PROJECT FINAL REPORT

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Project title: An integrated approach to diversify the genetic base, improve stress resistance, agronomic management and nutritional/processing quality of minor cereal crops for human nutrition in Europe

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Executive summary

Minor cereals spelt, rye, oat, emmer and einkorn are underutilised in Europe and yet may provide nutritional benefits, and new business opportunities for farmers and food processors, especially in remote rural areas. Gene banks have preserved many **minor cereal genotypes** (accessions, landraces and historical crop varieties) but these must be better characterised with modern research methods if they are to be exploited in crop breeding. Also, there is more to learn about how minor cereals can be best used in modern food production, and effectively promoted in the modern consumer-driven market. **HealthyMinorCereals**, a five year project aimed to enhance the utilisation of minor cereals in Europe, with a multi-actor approach involving breeders, farming and food production enterprises, as well as universities and research institutes.

The project has completed a **comprehensive phenotypic characterisation** of minor cereal genotypes, with more than 1700 evaluated at 6 locations in 5 different countries in the first year. A panel of traits has been analysed, including height, leaf and spike morphology, and salt tolerance, and a multivariate statistical analysis was performed to identify gene-environment interactions. The **disease resistance** of spelt, rye and oat genotypes was investigated in a separate set of field trials, involving either natural infection pressure or artificial infection techniques. Spelt genotypes have been tested for resistance against stem rust, leaf rust, yellow rust and common bunt, and fusarium head blight. Susceptibility to crown rust and stem rust was evaluated in oat genotypes, and to *Microdochium nivale* in rye. Based on these results, the development of new varieties of minor cereals has already been initiated, especially taking advantage of selected genotypes with higher disease resistance.

Over 600 minor cereals genotypes have also been analysed for **micronutrients** including zinc, iron and selenium, and **bioactive compounds** including phenolics, beta-glucan and total antioxidants, identifying significant differences between genotypes. Further experiments have tested seed extracts in human cell cultures to measure antioxidant effects compared to a positive control. In comparison to common wheat, significantly higher antioxidant activity was found, especially in rye.

Genetic characterisation of the available collections of spelt, rye, oat and wheat wild relatives was carried out using various genetic markers (SNPs, chloroplast SSR markers) to analyse genetic relatedness, in some cases identifying misclassified material, and investigating intra-species diversity. A genome wide association study of spelt quality and disease resistance traits has commenced, and an analysis of resistance genes in spelt crosses. This data will help identify genotypes with desirable traits for future breeding.

Ways to improve the **cultivation of minor cereals** has been investigated in a coordinated set of field trials in four countries. These have included use of various organic fertilizers, tillage and weed control practices, the use of variety mixtures, legume intercropping, and irrigation in semi-arid climates. These trials have identified promising strategies to maintain yield and reduce crop diseases especially for organic cultivation. Subsequently, farmer-participatory trials have been used to further demonstrate practical application.

Studies into the **use of minor cereals in modern food production** have addressed every stage of production for bakery goods, breakfast cereal, pasta and novel foods. Dehulling, milling and extrusion processes have been investigated and optimised, especially to minimise loss of nutritional content. Differences in baking quality between minor cereals have been found, and overall it is concluded that a mixture of grains provides the best baking quality. Scaling up these results has involved specification of industrial scale production and quality and safety systems, and production of test food products that have been evaluated by consumers.

Finally, social sciences research has been conducted into the **positive and negative factors affecting the introduction of minor cereals into the market**, including case studies in four countries, and an online consumer survey. Recommendations include the raising of awareness of minor cereals among consumers and producers, the development of producer cooperatives/networks and locally adapted business models, and appropriate public or private investment in training and processing equipment.

Results from the project are being published for use by breeders, gene banks, farmers and the food industry. Other dissemination activities include a commissioned film and open days, an international symposium, and public presentations at exhibitions in Brussels and Plovdiv.

Project context and objectives

The productivity of European and global agriculture has been vastly increased through focussing on a relatively small number of crop species, bred for high yields, and dependent on large inputs of mineral fertilizers and pesticides. However, this strategy has left agriculture with an over-reliance on these high inputs, and environmental damage. In the process of domestication, a crop goes through a genetic bottleneck, ending up with much less genetic variation than is available in the wild species. This genetic uniformity can make crops more vulnerable to biotic and abiotic stresses that are likely to increase with climate change. The current self-sufficiency of Europe in cereal crops may therefore be called into question, unless a more diversified and sustainable strategy for the arable sector can be developed.

Cereals grown in Europe such as common wheat and barley have been bred for mainly high yields, and are dependent on large inputs of artificial fertilizers, pesticides and energy. Other cereal species, including **spelt wheat** (*Triticum spelta*), **rye** (*Secale cereale*), **oat** (*Avena sativa*), **einkorn** (*T. monococcum*) and **emmer** (*T. dicoccum*) were important in the early development of agriculture, and spelt, rye and oat were widely grown in Europe prior to the Second World War. However, they are no longer widely grown in Europe and are now classified as "minor cereals" with small areas of cultivation.

Many minor cereal species retain characteristics that have been lost, to a large extent in modern common wheat varieties that have been bred to maximise yield. They often have higher resistance to crop diseases and are less dependent on fertilizer and pesticide inputs. Further genetic diversity can be found in wild wheat relatives that are also preserved in gene banks, but remain underutilized.

Policy makers and funding agencies are now recognising the value of indigenous biodiversity for diversification of farming systems. An expansion of minor cereals in the European arable sector would therefore be beneficial for the environmental impact and sustainability of agriculture, and the economic viability of farming enterprises, especially SMEs in marginal areas. Also, consumer demand for food products with good nutritional quality and with environmental benefits has increased in recent years, and foods based on minor cereals can certainly meet these criteria.

However, the minor cereals have been hardly developed as commercial crop varieties, with virtually no major investment in exploiting genetic diversity, breeding programmes, and optimising of agronomic and food industry processes. Typically, farmers have access to an insufficient number of varieties, and yields are not high enough in most cases to be competitive with other major cereals, limiting market penetration.

There is therefore considerable potential for improving yield, disease and drought resistance, nutritional quality, and suitability for various food products and other uses through exploiting the available genetic diversity of minor cereals, including crop wild relatives, within a dedicated, multi-disciplinary international research programme, HealthyMinorCereals.

The main objective of the HealthyMinorCereals project was to improve and diversify European production of these small grain cereals to increase their productivity, robustness, quality for various uses along with an improved adaptation to more variable environmental conditions and also their diversification and utilization for supporting their cultivation and broader introduction into farmers' fields and the market.

HealthyMinorCereals has, for the first time in Europe, applied a comprehensive approach to the improvement of minor cereals, covering spelt, emmer, einkorn, rye and oat. These 5 minor crop species selected for HealthyMinorCereals are traditional foodstuffs in many European countries e.g. oat in the UK, Estonia and Germany, rye in the Czech Republic, Estonia, Poland, Germany, and spelt in Germany, Austria, Switzerland. Einkorn and emmer are less well known but are cultivated in various regions of Europe, especially in mountainous areas where other cereal species are not productive, and in Turkey, where they originated. Wild wheat relatives have also been studied in the project for their potential to provide important traits for improving minor cereals, such as resistance/tolerance to biotic/abiotic stresses and nutritional value.

