Improvement of spelt wheat lines with low fermentable carbohydrate content (FODMAP) using modern and classical research methods

Extensive research has been initiated to investigate the FODMAP content of different cereal species with primary focus on spelt wheat (*Triticum spelta* L.), as the most promising based on current scientific literature. Based on the Plant Variety Database of the European Commission, 26 spelt genotypes were collected from different European countries, together with 5 Hungarian spelt varieties. These genotypes have been characterized together with 105 spelt lines from our own (CR Ltd.) gene bank. Additionally, all the registered aestivum and triticale varieties of the Cereal Research Ltd. and some sorghum lines, all together 44 genotypes, have been investigated. The Australian GWF spelt variety, described as a typical low FODMAP genotype, was used as control in this study. We have tested ancient type (27 genotypes) of 13 *Triticum* species (13 pcs) for fructan. The average fructan content varied between 1-2%, where the T. carthlicum showed the lowest and T. dicoccoides and T. durum gave the highest results. In some cases, we measured more than twofold difference between genotypes of one given species. Triticale and T. monococcum (einkorn) samples showed the highest and sorghum genotypes showed the lowest fructan content among the examined cereal samples. In case of T. aestivum genotypes, we have measured lower fructan contents than expected based on published data. Compared to the Australian control, all cultivated EU and Hungarian spelt varieties contain higher fructan content, except three cases. In case of spelt genotypes from our own gene bank, an the other hand, about 30 % of the CR Ltd. samples proved to be sufficient for further breeding to gain low fructan content. These 32 varieties have been selected for sowing again in order to examine the year effect on fructan content.

The year effect was significant, the average fructan content was 0.9%, 1.1% and 0.8% in consecutive years (2016-2018). Among the 32 genotypes there were two varieties, which gave <1% of fructan content in all experimental years. No correlation between phenotypic properties (f.e. shell colour) and fructan content have been found in these investigations.

As a complementary analysis, PCR-based allergen tests have been carried from the extracted total genomic DNA of spelt samples, containing the lowest fructan contents. Five genotypes from the examined twelve genotypes gave positive results and were selected for further crossing programme.
Cultivated spelt varieties (n=4, fructan content varied between 0,8-1,5%) were crossed (in diallele system) to determine the inheritance of fructan content. The F_1 generation and the parents were tested in the experiment. The fructan content was never lower in F_1 than in the parents, the results shown a medium value between the parents in the F_1 generations. The F_1 generation was sown again to keep on following the fructan content in F_1 generation. Crossing spelt wheat genotypes with low fructan content (<1%) were also carried out. In this case the fructan content remained low (<1%) in all cases, the results varied between 0,5-1%. In F_2 generation (inside the population) the average fructan content was higher than in F_1 generation (among the combinations) by 0,2%, and its deviation was much wider in F_2 than in F_1 generations. On the whole the results shows intermediere inheritance in case of fructan content.

More than 75 Australian common wheat varieties (from 2015 and 2016 harvest) were also measured and compared to Hungarian assortment. The year effect was also very strong in case

of Australian samples, and significant difference could be observed in the average fructan content between the Hungarian and Australian ones, where the Hungarian varieties have the lower fructan content.

Effect of agronomical factors on the fructan content such as using fertilizers in different amount was also analysed. Three wheat genotypes from a fertilizer experiment were tested where altogether 16 fertilizer combinations (4Nx4PK) were used. The field experiment was done in three replicates. 144 samples were measured for fructan. The results showed differences in fructan content between the treatments of different fertilizers, but the differences were not significant neither of N nor PK treatments. Differences between the tested varieties were significant.

The effect of abiotic stress such as drought on fructan content was tested with spelt and aestivum varieties. Twelve genotypes were selected, three plants were grown in one pot in four replicates for the irrigation and drought experiments too. The pots were irrigated with two water regime from the time of flowering. The control (non-stressed) pots were irrigated for 60% of the water capacity of the soil. The water withdrawal experiment included a 20% water capacity of the soil, from the day of flowering. The weight of biomass, the heading and plant height and the weight of produced kernel were measured beside the fructan content and the applied water amount. All these parameters were reduced significantly due to drought. The average weight of kernel was 5.2 g in case of well-watered plants, and 1,7 g in case of the stressed plants showing a dramatic effect of water withdrawal. Though the amount of kernel was reduced significantly, no trend could be observed in fructan content. In case of 50%, the fructan content has increased significantly, the rate of this increase was between 120-190 %. Reduced fructan content was measured only once, in the rest cases no significant changes were observed. There were also no correlation between the weight of kernel and fructan content. According to the results, the degree of changes in fructan content depends on spelt variety, but the experiments should be repeated with more varieties.

At the start of OTKA project, a very wild-ranging spelt wheat testing and breeding programme was launched. The application of biotechnology methods can effectively support the production of new varieties and hybrids in crop plants. One of these techniques is *in vitro* androgenesis, which can reduce the length of a breeding process. During this project, three different crossing programs were completed.

