömösközi, S. 2019. Possibilities and barriers in fibre-targeted breeding: Characterization of arabinoxylans in wheat varieties and the breeding lines. *Journal of Cereal Science* 86, 117-123.

¹⁴ Ceccarelli, S., Grando, S., Baum, M. 2007. Participatory plant breeding in water-limited environments. *Exp. Agric.* 43:411–435.

¹⁵ Østergård, H., Finckh, M.R., Fontaine, L., Goldringer, I., Hoad, S.P., Kristensen, K., Lammerts van Bueren, E.T., Mascher, F., Munk, L., Wolfe, M.S. 2009. Time for a shift in crop production: Embracing complexity through diversity at all levels. *J. of Agric. and Food Information* 89:1439–1445.

¹⁶ Wolfe, M.S., Baresel, J.P., Desclaux, D., Goldringer, I., Hoad, S., Kovacs, G., Löschenberger, F., Miedaner, T., Østergård, H., Lammerts van Bueren, E.T. 2008. Developments in breeding cereals for organic agriculture. *Euphytica* 163:323–346.

¹⁷ Finckh, M. R., Gacek, E. S., Goyeau, H., Lannou, C., Merz, U., Mundth, C. C., Munk, L., Nadziak, J., Newton, A., de Vallavieille-Pope, C., Wolfe, M. 2000. Cereal variety and species mixtures in practice, with emphasis on disease resistance. *Agronomie* 20:813-835.

¹⁸ Martinant, J. P., Cadelen, T., Billot, A. and Chartier, S. 1998. Genetic analysis of water-extractable arabinoxylans in bread wheat endosperm. *Theor Appl Genet.* 97:1069-75.

WE-AX content, the viscosity of the extract (which is determined to a great extent by the WE-AX content) and the arabinose-xylose ratio within WE-AX were recorded for the lines in these populations. A major QTL (the region of the chromosome where genes responsible for the quantitative trait are located) for all three traits was identified on the 1B chromosome. This QTL explained 32–37% of the variability for extract viscosity and 35–42% for the arabinose-xylose ratio. Quraishi et al. (2010¹⁹) reported the development of five further mapping populations: an RIL (recombinant inbred line) population containing 187 lines derived from a Courtot × Chinese Spring cross (Perretant et al., 2000²⁰), 241 DH lines originating from an Arche × Recital cross (Laperche et al., 2007²¹), 194 RIL lines from a Renan × Recital cross (Quraishi et al., 2009) 124 DH lines derived from a Valoris × Isengrain cross and 280 lines from a cross between RE006 and CF007 (Charmet et al., 2009²²). The parental genotypes in the last two populations differed widely for the viscosity caused by their WE-AX content. In the first step 12 QTLs were identified in the five populations, of which three proved to be meta-QTLs for WE-AX viscosity on chromosomes 1B, 3D and 6B. Martinant et al. (1998²³) identified a QTL on chromosome 1B, while Charmet et al. found that the QTL on 6B explained more than 59% of the variance in WE-AX viscosity in the Valoris/Isengrain and RE006/CF007 populations. Quarishi et al. (2010¹⁹) supplemented this meta-QTL analysis with the association genetic analysis of 156 wheat genotypes (Ward et al., 2008⁵), identifying seven loci linked to the viscosity caused by WE-AX. Three of these could be associated with the meta-QTLs on chromosomes 1B, 3D and 6B, while the other four were located on chromosomes 3A, 5B, 7A and 7B. Both approaches revealed that the QTL on chromosome 1B was the most important. Later Quarishi et al. (2010¹⁹) demonstrated that this region of the chromosome contained four DNA sections that were involved in the development of this trait. They also designed molecular markers linked to this locus that were responsible for WE-AX viscosity, and which could thus be suitable for use in plant breeding programmes.

Materials and methods

Yumai-34 was crossed with three European wheat varieties with good agronomic traits (Lupus, Mv Mambo and Ukrainka). The spikes of the F₂ segregating population were sown in spike progeny rows to produce the F₃ generation. The plants were then selected for agronomic traits and for high water-soluble pentosan content (measured as AX in the flour) in each of the following generations. After several rounds of selection 31 lines (12 Lupus/Yumai-34, 3 Mv Mambo/Yumai-34 and 16 Ukrainka/Yumai-34) were identified as having above-average water-soluble pentosan content combined with good agronomic traits. These were investigated in more detail in the F₇, F₈ and F₉ generations (2013–2015), using the parental wheat cultivars as the control. In order to develop competitive wheat breeding stock with good performance under organic and low-input conditions, six cropping populations were established in Austria (A),

¹⁹ Quraishi, U.-M., Murat, F., Abrouk, M., Pont, C., Confolent, C., Oury, F. X., Ward, J., Boros, D., Gebruers, K., Delcour, J. A., Courtin, C. M., Bedő, Z., Saulnier, L., Guillon, F., Balzergue, S., Shewry, P. R., Feuillet, C., Charmet, G., Salse, J. 2010. Combined meta-genomics analyses unravel candidate genes for the grain dietary fibre content in bread wheat (*Triticum aestivum* L.). *Funct Integ Genom.* 11:71-83. (doi 10.1007/s10142-010-0183-2).

