NEW ASPECTS IN WHEAT BREEDING: IMPROVEMENT OF BIOACTIVE COMPONENT COMPOSITION AND ITS EFFECTS

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

An important element of a healthy human diet is the fiber content in cereals. Of these, wheat (Triticum aestivum) contains predominantly arabinoxylan, while oats and barley contain higher levels of β-glucan. These components are mainly present in the wheat kernel, and are present in far less amounts in flour. As flour products represent a significant proportion of human nutrient sources, increasing the fiber content of flour may be of particular importance. The main sources of fiber in human diet are non-perishable cereal-based foods. As a result of research over the past two decades, a Chinese wheat variety, Yumai-34, has been identified, 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. The resultant increased fiber content (0.5% in WE-AX and 1.0% in TOT-AX content in dry matter of flour) is not only beneficial due to its health-promoting effect but also has a positive effect on processing properties (eg flour) by increasing its water uptake capacity (Tremmel-Bede et al. 2017, Tremmel-Bede 2020).

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

The heritability of various traits, the effects of environment (E.) and genotype (G) 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 × 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×E), but the heritability of TOT-pentosan proved to be much lower (0.341) than that of WE-pentosan (0.825).
The main component of pentosans, so the quantity and composition of arabinoxylans, was primarily determined by the environment, so the broad-sense heritability of these parameters was moderate (0.516 and 0.772). However, the genotype had a significant effect on the quantity and composition (A/X) of WE-AX, leading to much higher heritability of 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.

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.
Stability of the compositional traits of the selected genotypes was studied using GGE biplot analysis and the determination of CV (coefficient of variation) values. Lines with more stable thousand-kernel weight also had more stable starch content, so it was less sensitive for different environmental conditions. More stable starch content 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). 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 of the genotypes and the environments (Török et al. 2019, Tremmel-Bede 2020, Tremmel-Bede et al. 2020).

3. 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. The β-glucan, total pentosan, water-soluble pentosan, total arabinoxylan and water-extractable arabinoxylan quantities were measured. 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, similarly as was found in the Ukrainka/Yumai-34 population (Lovegrove et al. 2020). 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, 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 (seed coat and aleuron layers) were determined by markers on chromosomes 2A (single and multi-trait) and 5B (multi-trait), while the flour (starchy endosperm) 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 (Tremmel-Bede 2020).

4. Production of wheat lines with high amylose content for other alternative uses of wheat

Winter wheat lines mutant for all the three SGP alleles (starch granule protein, Sgp-A1B1D1) were crossed with three commercial wheat varieties (Ukrainka, Lona, Solstice). During the three backcrossings, the presence of mutant alleles was checked by molecular marker selection. The physical (thousand weight) and compositonal traits (protein, gluten, amylose) of the seed and starch viscosity (RVA) parameters of the selected lines were evaluated in the F3, F4 and F5 generations of BC3 and BC2xBC2 crosses. Ten high amylose (~ 40%) lines were identified among the BC2xBC2 lines, which also had high protein content (> 14%), while the gluten spread, the gluten index, the starch viscosity values, the thousand grain weight and the flour yield gave relatively low values. Two lines were identified among them which had moderately high amylose content (~ 30%) but also had outstanding gluten quality and good physical properties while giving different viscosity values then that of the normal wheat lines. We also made further crossings with these lines. Our experiments have shown that despite controlled molecular selection, it is very difficult to identify and maintain stable mutant lines. Another problem was the low thousand kernel weight and flour yield of the mutant genotypes and the high content of bran in the flour fraction. Despite the high amylose (and thus fiber) content achieved, the practical utility of the produced genotypes remains questionable due to their disadvantageous processing properties.

5. Experiments carried out with wild wheat species

To increase the fiber content of wheat, we investigated the changes in fiber content and composition in wild wheat species such as Ae. biuncialis and Ae. geniculata as potential gene sources. The effect of the addition of certain chromosomes of the U and M genomes on the content and composition of arabinoxylan and β-glucan in wheat was investigated. Subsequently, chromosomal localization of the orthologous Aegilops genes was identified by the major wheat genes determining the fiber content. Based on the results of spectrophotometric and anion exchange chromatography (HPAEC), both Aegilops species contained more arabinoxylans and β-glucans than wheat. Of the additions, the 5U, 7U and 7M chromosomes