HealthyMinorCereals has applied state of the art research, and practical agricultural and industrial knowhow to the evaluation, exploitation and development of these minor cereals, particularly for the benefit of SMEs. The consortium has involved SMEs as partners who are directly involved in crop breeding, farming, milling and food production/processing in various parts of Europe.

The specific objectives of HealthyMinorCereals

To improve the genetic characterisation of minor cereal species such as oat, rye, spelt, einkorn and emmer

Gene banks often maintain very similar genotypes and the genetic make-up of most genotypes and their origins are often poorly defined. A first step in the utilisation of existing culture collections of wild relatives/minor cereals is therefore to characterise their genetic diversity (e.g. relatedness) through the use of genetic markers. For breeding purposes it is necessary to also characterize their crop wild relatives, to increase the potential for the exploitation of selected traits such as tolerance/resistance to biotic/abiotic stresses as well as nutritional quality.

To exploit the availability and enable the transfer of specific alleles from different genetic resources and chromosome segments to improve the transferability of specific traits, e.g. disease resistance and nutritional quality by association analysis and gene sequencing

These studies were designed to identify genotypes with desirable markers/traits for future breeding of minor and major cereal species including introgression of suitable alleles with the help of functional markers. Main target traits were disease resistance genes such as Lr1, Lr10, Lr34/Yr18/Pm38, Yr36 and Mla-like genes.

In view of the requirements for crop breeders, to perform a detailed phenotyping of minor cereal genotypes and determine the potential for developing new commercial varieties and to obtain first crosses of selected minor crops and wild wheat relatives

Exact phenotyping and selection based on multi-environment field trials is traditionally one of the main methods used by plant breeders. In recent years, selection has been increasingly based on information derived from genetic approaches. However, only a few studies describe the phenotypic variation between available genotypes of minor cereal species, and these studies have concentrated mainly on agro-morphological traits. HealthyMinorCereals has therefore carried out an ambitious programme phenotyping of oats, rye, spelt and wild wheat relatives available in European gene banks, requiring a multiplication through several seasons to obtain sufficient seed for study in the project. Subsequently, the development of crosses between promising genotypes of minor cereals has been started.

To identify minor cereals genotypes exhibiting increased genetic resistance/tolerance towards biotic stress i.e. Fusarium, rust and bunt and others, and to localise some of these resistance genes

Disease resistance is an important and effective component of sustainable cereal production, because they minimise the mycotoxins content, the need for fungicide application and problems associated with fungicide resistance and environmental pollution. However, the available genotypes of minor cereals have not been investigated in detail for disease resistance traits/genes. HealthyMinorCereals has therefore performed multi-site field trials for a systematic evaluation of minor cereals disease resistance to stem rust, leaf rust, yellow rust, Fusarium head blight and common bunt in spelt, einkorn and emmer; crown rust and stem rust in oat; and *Microdochium nivale* in rye.

To evaluate and improve agronomic management practices of minor cereals for increased yield and quality for sustainable food production in four diverse climatic zones in Europe – including identification of genotypes tolerant to drought, for semi-arid regions of Europe.

There has been virtually no systematic R&D to improve agronomic management practices for minor cereal crops in Europe over the last 40 years. There is a particular need to develop innovative fertilisation regimes that allow new varieties to reach their yield potential. Studies with major cereals demonstrate that changes in N fertilisation may also affect grain composition (e.g. protein content), associated processing qualities and disease susceptibility patterns so these need to be assessed also for minor cereals. Improved performance of minor cereal crops may be achieved via improved rotation designs, intercropping (e.g. with legumes) and the use of variety mixtures. For example, wheat variety mixtures have been shown to reduce disease severity and lodging and stabilize grain yields. In HealthyMinorCereals, field trials in four countries on experimental and operating commercial farms have aimed to

- identify fertilisation regimes (with a particular focus on utilising organic fertilizers), tillage and intercropping protocols and/or the use of variety mixtures that improve performance of minor cereals cultivation
- understand the impact of genotype x environment interactions on the performance of minor cereal production systems

In addition, trials in Crete have investigated the effect of supplementary irrigation on the performance of spelt and oat genotypes, and the overall commercial viability of reintroducing small grain cereal production to this arid area.

To characterise nutritional composition of selected minor cereal genotypes for human consumption, considering both beneficial and anti-nutritive components, with identification of possible mechanisms for health promotion in human cell cultures, and plant mechanisms responsible for accumulation of beneficial nutrients

Consuming a diverse and antioxidant-rich diet contributes greatly to maintaining better human health. In this regard, cereal-based food can play a key role because they contain many unique and diverse nutritionally-valuable compounds and biologically-active antioxidants. Cereal grains with their high content of antioxidants could help reduce the risk of developing several chronic human diseases, including cancer, inflammation, cardiovascular and neurodegenerative diseases. Minor cereals have not been bred/selected to the same extent as the major cereals, and are thought to have retained a greater diversity and higher concentrations of these nutritionally relevant compounds. However, there has been limited analytical research to quantify profiles and/or concentrations of antioxidants, other nutritionally relevant secondary metabolites (e.g. beta-glucan), fibre and micro-nutrients (e.g. Zn and Fe) in minor cereal genotypes. Also, studies investigating the antioxidative effects of cereal species have often been conducted using only a small number of genotypes.

In HealthyMinorCereals we have analysed the available minor cereal genotypes for profiles/concentrations of nutritionally relevant micronutrients and secondary metabolites. Moreover, no study known to us has tested a high number of genotypes of a given cereal species for their effects to counteract oxidative stress and related cell damage in human cell cultures. Seed extracts of selected rye and oat genotypes have also been assessed for their effects on preventing apoptotic cell death and blocking oxidative degeneration in selected human cell cultures.

To develop and optimise processing/fortification strategies and undertake new food product development based on minor grain cereals to support their market potential and human health.

The higher nutritional quality and specific taste of minor cereals (rye, oat and hulled wheats: spelt, emmer, einkorn) is a major reason for their growing popularity. However, the range of processed food products based on minor cereals is currently very small. Apart from breakfast cereals and a few regional products, most minor cereals are currently processed into wholegrain flours and groats for bread-making. To further increase demand for minor cereals, it is therefore thought to be important to develop a wider range of minor cereal food products and associated processing protocols. In HealthyMinorCereals, we have therefore focused on developing food processing innovations leading to new products, especially identifying the production methods that maintain the most nutritional quality.

To investigate factors contributing to successful marketing strategies for minor cereals, taking into account regional differences in Europe

To support the market success of new or/and traditional minor cereals, it is essential to learn from successful introductions of major cereal varieties into local and regional markets. Data about consumer interest, awareness or willingness to pay linked to minor cereals are not available for SMEs, policy makers or other stakeholders. HealthyMinorCereals has therefore carried out research into the economic potential of minor cereals based on available literature, specially performed case studies and consumer research. The results have been used to make final recommendations on how to increase the market potential for minor cereals.