²⁰ Perretant, M.R., Cadalen, T., Charmet, G., Sourdille, P., Nicolas, P., Boeuf, C., Tixier, M. H., Branlard, G., Bernard, S. 2000. QTL analysis of bread-making quality in wheat using a doubled haploid population. *Theor Appl Genet.* 100:1167-75.

²¹ Laperche A, Brancourt-Hulmel M, Heumez E, Gardet O, Hanocq E, Devienne-Barret, F., Le Gouis, J. 2007. Using genotyp x nitrogen interaction variables to evaluate the QTL involved in wheat tolerance to nitrogen constraints. *Theor Appl Genet.* 115:399-415.

²² Charmet, G., Masood-Quarishi, U., Ravel, C., Romeuf, I., Balfourier, F., Perretant, M. R., Joseph, J. L., Rakszegi, M., Guillon, F., Sado, P. E., Bedő, Z., Saulnier, L. 2009. Genetics of dietary fibre in bread wheat. *Euphytica.* 170:155-68.

²³ Martinant, J. P., Cadalen, T., Billot, A. and Chartier, S. 1998. Genetic analysis of water-extractable arabinoxylans in bread wheat endosperm. *Theor Appl Genet.* 97:1069-75.

Hungary (H) and the United Kingdom (UK). These were designated as POP-AT (A), English Composite (H, ENG-CCP), Elite Composite (H, ELIT-CCP), Hungarian Composite (H, HUN/CCP), YQ-CCP (UK) and NIAB-Elite-CCP (UK, NIAB/CCP). These populations were examined together with three variety mixtures, designated as MIX-AT (A), YQ-MIX (UK) and NIAB-Elite-MIX (UK, NIAB-MIX), and a diverse population originating from France, designated as INRA-60parent-CCP (INRA-CCP). The development of YQ-CCP and YQ-MIX was reported in detail by Döring et al. (2015²⁴). A winter wheat (*Triticum aestivum* L.) cultivar, Mv Emese, was used as the control at all the locations. A list of the populations and cultivar mixes, with the year of testing and the locations, was published by Tremmel-Bede et al. (2016²⁵). For genetic analysis Mv Toborzó was crossed with the cultivar Tommi, after which a mapping population consisting of 240 recombinant inbred lines (RIL) was developed via eight cycles of self-fertilisation. The parents were bred under diverse ecological conditions. Mv Toborzó is an early maturing Hungarian cultivar, while the German cultivar Tommi counts as a very late cultivar in Hungary. Preliminary analysis showed that the two cultivars carried different alleles for the *PPD-D1* (2D) photoperiod sensitivity gene and for the dwarfing genes *Rht-B1* (4B), *Rht-D1* (4D) and *Rht8* (2D) (Kiss et al., 2014²⁶), so the heading date, plant height and yield components of the RIL lines exhibited great variability.

The physical, compositional and processing properties listed in Table 1 were investigated for the genotypes given above. The following genotypic analyses were performed on the Mv Toborzó/Tommi population: Extraction of genomic DNA from young leaves using a DNeasy Plant Mini Kit, 15K Infinium analysis using 13007 SNP markers from TraitGenetics GmbH (Gatersleben, Germany), construction of a linkage map using Joinmap 4.0 software (Kyazma, B.V., Wageningen, The Netherlands) and QTL analysis using the GenStat statistical program (VSN International Ltd.). The statistical methods used to evaluate the data were as follows: Microsoft Excel (least significant differences, correlation analysis), Statistica 6.0 (principal component analysis, comparison of yields), SPSS 16.0 (SPSS RT., Chicago IL, USA) (discriminant analysis, Tukey's post hoc test, linear mixed model analysis) and GenStat 17.0 (VSN International Ltd., Hemel Hemstead, UK) (GGE biplot analysis).