had a significant positive effect on the wheat β-glucan content, while the total amount of arabinoxylan in wheat was increased by the addition of 5Ug, 7Ug, 1Ub chromosomes. The amount of water-soluble arabinoxylan was mainly increased by 5U, 5M, 7M chromosomes, but also 3, 4, 6Ug and 2Mb chromosomes also had smaller effects. The structure of wheat arabinoxylan was also influenced by 5Ug and 7Mb chromosomes, which were determined from the oligosaccharide pattern obtained after endoxylanase digestion. We found that Aegilops homologues of the genes responsible for β-glucan synthesis are located on the same homologous chromosome group (I, II, V and VII) as in wheat. This is also true, with a few exceptions, of arabinoxylan synthesis genes (II, III, IV, VII). These results contribute to mapping the genetic regions responsible for fiber content in Aegilops species and to more efficient transfer of alleles of wild species in breeding programs where the primary goal is to produce healthier cereals (Rakszegi et al. 2017). Wild species of wheat, such as Aegilops, can also be an important source for increasing the resistance of plants against environmental stress. In our investigations we found that the protein and β-glucan content of the Aegilops parents as well as the water solubility of arabinoxylan components remained outstanding even under the influence of drought stress. Neither protein nor total (TOT) pentosan, water soluble (WE) pentosan, TOT pentosan / β-glucan, TOT-AX oligosaccharides (AXOS), and US / M + D were affected by the presence of foreign chromosome in the addition lines during drought. Overall, the effect of 2Ug and 7Ug chromosomes contributed to the joint, stable preservation of wheat grain size and composition during drought stress (Rakszegi et al. 2019).

6. Methodology developments in dough modell systems

We have developed and applied a methodology for studying the effects of arabinoxylans on dough rheological properties in addition and reconstitution model systems. During this process, protein and starch isolates were prepared from different wheat flours of different varieties using the Glustar System combined gluten washing equipment developed at the department. The rheological parameters of the dough model systems made from native flour and starch and protein isolates prepared in my experiments were examined with a micro-doughLAB device. Subsequently, the effect of arabinoxylans on the rheological properties of the dough was studied by addition and reconstitution experiments. We found that although there are differences in the rheological behavior of the two model systems, the changes between the samples are similar in both dough systems. It has been found that the addition of arabinoxylan to the flour system results a consistent increase in the proportion of AX addition. The gluten- starch model system shows lower consistency than in case of the flour-based samples. During the isolation process, changes may occur in the properties of the macromolecules, resulting in interactions of varying degrees and nature. To further elucidate the role of AX, we plan to investigate the composition and structure of the two dough structures and to investigate the network formation potential of macromolecules by reduction and reoxidation experiments (Jaksics et al. 2020).

Main results and conclusions:

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).
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.

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. 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.
5. QTLs correlated to fibre content were identified in a Mv Toborzó/Tommi RIL population containing 240 lines. 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.
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.
7. It has been observed that grain composition, such as fiber content, is greatly influenced by factors associated with plant development. These markets (Rht1, Rht2, Ppd-D1, etc.) are located on the 2D, 4B, and 4D chromosomes, close to the alleles defining the fiber content.
8. Wheat genotypes with high amylose (fiber) content have been produced and it has been found that the ratio of amylose / amylopectin molecules in starch can only be modified to a certain extent without compromising processing properties.
9. We found that the 5U, 7U and 7M chromosomes of Aegilops had a significant positive effect on the β-glucan content of wheat, while the addition of 5Ug, 7Ug and 1Ub chromosomes increased the total amount of arabinoxylan in wheat.
10. We found that Aegilops homologues of the genes responsible for β-glucan synthesis are located on the same homologous chromosome group (I, II, V and VII) as in wheat. This is true, with a few exceptions, of arabinoxylan synthesis genes (II, III, IV, VII).
11. Overall, the effect of 2Ug and 7Ug chromosomes contributed to the joint, stable preservation of wheat grain size and composition during drought stress.
12. We have developed and applied a methodology for studying the effects of arabinoxylans on dough rheological properties in additional and reconstitution model systems.
13. It has been found that the dough consistency increases with the addition of arabinoxylan in different flour systems. However, when the components of the dough system are broken down to its elements, there are changes in the properties of each macromolecule resulting in different levels and types of interactions and dough-forming properties.

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