To demonstrate and disseminate best genotypes, production systems, processing and food products, optimization for perspective stakeholders

Building on the research activities, the applicability of the results to farming and industrial food production has been demonstrated, and project results disseminated through open days, videos and exhibitions, as well as scientific publications and an international symposium.

The HealthyMinorCereals is unique in combining within a single project, research on molecular biology, crop breeding, agronomy, human nutrition, food technology and marketing to deliver an integrated solution for the advancement of minor cereals utilisation in Europe.

HealthyMinorCereals results

Phenotyping of minor cereals to determine the potential for developing new commercial varieties

Leader: University of Natural Resources and Life Sciences (BOKU)
Other partners involved: CRI, GZPK, ETKI and Selgen

In this part of the project, a comprehensive set of data on the characteristics (phenotype) of minor cereal genotypes, preserved in gene banks, was developed through extensive field trials involving partners BOKU, CRI, GZPK, ETKI and Selgen. Multivariate statistical analysis was used for a combined analysis of all data and the development of new varieties was initiated.

Multiplication of genetic resources and breeding material

A key challenge for studying gene bank material is that the amount of material stored for each genotype is usually very limited, sometimes even just a few seeds. Multiplication of this material through one or more seasons of cultivation was essential to obtain sufficient amounts for this research.

Multiplication and distribution of **spelt** genetic resources was carried by BOKU. In total, more than 300 genotypes were obtained from various gene banks in Austria, Czech Republic (CRI), Switzerland, Germany, Hungary, Sweden, Georgia and the USA, while commercial cultivars were provided by partners CRI, GZPK, Selgen and some external companies. GZPK also provided some of their spelt breeding lines. After removing duplicates and spring spelt genotypes, about 180 genotypes were further distributed to partners ETKI and GZPK for phenotyping. Seed material of more than 200 spelt genotypes and some crosses were provided to partner University of Kassel for genotyping (see page 11) including progenies of crosses between spelt and common wheat and Georgian spelt, respectively.

BOKU multiplied also about 30 genotypes and/or breeding lines each of **einkorn** and **emmer wheat** originating from several gene banks, while CRI multiplied genetic resources of **wheat wild relatives** (mainly *Aegilops* species and wild einkorn wheats) originating from their germplasm collection for phenotyping. Selgen multiplied 264 genotypes of **oats** originating worldwide from their own collection. These were mostly European (incl. Russian) hulled oat genotypes, with 14 black glumed and 21 naked (i.e. free-threshing) genotypes, all of which were used for genotyping and phenotyping in the project. **Rye** genotypes obtained mostly of Scandinavian and Baltic origin were multiplied by ETKI and CRI. In this case, multiplication suffered from low germination capacity, winter damage, low weed competitiveness and severe lodging. Therefore, two additional seasons of seed multiplication were necessary to have enough material for phenotyping. A set of 200 genotypes was selected by CRI for genotyping (see page 11).

Extensive phenotyping in field trials

A common list of physical descriptors and traits was established for oats, rye and spelt, drawing on various internationally established protocols. In the first year more than 1700 accessions were evaluated on 6 locations in 5 different countries.

The **spelt** material was scored for winter damage/snow mould, growth habit, growth stages, glaucosity, ear habit, ear snapping, presence of awns, plant height, lodging, ear colour, culm colour during yellow ripening, susceptibility to powdery mildew, yellow rust, leaf rust. A heavy epidemic of yellow rust in 2015 affected the spelt wheat trials (except in Estonia). A high percentage of genotypes were heavily susceptible to yellow rust and, therefore, some other fungal leaf diseases were not observable. Based on the results from 2014 the spelt wheat diversity panel was reduced to 80 genotypes. This core spelt collection was used in further trials of disease resistance (see page 13) and phenotyped more intensively during the rest of the project.

Observations of **oats** at Selgen and ETKI were done for heading, plant height, lodging, powdery mildew resistance. In 2014 a few winter oat types were observed in diversity panel which were excluded for the next seasons. A selection of 179 husked oat samples cultivated by SELGEN were dehulled by BOKU and delivered to Sabanci University for their nutritional analysis of composition. The oat diversity panel was reduced from 264 to 112 genotypes in 2015, to create our core collection.

Figure 1. Phenotyping of oat in Estonia (Ilmar Tamm, ETKI)

For **rye**, problems with seed multiplication limited the phenotypic field trials that could be completed by the end of the project, but work is continuing in 2018.

Wheat wild relatives were evaluated at CRI for field emergence, heading, flowering and maturity, plant height, culm and leaf colour, glaucosity, ear length and colour, awn length and colour, number of spikelets, spike and awn colour at maturity, winter hardiness, resistance to yellow, leaf and stem rust, powdery mildew and viruses. The heavy yellow rust attack in 2015 affected mainly *Ae. cylindrica* accessions.

A screening study was conducted at Sabanci University using 8 spelt genotypes to examine their response to drought/salt stress. The results obtained showed that there was no clear difference in drought stress tolerance among the genotypes. However, the genotypes differed markedly in their salt stress tolerance. It is known that cereal genotypes having higher K and Ca and lower Na concentrations (thus higher K/Na ratios) are usually ranked as salt-tolerant genotypes. The spelt genotypes **Sp96 (tolerant)** and **Sp67 (susceptible)** were selected as the most contrasting genotypes in terms of their K, Ca and Na concentrations and K/Na ratio.

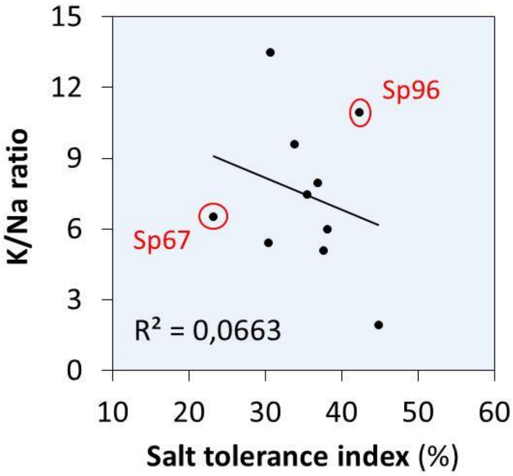


Figure 2: Relationship between shoot K/Na ratio and salt tolerance index

As shown in Figure 2, there was no relationship between salt tolerance index and K/Na ratio among the genotypes. However, the results indicate that in case of the most salt tolerant (Sp96) and most salt susceptible (Sp67) spelt genotypes, **higher K concentration and higher K/Na ratio** in plant tissues are closely related with differential tolerance to salt.

Multivariate statistical analysis of phenotypic data

Multivariate analysis was performed to combine and analyse data obtained for the same genotypes in different locations. First, trait variability in the individual test environments was described by creating boxplots. Subsequently, linear mixed model analyses of variance were applied using the genotype as fixed and the test environment and genotype x environment interaction as random effects. The resulting data was submitted to principal components analysis (PCA). In brief, the results are summarised below.