TRAIT		METHOD	INSTRUMENT
Physical properties	Thousand-kernel weight	MSZ 6367/4-86	Marvin Seed Analyser
	Test weight	AACC39-25	Foss Tecator 1241
	Hardness Index	AACC 55-31	Perten Falling Number System
	Kernel width and length		Marvin Digital Seed Analyzer
Compositional properties	Protein content	ICC 105/2	Kjeltec 1035 Analyzer

²⁴ Döring, T. F., Annicchiarico, P., Clarke, S., Haigh, Z., Jones, H. E., Pearce, H., Snape, J., Zhan, J., Wolfe, M. S. 2015. Comparative analysis of performance and stability among composite cross populations, variety mixtures and pure lines of winter wheat in organic and conventional cropping systems. *Field Crops Res.* 183:235–245.

²⁵ Tremmel-Bede K , Mikó P , Megyeri M , Kovács G , Howlett S , Pearce B , Wolfe M , Löschenberger F , Lorentz B , Láng L , Bedo Z , Rakszegi M. 2016. Stability analysis of wheat populations and mixtures based on the physical, compositional and processing properties of the seed. *Cereal Research Communications.* 44:(4) pp. 694-705. (2016)

²⁶ Tibor Kiss, Krisztina Balla, Otto´ Veisz, La´szlo´ La´ng, Zolta´n Bedo, Simon Griffiths, Peter Isaac, Ildiko´ Karsai. 2014. Allele frequencies in the VRN-A1, VRN-B1 and VRN-D1 vernalization response and PPD-B1 and PPD-D1 photoperiod sensitivity genes, and their effects on heading in a diverse set of wheat cultivars (*Triticum aestivum* L.). DOI 10.1007/s11032-014-0034-2

	Gluten content	ICC 137/1	Perten Glutomatic 2200
	Pentosan content	Douglas method (1981)	Spectrophotometer
	Arabinoxylan content	Gebruers et al., 2009	GC
	β -glucan content	AACC 32-23.01	Spectrophotometer
Processing properties	Gluten index	ICC 155	Perten Glutomatic 2200
	Gluten spread	MSZ 6369/5-87 (1987)	
	Zeleny sedimentation	ICC 116/1	SediCom System
	Farinograph parameters	ICC 115/1	Brabender Farinograph

Table 1: Physical, chemical and processing properties tested in the experiment

Results

Development and characterisation of wheat lines with high fibre (arabinoxylan) content

The enhancement of the nutritional quality and health aspects of foodstuffs has received increasing attention all over the world in recent years. The main sources of fibre in the human diet are non-perishable cereal-based foods. Recent research has identified a wheat cultivar, the Chinese Yumai-34, which has an unusually high water-extractable (WE-AX) and total (TOT-AX) arabinoxylan, i.e. fibre content. After crossing this Chinese cultivar with three Central European wheat cultivars (Lupus, Mv Mambo, Ukrainka), the physical (test weight, thousand-kernel weight, flour yield, Hardness Index), compositional (protein, gluten, WEAX, TOTAX) and processing (gluten index, Zeleny sedimentation, Farinograph parameters) properties of 31 selected lines with high AX content were compared in the F₇–F₉ generations over three years (2013–2015). Increases of 42.37% in the WE-AX content and 24.09% in the TOT-AX content of the flour were achieved, together with an improvement in the dough properties. The thousand-kernel weight, protein content, gluten content, Zeleny sedimentation and flour water absorption also rose in numerous lines, three of which also had grain yield quantity that was competitive with that of the official control cultivars.

It was demonstrated that conventional breeding is capable of increasing the water-extractable and/or water-unextractable dietary fibre content in white wheat flour without having to choose between yield and quality. This higher fibre content could not only have a positive on human health, but could also improve processing properties, for instance by increasing the water absorption capacity of the flour.

Analysis of the heritability, environmental sensitivity and stability of the AX content

The heritability of various traits, the effects of environment and genotype and the stability of the traits, with special regard to chemical components, were analysed for the same group of genotypes. Both the protein and starch content were found to be determined strongly by the genotype, so the heritability of these chemical traits was very high (0.851 and 0.828, respectively). In addition a significant year effect and G \times E effect was observed for the starch content. The total and water-soluble pentosan content was significantly influenced by all three factors (G, E, G \times E), but the heritability of TOT-pentosan proved to be much lower (0.341) than that of WE-pentosan (0.825). This result could be attributed to the methodological limitations