In the first, the protocols of *in vitro* adrogenesis was modified and developed for spelt wheat androgenesis induction. The two latest crossing programs of them were fitted to the developed in vitro androgenesis protocol and about one-thousand fertile laboratory-derived DH_0 plants were obtained and harvested, selected and propagated in the project.

The main goals of our first experiments were the androgenesis induction in anther culture and isolated microspore culture of spelt wheat genotypes. In anther culture, the cold pre-treatment (appr. two weeks, at 2-4 °C) increased significantly the efficiency of method (number of in vitro green plantlets). Hundreds of green plantlets were produced via anther culture. In isolated microspore culture experiments, different stresses (cold pre-treatment, starvation, 32 °C heat shock) were applied for induction of in vitro androgenesis. Viable microspores were collected by gradient centrifugation (mannit/maltose cushion). The ovary co-culture supported the development of ELS in spelt wheat genotypes. The presence of growth regulators increased the number of ELS, green and albino plantlets. The albinism was the main bottleneck of isolated

microspore culture in spelt wheat, but few green plantlets were produced from spelt genotypes. The anther culture method was proved as a promising method for DH plant production. A diallele-, low × low fructan content- and low × high fructan content combinations were generated and used in these anther culture experiments. We induced androgenesis in anther culture of these three populations.

From the anther culture-derived regenerants of the diallel population, 912 fertile DH lines were harvested. After the first selection, more than 400 DH lines were sown at next autumn for further propagation, test and selection. Later, the fructan content of the most promising lines were measured, and these values were higher than the main goal of the project. The best lines can be used in our conventional spelt breeding programme.

Ten selected combinations (low × low fructan content) and some more combination (low × high fructan content) were used for further improvement of *in vitro* anther culture experiments. The means of ELS production ranged from 19.5 to 183.47 ELS/100 anthers, while the *in vitro* regenerated green plantlets was 28.28 green plantlets/100 anthers (6.3-51.00 green plantlets/100 anthers depending on genotype). The acclimatised green plantlets were transplanted in the DH garden of the nursery in October. Altogether, 1535 transplanted plants were grown up till harvest, and 436 fertile spelt wheats were identified based on the seed production. The values of spontaneous rediploidization ranged from 9.76 % to 54.24 % depending on genotype. The phenomenon of albinism was observed in the experiments. However, the number of albinos was mitigated, it didn't hinder the production of green plantlets and DH lines. The method of *in vitro* anther culture has been established for practical breeding and applied research. For the end of the project – after our improvements – the spelt wheat *in vitro* DH production could be developed for professional level.

At the time of this report, three lines are in large scale propagation (one of them in 1st-year national test, NÉBIH) and they are ready for realising as new variety with special FODMAP values (low fructan content, good bread making quality), but two lines have been still tested during different environment conditions. Over these one + two advanced lines, we have 66 project-generated lines after fructan and agronomy tests and selection. They are in this season in four-replicated yield trial test. Over these 3 + 66 lines, in this season we will test 432 newly improved FODMAP-origin DH lines in DH_1 generation in breeding nursery. After their fructan and agronomy test, they will be involved in breeding program for a similar breeding and test programs as the previously mentioned advanced DH lines (3 + 66).

Some spelt genotypes of DH breeding and registered varieties (n=11) were tested for baking technological parameters. As the amount of DH spelt kernels were limited for analyses, micro-doughLAB and micro Zeleny tests were used. Wholemeal flour and white flour were made from the samples, and commercial BL-80 wheat flour was used as standard. Water absorption, development time, stability and degree of softening were measured during the micro-dough LAB test, and Zeleny sedimentation index was determined by micro Zeleny test. The spelt varieties showed similar technological parameters: the average water absorption of white spelt flours was 55%, the development time was between 1-2 minutes, the stability was between 1-4% and the degree of softening was above 100 FU. These results correspond to the literature, as the spelt varieties had shorter development time and stability and higher degree of softening than wheat genotypes. The wholemeal flours showed more similar pharinographic properties than the standard wheat flours, especially the SPC 37 DH line had far more similar pharinograph

curve than the wheat standard. According to the micro Zeleny index, the spelt genotypes resulted in poor quality. The Zeleny index of the wheat standard was 4.2 ml, the spelt varieties had significantly lower Zeleny values with average value of 3 ml in case of white spelt flour, and 1.6 ml in case of wholemeal flour. It was also proved in this experiment that correlation of Zeleny index with other technological parameters is poor when wholemeal flour is used, but white flour can be recommended in micro Zeleny test to predict the dough technological quality of spelt flour.

This short research, breeding and utilization summary shows for the end of the project we have three generations spelt DH lines breeding materials and we believe in releasing new varieties and/or patented varieties in close future.