For **spelt**, the following traits were included: powdery mildew, leaf rust and yellow rust resistance, plant height and lodging tolerance, heading date, spike length, spike weight, number of spikelets per spike, number of sterile spikelets per spike, number of grains per spikelet, thousand grain weight, percentage of grains, hulled grain yield.

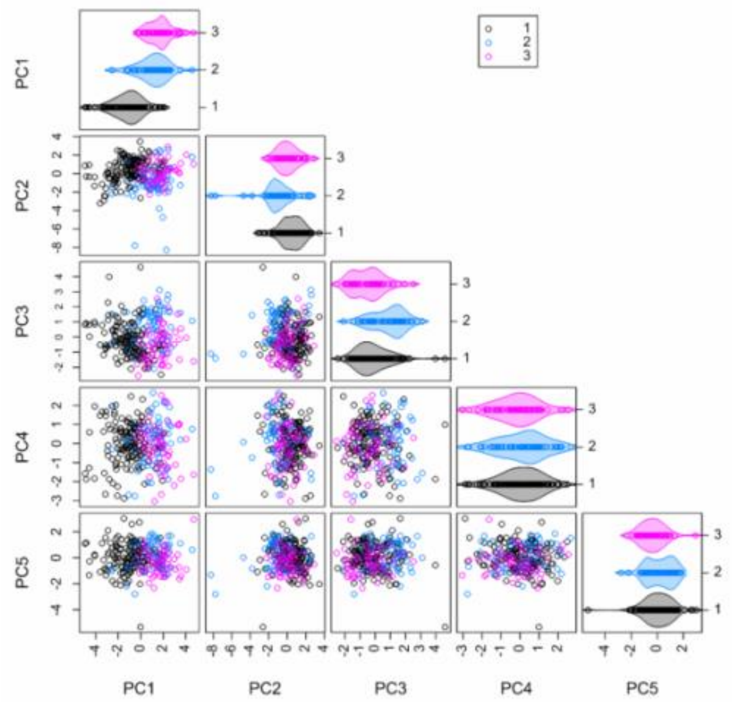


Figure 3: Scatterplot matrix of the first five principal components and density plots of the three different spelt wheat groups (1=landraces and traditional varieties; 2=modern varieties; 3=modern organic breeding lines. This shows how the genotypes cluster according to different phenotypic parameters, correlate this to their origin and determine respective data relationships.

From Figure 3 we can see that the spelt core collection differentiated according to 'breeding period': group 1 represents old landraces and traditional varieties which showed negative PC1 scores, whereas group 2 (modern semi-dwarf varieties) and group 3 (modern organic varieties/breeding lines from GZPK) were overlapping. Group 1 genotypes were associated with tall plant height, low lodging tolerance, and low yield. Modern varieties and breeding lines derived from the organic breeding programme of GZPK are more similar to modern semi-dwarf varieties with respect to lodging tolerance and yield potential, but are also similar to traditional varieties with respect to plant height, spike morphology and grain characteristics.

For **oat**, the following traits were submitted to PCA: percentage of grading >2.0 mm, plant height, hectolitre (test) weight, grain yield, leaf blotch, panicle shape, panicle length, heading and maturity date, and thousand grain weight. It was obvious from the PCA that the naked forms are a different group, exhibiting usually lower yields (-32%), grain mass (-22%) and grading (-13%), but higher test weight (+24%). Grouping according to geographic origin did not show a clear pattern along any PC, however, revealed that the diversity within Austrian and Czech varieties is significantly smaller as compared to French, German or Scandinavian countries as the varieties grouped much more together. Breeding for a smaller markets and the existence of only one breeding programme per country seems to have effects on the phenotypic diversity of the varieties.

Due to problems experienced with **rye** experiments, the obtained phenotypic data was more limited. PCA based on 15 traits revealed a strong discriminant influence of grain size, grain yield, thousand grain weight, lodging and plant height. An astonishing finding was that genotypes from Northern Europe (mainly Finland) differentiate significantly from Estonian genotypes although the geographic distance between these regions is low. It can be assumed that this material is highly adapted to the prevalent climatic conditions in its immediate geographical neighborhood. German and central European (mainly Austrian) material was very similar, which is not surprising as the Austrian rye breeders often use German varieties for crossing.

Crosses between selected minor cereal varieties

Several partners are actively running breeding programmes and have initiated new breeding lines in the project to improve the performance of minor cereal varieties using genotypes with interesting traits selected from the phenotyping trials. For more information on the exploitation of these breeding lines, see page 32.

At CRI three rust resistant genotypes of **wild wheats** *Ae. tauschii* and *Ae. cylindrica* were selected for crossing, however none of these crosses resulted in successful F_1 progenies. BOKU carried out crosses between breeding lines of emmer wheat with progenies of an interspecific tetraploid hybrid.

At BOKU, crosses were carried out in 2014 from the **spelt** core collection, with Georgian spelt wheat. In 2016, individual plant progenies from these crosses were crossed to 11 spelt genotypes from our project collection. At ETKI, spelt wheat crosses were also carried out, with some between *T. spelta* and *T. aestivum*. In 2018, over 20 crosses including F_1 to F_4 generations are in development.

GZPK started spelt crosses between their own breeding lines and material selected by HealthyMinorCereals in 2016. More than 50 crosses were carried out. From three crosses (i.e. REPSA.18/ZMA.6, REPSA.18/ZDREP.8, ZSO.9/ZMA.6) 200 single spike progenies each were sown by GZPK in 2017, and the resulting phenotyping data was provided to partner UNI KASSEL for gene association mapping (see below). In 2018 GZPK has sown 21 breeding lines and 39 spelt genotypes in preparation for the creation of possible new breeding lines.

SELGEN developed **oat** crosses from selected oat genotypes in 2015 and back-crosses in 2016. All crosses and back-crosses included Seldon, a variety bred by Selgen and released in Austria in 2012. In 2018, progenies of 15 crosses have been tested in F_2 or F_3 generation. At ETKI, cross combinations of **rye** involving genotypes from the rye diversity panel were carried out. By 2017, 59 new rye crosses were successful.

Figure 4. Various spelt crosses obtained in HealthyMinorCereals

Genomic characterisation and analysis of minor cereal genotypes

Leader: University of Kassel
Other partners involved: CRI

The first step to analyse crop genetic resources is to get an overview on the structure of genetic relatedness in groups of genotypes (lines or populations as available in genebanks) identified as a possible useful resource for future breeding: how related are those genotypes, do they group together, and do those groups relate to phenotypic features, or geographical origin? Subsequently, the traits most interesting for breeding have to be genotyped to make them useful for breeding.

In order to simplify breeding for interesting traits from spelt wheat genetic resources by introgression of different alleles, genomic techniques have been used to localise the respective gene loci. This includes genome-wide association analysis (GWAS) both for agronomic traits, diseases and quality traits, sequence comparisons for six known resistance gene loci in the HealthyMinorCereals spelt collection, and genotyping of three spelt crosses. All these results are in the process of further analysis prior to publication, expected during 2019-2020.

Genotypic characterization of minor cereals and wild relatives

The genotypic characterisation of genotypes of oat, rye, spelt wheat and wheat wild relatives collected for HealthyMinorCereals firstly involved the analysis of genetic markers (microsatellites or SNPs), and then data analysis including Principal Component Analysis to analyse their genetic relatedness.