of the spectrophotometric technique. The quantity and composition of arabinoxylan, the chief component of pentosans, was primarily determined by the environment, so the broad-sense heritability of these parameters was only moderate (0.516 and 0.772). However, the genotype had a significant effect on the quantity and composition (A/X) of water-unextractable arabinoxylan, leading to much higher heritability for these traits (0.840 and 0.721). The low genetic determination of TOT-AX could probably be attributed to the fact that the majority of the 31 genotypes selected for the experiment were lines with high AX content, resulting in smaller variability with respect to fibre. As WE-AX has high heritability, it could be a suitable component for the achievement of breeding aims. The results are still contradictory, however, for the TOT-AX content, as the heritability values reported by various authors exhibit considerable variability. This could be partly due to differences in the methodology and partly to the diverse cultivars tested. Water absorption was found to be a genetically determined trait with a heritability of 0.829; the genotype explained 38.67% of the total variance. Lines LU/YU_8, 9 and 10 had the greatest water absorption capacity, and the protein and pentosan contents of these lines were also amongst the highest values. Both pentosans and proteins are able to bind large quantities of water. The effect of these components on processing properties is thus partly due to changes in water absorption. The high water-binding capacity of the fibre components results in greater Farinograph water absorption, which could, to a certain extent, be advantageous for the processing industry, particularly for breadmaking. This may also be accompanied by changes in the gelling properties of the starch.

The chemical content stability of the selected genotypes was studied using GGE biplot analysis and the determination of CV values. Lines with more stable thousand-kernel weight also had more stable starch content, which in turn led to an increase in protein content stability. The closest correlation was found between the stability of total and water-soluble pentosans, indicating that the WE-pentosan content of genotypes with more stable TOT-pentosan content was also more stable than that of the other lines. There was also a positive correlation between the stability of the WE-pentosan and WE-AX contents. In addition, the stability of the WE-AX content was closely correlated to that of its composition (A/X). The stability of flour water absorption, on the other hand, exhibited a negative correlation with the stability of the WE-AX content and composition (A/X). A large number of lines were concentrated in a single group on the GGE biplot, suggesting that there was no significant difference in stability between these lines. For each trait, only a few lines or varieties could be identified as having significantly different stability compared with the mean for the genotypes and environments.

Analysis of the stability of AX content in wheat composite cross populations and variety mixtures

The physical, compositional and processing properties of seven populations and three variety mixtures developed from winter wheat cultivars in European countries including Hungary were studied and evaluated in a three-year experiment (2011–2013) set up under different climatic conditions with various field management strategies. The desirable compositional and nutritional traits of the populations were determined, followed by the statistical analysis of the effect of genetic diversity on the stability of these traits, with special regard to fibre content. Under low-input and organic farming conditions no significant difference was found in the physical, compositional and processing properties of the populations/mixtures, only in their stability. Most of the populations exhibited greater stability than the control wheat cultivars, and the populations that were developed longest ago were more stable than those developed later. A few populations were particularly unstable for certain traits at certain locations (especially at organic locations in Austria, Switzerland and the UK). All the populations/mixtures had similarly high protein content (13.0–14.7%), but considerable variability was detected for the gluten content (28–36%) and, among the fibre components, for the

arabinoxylan content (14.6–20.3 mg/g). The best population with regard to both protein and arabinoxylan content was the ELIT-CCP population developed in Hungary. The final conclusion was that diversity had a quality-stabilising effect and that quality stability was more reliable in low-input systems than in organic systems. In the long term these results could be favourable not only for breeders but also for consumers.

Identification of QTLs related to fibre content in the Mv Toborzó/Tommi population, with the aim of developing molecular markers

In agreement with earlier studies, it was established that water-extractable arabinoxylan content is a highly heritable trait and is thus suitable for breeding purposes. There are limits, however, to how much the fibre content of cereals can be increased, as there are still no molecular markers available for selection, and biochemical selection is both time-consuming and expensive. The fibre content diversity of the Mv Toborzó/Tommi RIL population, consisting of 240 lines originating from a Mv Toborzó × Tommi cross, was therefore investigated in order to identify first QTLs and then molecular markers that would make selection easier for breeders. A record was made of the β -glucan, total pentosan, water-soluble pentosan, total arabinoxylan and water-extractable arabinoxylan quantities. Although the significant differences expected to be found for these traits between the parental Mv Toborzó and Tommi genotypes failed to be detected, the fibre contents of the lines in the population exhibited a much wider range than that of the parents, providing sufficient variability for genetic analysis. As wheat generally contains larger quantities of arabinoxylan, and our knowledge on this component is more limited than for β -glucan, arabinoxylan was in the centre of interest in these studies. The strongest QTL for arabinoxylan content and composition, especially as regards the water-extractable fraction, was detected on chromosome 1B. Further QTLs were identified on chromosomes 2A, 2D, 4D, 3B, 5A and 6B. Among the markers on the 1B chromosome, the phenotypic variance was determined to the greatest extent by markers correlated to the water-soluble pentosan and arabinoxylan content and composition (A/X). While the greater quantity of arabinoxylans could be attributed, based on the values for the additive effect, to the Mv Toborzó parent, values related to the composition (R.) were increased by the effect of the Tommi cultivar. The marker identified on chromosome 6B was also related to the WE-pentosan content, and the effect was increased by the higher fibre content of Mv Toborzó in the case of the flour. The joint consideration of several traits and fractions led to the identification of fibre-related markers on the 1D, 3A, 4B and 5B chromosomes. In the present case, the allele on chromosome 3A correlated with the arabinose/xylose ratio of the flour and bran, i.e. with the composition (A/X) of TOT-AX, the quantity of which was increased by Mv Toborzó 162, while the allele on chromosome 5B determined the TOT-AX quantity in the bran, which was determined to a greater extent by Tommi. The composition and properties of arabinoxylan are known to differ in various wheat fractions, being determined by different alleles. It could be seen in the present analysis that the arabinoxylan properties of the bran were determined by markers on chromosomes 2A (single and multi-trait) and 5B (multi-trait), while the flour properties were influenced by chromosome 1B. Markers where Tommi had a greater effect on the fibre content than Mv Toborzó, despite its higher fibre content, were found on chromosomes 2D and 4D. This could be explained by the fact that the markers were located very close to alleles decisive for plant development and kernel size (Rht1, Rht2, Ppd-D1, etc.), suggesting that the chemical composition of the kernels may be greatly influenced by factors related to plant development. It is therefore necessary to consider the results of several years and to perform separate analysis on genotypes with different types of plant development genes if correct conclusions are to be drawn.

Main points of the thesis

1. It was demonstrated that the content of water-extractable and/or -unextractable dietary fibre can be increased in white wheat flour by means of conventional breeding without having to make a choice between yield and quality. An average increase of 42.37% was achieved in the WE-AX value of wheat kernels and 24.09% in the TOT-AX content (in terms of flour dry matter). (1.)
2. The genotype had a significant effect on the quantity and composition of water-extractable arabinoxylan, so the heritability of these traits was high, confirming that they could be suitable selection components for breeding purposes. On the other hand, the heritability of TOT-pentosan was found to be considerably lower than that of WE-pentosan. (4.)
3. A close correlation was found between the genetic stability of total and water-soluble pentosan: genotypes with more stable TOT-pentosan content also had more stable WE-pentosan content. (4.)
4. The analysis of wheat composite cross populations and variety mixtures revealed that genetic diversity had a stabilising effect on quality and fibre content, and that this was more pronounced for the low-input technology than for organic systems. (2)
5. QTLs correlated to fibre content were identified in a Mv Toborzó/Tommi RIL population. The strongest QTL for arabinoxylan content and composition, especially the water-extractable fraction, was detected on chromosome 1B. Further QTLs were found on chromosomes 2A, 2D, 4D, 3B, 5A and 6B. When several traits and fractions were jointly considered, markers were also identified on chromosomes 1D, 3A, 4B and 5B. (3.)
6. It was shown in the analysis that markers on chromosomes 2A (single and multi-trait) and 5B (multi-trait) determined the characteristics of bran arabinoxylan, while chromosome 1B had more influence on flour properties. (3.)

Possibilities for application

Cereals contain large numbers of bioactive components, including fibres, the advantages of which have only partially been exploited up till now, if at all. Most consumers still prefer bakery products made from white flour, and there is less demand for products made from wholemeal flour. This makes it especially important to develop cereals containing a greater quantity of fibre in the white flour. The present results will thus be of use primarily to breeders, after which new cultivars with flour rich in fibre will allow farmers to produce value-added raw materials for the development of healthier bakery products. This will then contribute to a healthier diet for consumers, and thus to a healthier society.

In addition, the identification of QTLs related to high fibre content could lead to the development of markers that will make the selection and development of value-added genotypes more efficient, faster and less costly.