For **oat**, there was only a weak genetic structure in the 262 genotypes analysed. Not even naked oats or black oats formed a genetically distinctive group. Only oat genotypes from Canada and the US separated slightly from all other oat genotypes on the basis of their genetic markers, as may be expected considering the separate breeding programmes established in North America and Europe.

In contrast, the 218 **rye** genotypes genetically clearly separated into three groups. Especially one group of experimental varieties included in this experiment was clearly distinct from the other genotypes. Figure 5 shows these results, each point representing an accession, the distance between the genotypes representing the genetic distance between them.

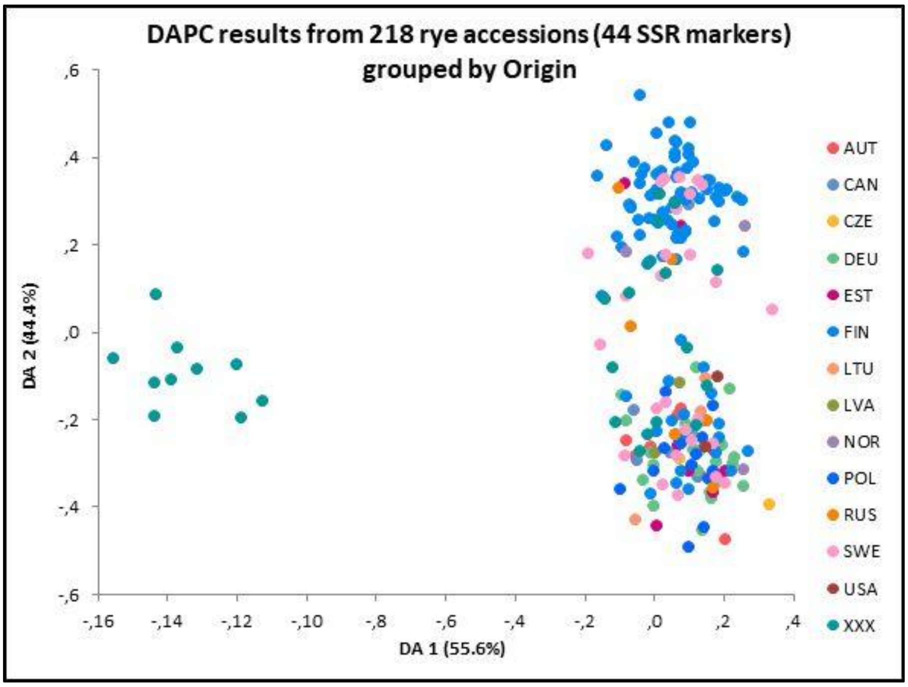


Figure 5. Genetic diversity from results of the analysis of 218 rye genotypes.

For **spelt**, a core of genotypes clustering closely to each other in the data represent the old, traditional spelt wheats, while the modern spelt wheats are genetically more diverse. Partly, this might be caused by crossing with common wheat to increase yield and baking qualitity, and to decrease plant height. In rare cases, this also could be caused by the reconstruction of wheat by *de novo* species hybridisations.

For the analysis of different species of **wild wheat relatives,** chloroplast markers, that change slowly, were used instead of genomic markers. The results of a non-parametric scaling indicates the genetic relationship between the species, as shown in Figure 6.

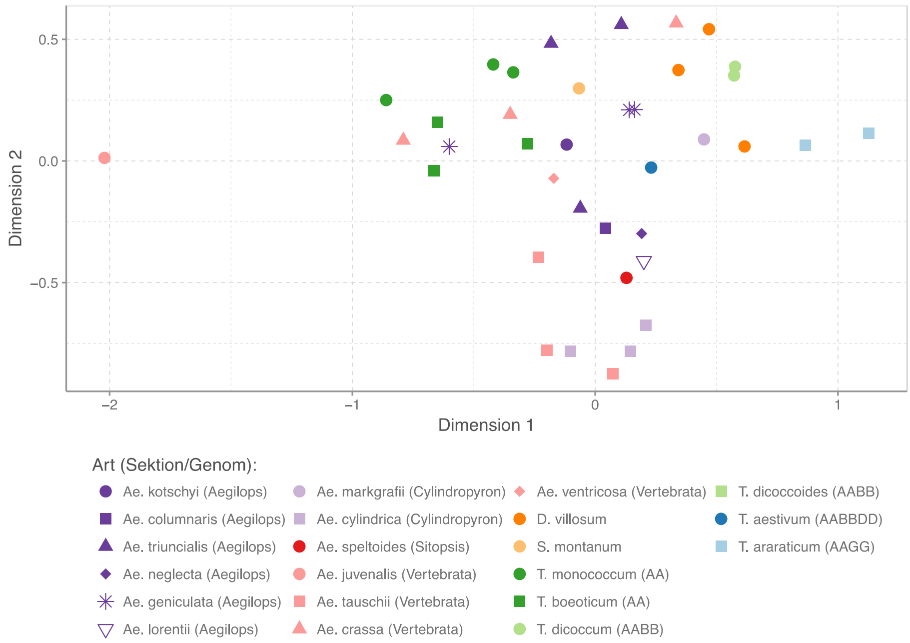


Figure 6. Analysis of genetic diversity of wild wheat relatives using chloroplast markers (distance of the species relates to genetic distance). Species closer to spelt are easier to use as genetic resources for breeding.

Association analysis of spelt for disease resistance and quality traits

A genome-wide association study was performed in order to determine sections of the plant genome which might be responsible directly or indirectly for the phenotypic traits observed in field trials and nutritional analysis performed by other partners. An example is the association of specific genomic regions to the mean of plant height. Phenotypic data of 44 agronomic, morphological and disease traits, and 10 nutritional quality traits measured in the spelt genotypes were obtained from project partners. For GWAS, 23 agronomic, morphological and disease traits and 9 nutritional quality traits were chosen. DNA samples of 184 genetically distant spelt genotypes were analysed by a 15 k Infinium SNP array. Association analysis of spelt for disease resistance and quality traits is now being finalized at University of Kassel.

Analysis of selected wheat resistance genes

The sequencing of six different disease resistance genes of 186 spelt genotypes is being performed at University of Kassel in order to identify allelic groups within those resistance genes, and also which genotypes may provide sources of genetic disease resistance. The analysis of wheat resistance genes was performed by targeted Sanger-sequencing. The following target genes were identified for analysis, from a literature and database search: Leaf rust resistance: LR10, LR21; stem rust: SR13, SR22; yellow or stripe rust: YR10; powdery mildew: PM3. University of Kassel completed PCR primer design and have generated PCR products for Sanger sequencing by an external company. Following receipt of the data, sequence analysis will be performed to determine expression of the 6 selected genes in the spelt collection.

Location of qualitative resistance genes in spelt crosses

Three spelt crosses developed by GZPK (F4 descendants, see page 10) are now being analysed by University of Kassel to identify genes that could be responsible for their resistance to leaf rust, stem rust and yellow rust, respectively. So far the leaf biomass has been sent to a genomics service company for DNA extraction and microarray genotyping using a 15k + 5k SNP array chip developed for common wheat.