Publications

Papers forming the basis of the thesis, published in scientific journals

1. K. Tremmel-Bede, L. Láng, K. Török, S. Tömösközi, G. Vida, P. R. Shewry, Z. Bedő, M. Rakszegi: Development and characterization of wheat lines with increased levels of arabinoxylan. *EUPHYTICA*, **2017** 213:(12) Paper 291. 15 p. IF: 1,626
2. K. Tremmel-Bede, P. Mikó, M. Megyeri, G. Kovács, S. Howlett, B. Pearce, M. Wolfe, F. Löschenberger, B. Lorentz, L. Láng, Z. Bedo, M. Rakszegi: Stability analysis of wheat populations and mixtures based on the physical, compositional and processing

properties of the seeds. *CEREAL RESEARCH COMMUNICATIONS*, **2016** 4:(4) pp. 694-705. IF: 0,515

3. A. Lovegrove, L. U. Wingen, A. Plummer, A. Wood, D. Passmore, O. Kosik, J. Freeman, R. A. C. Mitchell, M. Ulker, K. Tremmel-Bede, M. Rakszegi, Z. Bedő, M.-R. Pletterant, G. Charmet, M. Leverington Waite, S. Orford, A. BurrIDGE, T. K. Pellny, P. R. Shewry, S. Griffiths: Identification of a major QTL and associated marker for high arabinoxylan fibre in white wheat flour. *PLOS ONE*, **2020** <https://doi.org/10.1371/journal.pone.0227826>
4. K. Török, M. Szentmiklossy, K. Tremmel-Bede, M. Rakszegi, S. Tömösközi: Possibilities and barriers in fibre-targeted breeding: Characterization of arabinoxylans in wheat varieties and the breeding lines. *JOURNAL OF CEREAL SCIENCE*, **2019** 86, 117-123 IF: 2,452.

Papers published in scientific journals

M. Rakszegi, P. Mikó, F. Löschenberger, J. Hiltbrunner, R. Aebi, S. Knapp, K. Tremmel-Bede, M. Megyeri, G. Kovács, M. Molnár-Láng, G. Vida, L. Láng, Z. Bedő: Comparison of quality parameters of wheat varieties with different breeding origin under organic and low-input conventional conditions. *JOURNAL OF CEREAL SCIENCE*, **2016**, 69:pp. 297-305.

Oral presentations

Tremmel-Bede Karolina, Török Kitti, Tömösközi Sándor, Vida Gyula, Karsai Ildikó, Rakszegi Marianna

Diversity analysis on the chemical properties of a wheat mapping population.

25th Plant Breeding Conference. Budapest, Hungary, 6–7 Mar. 2019. ISBN: 978-963-8351-45-6. (Ed. Ildikó Karsai)

Tremmel-Bede Karolina, Láng László, Török Kitti, Tömösközi Sándor, Vida Gyula, Shewry Peter R, Bedő Zoltán, Rakszegi Marianna

Development and characterisation of wheat genotypes with high fibre content.

24th Plant Breeding Conference. Eds. Ildikó Karsai and Zsolt Polgár. ISBN 978-615-00-1469-2. Abstracts. p. 36. Hungarian Academy of Sciences, Budapest, Hungary.

Proceedings

In English

Tremmel-Bede K, Láng L, Bedő Z , Vida G, Rakszegi M. Identification and development of wheat lines with increased level of arabinoxylan. In: Rapkova R , Copikova J , Sarka E (Ed.) Proceedings of the 12th International Conference on Polysaccharides-Glycoscience .Konferencia helye, ideje: Prague , Cheh Republic, 19-21 Oct. 2016. (Czech Chemical Society) Prague: Czech Chemical Society, 2016. pp. 103-107. (ISBN:978-80-86838-59-3) - poster

Bede K, Rakszegi M, Mikó P, Megyeri M, Láng L, Bedő Z. The identification of wheat genetic resources with high dietary fiber content. In: Chable V , Goldringer I , Howlett SA , Barberi P , Mikó P , Mendes-Moreira PMR , Rakszegi M , Østergård H , Borgen A , Finckh MR , Pedersen T , Bocci R (Ed.) Diversity strategies for organic and low input agricultures and their food systems: Book of Abstracts. 155 p. Nantes, France, 7-9 Jul. 2014.pp. 83-84. - poster

Rakszegi M, Bede K, Mecha E, Bento da Silva A, Carbas B, Brites C, VazPatto MC, Bronze MR, Mendes-Moreira P, Aebi R, Hiltbrunner J, Windras C, Taupier-Letage B, Chable V. Diversity for quality in organic systems "soil to fork": GxExM effects on organoleptic, nutritional and end-use quality. In: Chable V, Goldringer I, Howlett SA, Barberi P, Mikó P, Mendes-Moreira PMR, Rakszegi M, Østergård H, Borgen A, Finckh MR, Pedersen T, Bocci R (szerk.) Diversity strategies for organic and low input agricultures and their food systems: Book of Abstracts. 155 p. Nantes, France, 7-9 Jul. 2014. pp. 71-73.- oral presentation

Rakszegi M, Kisgyörgy BN, Bede K, Lang L, Bedo Z. PRODUCTION OF WHEAT WITH ALTERED POLYSACCHARIDE CONTENT FOR HEALTHIER HUMAN CONSUMPTION. In: Radmila Rápková (szerk.). Proceedings of the 9th International Conference on Polysaccharides-Glycoscience. Prague, Csehország, 8 Nov. 2013. Prague: Institute of Chemical Technology, 2013. pp. 41-45. (ISBN:978-80-86238-58-6)-oral presentation.

In Hungarian

Bede K, Rakszegi M, Mikó P, Megyeri M, Wolfe M, Howlett S, Löschenberger F, Láng L, Bedő Z. Nagy rostanyag tartalmú búza génforrások azonosítása organikus nemesítési programokban In: Veisz Ottó (szerk.) Növénynevelés a megújuló mezőgazdaságban: XX. Növénynevelési Tudományos Nap. 522 p. Konferencia helye, ideje: Budapest , Magyarország , 2014.03.18 Budapest: MTA Agrártudományok Osztálya Növénynevelési Tudományos Bizottság, 2014. pp. 70-74. (ISBN:978-963-8351-42-5)- poszter

Némethné KisgyörgyB , Bede K, Láng L , Bedő Z , Rakszegi M. Magas amidon tartalmú búzatörzsek (*Triticum aestivum* L) nemesítése In: Veisz Ottó (szerk.) Növénynevelés a megújuló mezőgazdaságban: XX. Növénynevelési Tudományos Nap. 522 p. Konferencia helye, ideje: Budapest, Magyarország, 2014.03.18 Budapest: MTA Agrártudományok Osztálya Növénynevelési Tudományos Bizottság, 2014. pp. 330-334. (ISBN:978-963-8351-42-5)- poszter

Rakszegi M, Bede K, Mikó P , Megyeri M , Kovács G , Hiltbrunner J , Löschenberger F , Láng L , Bedő Z. A búza nemesítési stratégiáinak összehasonlítása 'low-input' és organikus rendszerekben agronómiai és technológiai tulajdonságok alapjánIn: Veisz Ottó (szerk.) Növénynevelés a megújuló mezőgazdaságban: XX. Növénynevelési Tudományos Nap. 522 p. Konferencia helye, ideje: Budapest, Magyarország, 2014.03.18 Budapest: MTA Agrártudományok Osztálya Növénynevelési Tudományos Bizottság, 2014. pp. 379-383. (ISBN:978-963-8351-42-5)- előadás

Rakszegi M , Mikó P , Megyeri M , Tremmel-Bede K, Láng L , Kovács G , Lángné Molnár MNemesítési és szántóföldi menedzsment stratégiák organikus és "low-input" körülményekre. In: Janda T (szerk.)III. ATK Tudományos Nap: A jövőt alakító Európai Unió agrárkutatói programok az MTA ATK-ban. 51 p. Konferencia helye, ideje: 2014.11.13 Martonvásár: MTA ATK, 2014. pp. 41-45. (ISBN:978-615-5387-03-6)- előadás

Conference abstracts

In English

Török K., Tremmel-Bede K., Szentmiklóssy M., Rakszegi M., Tömösközi S. The effects of genetic and environmental factors on arabinoxylan content of special Hungarian wheat lines. 2018. 7th International Dietary Fibre Conference, The Netherlands.

Török K., Tremmel-Bede K., Rakszegi M., Tömösközi S. Investigation of the variability of arabinoxylans in wheat varieties and breeding lines. In: LACC/IGW, 4th Latin American Cereals Conference, Book of Abstracts. 2018. pp. 127.

Török K., Tremmel-Bede K., Szentmiklóssy M., Gergely S., Rakszegi M., Tömösközi S. Arabinoxylans as main dietary fibre components in old Hungarian wheat landraces and in new lines. pp. 43. In EuroFoodChem XIX Conference Central Role of Food Chemistry within the Food Science. Budapest, Hungary, Hungarian Chemical Society, 2017.