Evaluation of the resistance of minor cereals to crop diseases

Leader: Crop Research Institute
Other partners involved: Selgen, GZPK, BOKU, ETKI

Resistance to diseases in the minor cereals spelt, oats and rye, has been studied in field trials in the Czech Republic (CRI, Selgen), Switzerland (GZPK), Austria (BOKU) and Estonia (ETKI). Crop diseases studied included rusts in spelt and oats, common bunt and Fusarium head blight (FHB) in spelt, and *Microdochium nivale* in rye, aiming to obtain at least two years of data for each experiment.

The field trials employed either natural or artificial infections. These are complementary approaches. Artificial infection experiments in nurseries are more controllable and accurate, but use pathogens isolated in the previous year in the respective countries. Natural infection experiments rely on natural levels of crop disease, but relate directly to the pathogen strains circulating in the current year. The analysis of infection was done on the basis of visual scoring, on a scale of 1 to 9 (where 1 = no infection, 9 = all spikelets infected).

Figure 7. Artificial infection with Fusarium head blight for 24 h in polythene bags (CRI)

Figure 8. Preparation of inoculum for artificial infection in the greenhouse (CRI)

Resistance of spelt to stem rust, leaf rust, yellow rust and common bunt

Rust diseases cause a decrease in both the yield as well as the quality of production. Resistance to common bunt has a particular importance in spelt cultivation. Common bunt produces a fetid trimethylamine odour which can devalue the grain product and its use as human food and animal feed. Infection by common bunt can also cause 50% losses in yield, and it is carried by seed. HealthyMinorCereals has established a new experimental procedure for evaluating common bunt resistance.

The evaluation of resistance to yellow rust and leaf rust by natural infection was very much affected by a major natural outbreak of yellow rust in central Europe in 2015. In 2015, all the spelt varieties monitored were attacked by yellow rust, as a result of this epidemic. Our evaluation of resistance against rusts in spelt (and also in oats) was also influenced by different pathogen strains occurring in the Czech Republic, Switzerland, Austria and Estonia.

In total 80 spelt genotypes were studied in HealthyMinorCereals. Several of them showed a high level of resistance to yellow rust, but only a few showed a high resistance to common bunt, stem rust or leaf rust. Sofia 1, a genotype originally from Belgium, was identified as resistance source to several crop diseases (high levels of resistance against common bunt and stem rust; moderate resistance against FHB, leaf rust and yellow rust). With respect to stem rust, almost all spelt genotypes showed a high susceptibility.

Figure 9. Left: Yellow rust on spelt. Centre: Stem rust –nurseries with spreader rows. Right: Stem rust on spelt (Martina Eiseltova, CRI)

Resistance of oat to crown rust and stem rust

In oat, 112 varieties were evaluated for resistance against rusts. Varieties Dumont and AC Preaknes showed the highest resistance to crown rust and Dumont was also resistant to stem rust. It is interesting to note that Dumont also had the highest total grain phenolics content out of over 600 minor cereals genotypes tested.

Figure 10. Oat infected with *Puccinia coronata* (crown rust) (Martina Eiseltová, CRI)

Resistance of spelt to fusarium head blight

Artificial infection of the 80 spelt genotypes was performed in CRU, GZPK and BOKU. In addition to visual assessments, all seeds from infected spikes were analysed for the myxotoxin deoxynivalenol (DON) in CRI. The contents of DON (and other mycotoxins T2 and HT-2) were evaluated by competitive enzyme-linked immunosorbent assay (ELISA) using commercially-available kits.

The results showed large differences in susceptibility to FHB among the 80 spelt genotypes. On the basis of the first results from the Czech Republic, Austria and Switzerland, the following genotypes were identified as most resistant with the lowest content of deoxynivalenol (DON): Toess 6D, Farnsburger Rotkorn, Roter Schlegeldinkel, Gugg 4E, Ebners Rotkorn, Rottweiler Dinkel St. 6, and LW12 Nürtingen. It is interesting to note that all these genotypes represent old landraces and/or traditional tall varieties, whereas the highest accumulation of DON was detected in the modern varieties Badenkrone and Alkor, showing a clear link with plant height (Figure 11).

The resistance of spelt varieties was evaluated also in conditions of natural infection after maize as a preceding crop and reduced tillage. Here, the mycotoxin content was not determined and the results are only supplementary.

Figure 11: Alkor (far left) and Sofia 1 (left) after artificial infection with *Fusarium cumorum* (CRI, 2016). Above: Snow mould experiments (ETKI).

Resistance of rye to *Microdochium nivale*

Tolerance to snow moulds is one of the most important factors determining winter survival in cold and moderate climates. *Microdochium nivale* is a widespread fungus causing pink snow mould on cereal seedlings. Resistance to snow mould is determined by multiple genes and the determination of resistance is therefore difficult as it requires investigations over long periods of time. An assessment of resistance in natural conditions is not always reliable.

Two rye varieties, the Estonian variety Tulvi and the Finnish variety Riihi, out of 104 tested genotypes were not infected by *M. nivale* in the applied artificial test and showed the best resistance.

Overall conclusions

Through these field trials, HealthyMinorCereals has provided important information on the disease resistance of genetic resources of the minor cereals spelt wheat, oats and rye. Results are already published and presented at a conference (see page 40). Landraces and old varieties were generally less productive with respect to grain yield and showed a tendency to lodging due to their taller plant height. However, useful levels of resistance to several diseases have been identified in several genotypes, which are already being used for crosses by the respective project partners with breeding activities in minor cereals.

Effect of agronomic management practices on minor cereals cultivation

Leader: Newcastle University
Other partners involved: CRI, PRO-BIO, Gilchesters, ETKI, Nikolaos Volakakis

Field trials in HealthyMinorCereals have been performed to study the effect of agronomic management practices on the performance of minor cereals. These have mainly involved small plot trials with four replicates, measuring grain yield, yield components, disease levels and grain quality. They have differing designs depending on what is being studied, but are generally multi-factorial. The last stage of these studies has involved farmer participatory trials to better reflect real-world conditions. For all these trials it was decided to use four representative of each minor cereal species, including old varieties/landraces together with new varieties recently developed by partners GZPK (for spelt) and Selgen (for oat).

Effects of fertilizer input type and level on minor cereals performance

Mineral N is the most commonly used nitrogen source in conventional systems, while manure and compost are the preferred N sources in organic farming. Digestate is a relatively new organic fertiliser which is attracting particular interest because of its high readily available source of N and from an increasing uptake of the biogas technology across Europe. There is a clear need to identify crop varieties that are more responsive to organic fertilisers and are adapted to specific regional/climatic conditions.

Field trials were established in 2014/15 and 2015/16 of the minor cereals in the UK (spelt and rye), Estonia (rye and oat) and Czech Republic (spelt and oat). The trials used 4 commercial varieties of each species which included older varieties/landraces and modern varieties from across Europe. Four different fertilisers were evaluated in the study: composted farmyard manure (FYM), mineral N, cattle slurry and biogas digestate. Composted FYM was incorporated into the soil prior to cultivation while all other N types were applied in the spring. The aims of this study were to evaluate the effects of contrasting genotypes and fertilisation regimes on the performance of the minor cereals across different climatic zones of Europe.