Tremmel-Bede K, Rakszegi M, Láng L, Bedő Z. Characterization of wheat genotypes with high dietary fiber content In: Breeding Research, Japanese Breeding Days . Konferencia helye, ideje: Kanazawa , Japan, 21-22 Mar. 2016. Tokyo: p. 128.-poster

Némethné Kisgyörgy B, Bede K, Kiss T, Sestili F, Lafiandra D, Láng L, Bedő Z, Rakszegi M. Development and characterization of high and low amylose wheat lines. In: Weegels P ,Tömösközi S, Auger F (Ed.) CEREALS&EUROPE SPRING MEETING: FINAL PROGRAMME. Konferencia helye, ideje: Budapest , Hungary , 27-29 Apr. 2015. Budapest: Budapest University of Technology and Economics, p. 32.-poster

Kitti Török, Karolina Tremmel-Bede, Marietta Szentmiklóssy, Szilveszter Gergely, Marianna Rakszegi, Sándor Tömösközi. Arabinoxylans as main dietary fibre components in old Hungarian wheat landraces and in new lines. In: Euro Food Chem XIX Conference: Central Role of Food Chemistry with in the Food Science. Konferencia helye, ideje: Budapest, Hungary, 4-6 Oct. 2017. Budapest: Hungarian Chemical Society, p. 43. – előadás

Rakszegi M, Bede K, Mikó P, Howlett S, Pearce B, Wolfe M, Löschenberger F, Láng L, Bedő Z. Increasing the biodiversity of wheat for quality and nutrition In: Wheat – genetic improvement of end use quality, AAB Conference . Konferencia helye, ideje: Harpenden , UK, 9-10 Feb. 2016. Warwick: Association of Applied Biologists, pp. 9-10.-oral presentation

Rakszegi M, Némethné Kisgyörgy B, Bede K, Láng L, Bedő Z. Altering the dietary fiber content of wheat flour by breeding to improve food nutritional value. In: Brandstetter A, Geppner M, Grausgruber H (Ed.) Tagungsband der 66. Jahrestagung der Vereinigung der Pflanzzüchter und Saatgutkaufleute Österreichs, 23.-25. November, Raumberg-Gumpenstein . Raumberg-Gumpenstein , Austria , 2015.11.23 -2015.11.25. Tulln: Universität für Bodenkultur Wien, 2016. p. 21.(ISBN:978-3-900932-36-7)-oral presentation

Rakszegi M, Némethné Kisgyörgy B, Bede K, Láng L, Bedő Z. Quality-oriented wheat breeding targets in Hungary. In: Shewry PR (szerk.) Wheat Breeding2014: Tools, targets & progress. Konferencia helye, ideje: Harpenden , Egyesült Királyság / Anglia , 2014.01.29 - 2014.01.30. Association of Applied Biologists, pp. 19-20.-előadás

Rakszegi M, Németh-Kisgyörgy B, Bede K, Láng L, Bedő Z. Production of wheat with altered polysaccharide content for healthier human consumption. *CHEMICKE LISTY* 107:(9) p. 743. (2013). oral presentation

Karolina Bede, Péter Mikó, Mária Megyeri, Géza Kovács, Mariann Rakszegi: Comparison of the varieties, growing sites and crop management systems based on the physical and breadmaking quality traits of cereals. Shaping the future of agriculture: The role of diversity in low-input and organic cropping systems. SOLIBAM Stakeholder Congress April 19 and 20, 2012 San Nilo Abbey (Grottaferrata-Rome Italy)

In Hungarian

Tremmel-Bede K, Rakszegi M , Láng L , Bedő Z Nagy rostanyag tartalmú búza genotípusok jellemzése In: Veisz Ottó (szerk.)XXI. Növénynevelési Tudományos Napok: Összefoglalók, 155 p. Konferencia helye, ideje: Martonvásár, Magyarország, 2015.03.11 -2015.03.12. Martonvásár: MTA ATK, 2015. p. 132.(ISBN:978-963-8351-43-2)- poszter

Papers in non-scientific publications

Bede K, Rakszegi M. Gabona és egészség: Számos hiánybetegség megelőzését segítenék a tartalmasabb magok, termékek *AGRÁRÁGAZAT* 7:(1) pp. 52-54. (2016)

Mikó P, Rakszegi M, Tremmel-Bede K, Megyeri M. Búzanemesítési stratégiák ökológiai és low-input gazdálkodásra *BIOKULTÚRA* 27:(2-3) pp. 20-22. (2016)

Rakszegi M, Tremmel-Bede K. Régi magyar búzafajták újrahasznosítása nemesítéssel *AGRÁRÁGAZAT* 17:(4) pp. 36-40. (2016)

Rakszegi M, Bede K, Mikó P, Megyeri M, Lángné Molnár M, Láng L, Bedő Z. Organikus és 'low-input' nemesítés hatása a búza minőségére *MARTONVÁSÁR* 25:(1) pp. 10-12. (2013)