Figure 12. Spelt varieties showing clear differences in susceptibility to yellow rust (Puccinia striformis) in the UK in 2015

All three crop species showed clear potential in low-input/organic production systems (especially rye and oat due to their lower disease susceptibility) but clear differences were identified between species, sites and seasons. There was considerable variation in the ranking of varieties in different environments which was most noticeable for spelt in the levels of yellow rust and hence yield recorded. The modern semi-dwarf (wheat x spelt cross) Filderstolz was the best performing variety in both seasons in the Czech Republic (with a 0.75 t/ha increased yield over the landrace Oberkulmer) where disease levels were generally low. By contrast in the UK, Oberkulmer had much lower yellow rust and leaf blotch (*Septoria tritici*) than the other varieties with Filderstolz being particularly susceptible to yellow rust (*Puccinia striformis*) (Figure 12). The differences in disease susceptibility were clearly reflected in the final yields where Oberkulmer produced a 1.15 t/ha increase when averaged across both seasons.

Biogas digestate produced a grain yield which was generally on a par with mineral N fertiliser but in the UK produced a 0.5 t/ha increase in spelt yield, providing a clear economic benefit to farmers where this material is becoming increasingly available. The more readily available N fertilisers, biogas digestate and mineral N, resulted in higher protein levels than other fertility types. Spelt also had the potential to produce high protein contents even with reduced N fertility amounts and in a year with notably high protein content in UK-grown milling wheat, the spelt trials produced grains with exceptionally high protein levels.

Interest in minor cereals as alternative grain crops continues to grow and the field trial results support the use of milling-quality varieties (spelt and rye) in arable rotations. Results encourage the continued adoption of biogas digestate as an alternative fertility source for low-nutrient requirement crops and for use in organic production systems where availability of N for grain fill is often limited. Biogas digestate provides a clear opportunity to increase the productivity of minor cereals by matching N supply and demand particularly in organic production systems.

Effects of tillage and weed control practices on minor cereals performance

Despite the advantages associated with conventional tillage, there are some clear drawbacks e.g. high energy use, potential increases in soil compaction and erosion but particularly reductions in soil organic matter and health. Organic farmers have shown less interest in non-inversion tillage practices because they are associated with increased weed pressure. The purpose of the study was to examine the effect of tillage, weed control and nitrogen (N)-availability, weed pressure and disease development on contrasting varieties (selected particularly for differences in growth habit) of the minor cereals (spelt rye and oat) in the UK and Czech Republic. A key question is how these species respond to a minimum tillage cultivation system.

For both spelt and rye grown in the UK in 2016 and 2017 a conventional plough based cultivation system had a 0.8 and 0.7 t/ha yield increase compared with minimum tillage with a consistent response across both species. The increased yield was reflected in higher leaf greenness throughout the season indicating increased mineralisation and uptake of available soil nitrogen. There were no consistent effects of cultivation treatment on disease levels but the conventional tillage treatment increased grain protein from 12.4 to 13.1% in spelt and 10.1 to 10.5% in rye where compared with minimum tillage when averaged across the two seasons. Conventional tillage encourages N mineralisation in soils, thus high amounts of N become available for plant uptake, leading to higher protein contents in both crop species. The increased protein content and reduced establishment costs make minimum tillage a worthwhile consideration for minor cereals.

In contrast, results from the Czech Republic showed no effect of cultivation treatment on the grain yield of either spelt or oat when averaged across the 2 years that the trials were carried out. However for oat, the results were different in each year: conventional cultivation gave higher grain yield in 2017 (5.0 vs 4.7 t/ha) but minimum tillage had the highest grain yield (4.1 vs 3.8 t/ha) in 2018.

Effects of variety mixtures protocols on minor cereals performance

Increased crop genetic diversity has long been recognized as a way to increase stability of performance due to the buffering, compensation and complementation effects of diverse crop material. Genetically heterogeneous material has been shown to; provide greater yield stability, buffer variation in soil, climate and weed pressures and especially to restrict the spread of fungal diseases. However, widespread application of variety mixtures is constrained by the concerns of farmers and processors re negative effects of heterogeneity on grain quality and marketing which can be avoided by careful selection of the varieties to be used.

Figure 13. Spelt varietal mixture and pure-line varieties showing differences in yellow rust levels in the UK in 2016

The performance of three single pure line varieties of the minor cereals (spelt, rye and oat) was compared with a varietal mixture (physical mixture of all 3 varieties at the same seed rate) of all three cultivars across contrasting environments in the UK and Estonia. For rye the modern Polish variety Dankowskie Amber had the highest yield in both the UK (rye and spelt) and Estonia (rye and oat) in both seasons. The yield of the variety mixture was higher than the mean of the three pure-line varieties in the UK by 0.19 t/ha and in Estonia by 0.24t/ha when averaged across the 2016 and 2017 growing seasons. For both spelt and oat grown in the UK and Estonia respectively the yield of the variety mixture was on a par with the mean of the three pure-line varieties.

The advantage of cereal variety mixtures has previously been demonstrated, especially in terms of plant disease restriction. The use of the pure-line varieties encouraged differing disease levels and it was only in oat in 2016 where the varietal mixture showed lower crown rust and stem rust than all of the pure-line varieties. With clear differences in the susceptibility of the pure line spelt varieties (Figure 13) to yellow rust (*Puccinia striformis*) the varietal mixture in the UK produced consistently lower levels of disease than the mean of the three pure-line varieties on the flag leaf and Leaf 2 in both 2016 and 2017. The results of this study show clear potential of varietal mixtures to produce a greater consistency in yield with reduced levels of disease in minor cereals

Effects of legume intercrops and fertilizer types on minor cereals performance

Intercropping is one of the oldest farming systems in the world and involves cultivation of at least two crops in the same field, at the same time. Intercropping experiments were carried out at Livadopa Field Station, operated by Nikolaos Volakakis in Crete, in 2016 and 2017 to evaluate the performance of spelt and oat with no intercrop, vetch, vetch+barley and chickpeas. The use of an intercrop had no significant effect on grain yield or crop health in either season, although intercropping with chickpea produced the highest grain yield in both seasons.

Farmer participatory trials

In agricultural research, Farmer Participatory Trials (FPT) are a common strategy to introduce new technologies/management practices to farmers. They can be laid out in replicated experimental design thereby closely mimicking what would be used at research stations but alternatively can be larger non-replicated trials. They are used to match farmer conditions and are much closer to farming reality compared to on-station small-scale micromanaged research trials. Sometimes these type of trials are referred to as 'demonstration trials', as these experiments are geared towards technology dissemination via farmer training and field days. Researchers usually support the farmers in the management/data collection.

Farmer Participatory Trials of minor cereals under both organic and conventional management systems were established i.e. spelt (UK and Czech Republic), Rye (UK and Estonia) and oat (Estonia and Czech Republic) in the 2016-17 and 2017-18 growing seasons. This involved strip-plot trials (minimum of 100m x 3m) in farmer's fields (4 organic plus 4 conventional farms for each species in each country) differing in climate/soil characteristics, rotational position, management practices etc. The varieties used were the ones also used in previous trials, but with the farmer comparing also with a variety that they were currently growing. The FPTs also enabled a comparison between fertility management systems, comparing the approach identified in previous trials in HealthyMinorCereals based on the use of biogas digestate to a farmers existing fertility management system which was generally Mineral N for conventional farms and FYM/cattle slurry for organic farms. The conclusions of the Farmer Participatory Trials are as follows:

- For both oat and spelt in Czech Republic, oat and rye in Estonia, conventional management gave grain yields that were significantly higher than the organic management, with differences ranging from 25-45%, consistent with a number of previous studies.
- The Mineral N treatment was superior to the biogas digestate and FYM treatments for both autumn-sown spelt and spring-sown oat in FPT using conventional management in the Czech Republic. This was also the case for spelt in the UK in 2017-18 whereas for rye there was no difference between the fertility treatments. In Estonia, biogas digestate resulted in a slight increase in grain yield of both rye and oats when compared with the established fertilizers (FYM on organic farms and mineral N on conventional farms) when averaged across both farming systems

- Clear genotype x environment interactions were observed for the performance of the different minor cereal species across sites and seasons. The performance of oat, spelt and rye varieties in the FPT matched well with project results obtained previously (see above). Filderstolz was the highest yielding spelt variety in CZ with the landrace Oberkulmer producing the highest yield in 2016-17. For rye, Elias followed by Dankowskie Amber were the best performing varieties in the UK while in Estonia Elvi was the best performing variety having been bred in that country. The naked oat variety Saul had produced a yield well below other varieties in both Estonia and Czech Republic in previous project trials, which was confirmed by the FPT in Estonia. However, in Czech Republic the yield of Saul was only slightly lower than the hulled variety Kalle.
- Poor establishment of rye in the autumn of 2016 (largely resulting from slug damage) was responsible for poor performance in the UK FPT.

Spelt cultivation in a semi-arid region of the Mediterranean with supplementary irrigation

Although Spelt cultivation has become more common in recent years in many semi-arid regions of the Mediterranean, arable farmers lack information about optimum agronomic protocols and the performance of available spelt varieties.

The main objective of the study performed by Nikolaos Volakakis was therefore to assess the effect of different fertiliser types an supplementary irrigation on the performance of four contrasting spelt varieties under semi-arid conditions in Crete.

The use of supplementary irrigation was found to significantly increase grain yield in the seasons with low winter rainfall only, while it had no effect on grain yields in the season with relatively high winter rainfall.

Chicken and sheep manure pellets resulted in similar or higher grain yields and lower lodging levels than mineral NPK fertiliser. Results also show that spelt varieties that were recently developed for the organic (ZOR) and conventional (Filderstolz) arable sector had higher yields, N use efficiency, and lodging resistance, but lower protein concentrations than traditional varieties from Switzerland and the Czech Republic (Oberkulmer or Rubiota).

Figure 14. Spelt plots with different fertility and irrigation regimes at Livadopa field station, southern Crete (Nikolaos Volkakis/Geokomi)

Results suggest that future variety selection programs should focus on increasing yield potential under organic-based fertilisation regimes by focusing on traits such as high N uptake and use efficiency as well as increased drought resistance.

Optimising food production and product development strategies

Leader: Institut für Lebensmittel-und Umweltforschung (ILU)
Other partners involved: SB, BGK, IGV, PRO-BIO

Introduction

Germany food research institute ILU has led research in HealthyMinorCereals on how the food industry can better process minor cereals grains with the best possible efficiency and maintenance of nutritional content, focusing particularly on spelt, einkorn and emmer which are less developed in the food industry than rye and oat. In this research ILU have been working particularly with Germany artisan bakery Stoltzenberger's Bakery, Polish breakfast cereals company BGK and the food technology institute IGV. These partners have also worked on the development and evaluation of new food products containing minor cereals, how to best process minor cereals that are not as easy to process as common wheat and the evaluation of minor cereals processing in an industrial scale and environment.

A particular challenge in HealthyMinorCereals has been to perform these studies with small amounts of grain, especially when working with samples from field trials and breeding programmes. ILU has access to specialised techniques including microbaking and Mixolab to get results from small quantities of grain and dough. Work commenced with commercial supplies of minor cereals before samples were available from the other project partners.

To get a final product that retains the high nutritional potential of its grain, the processing of the grain in all its stages, such as milling, baking, etc., has to be optimised. Examples from the results obtained over 5 years are presented below, from the first small scale trials through to optimisation at industrial scale.

Dehulling

Common wheat and rye and some oat varieties (naked oats) are free-threshing (or naked) and can be directly milled into flour; in contrast, spelt, emmer and einkorn are hulled wheats, meaning that their grains are tightly imbedded in thin, dry, scaly glumes forming a dry hull (or husk) that has to be removed prior to grain milling. Hulled-oat varieties have to be dehulled prior to milling into flour, as well.

Important criteria for hulled cereals are a high-peeling yield and good peelability. As an example, results are presented of the levels of spelt yield after each step in the process of dehulling/husking. The spelt grain yield is the ratio of spelt, husk and the unpeelable portion. To achieve reasonable economic returns for farmers, high crop yields and high peeling yields are necessary.

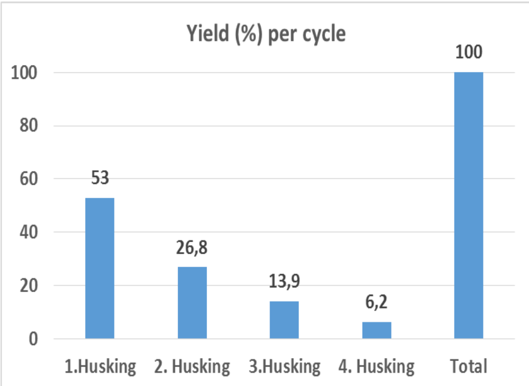


Figure 15. Spelt yield per dehulling cycle

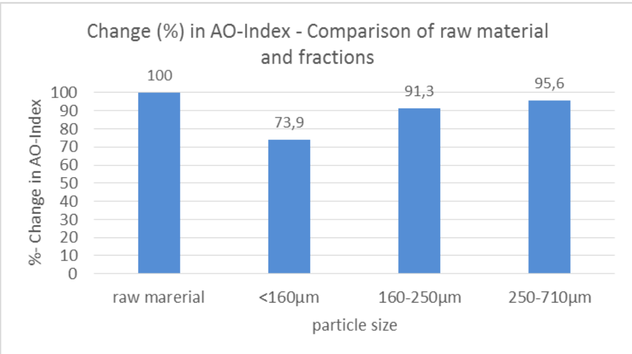
Figure 15 shows how each dehulling (husking) step increases the final yield, but to a decreasing extent. It took three to four steps to remove the husk from all grains completely.

In practice, we found that different varieties have different peeling properties (good, medium and poor peelability). For practical reasons, the 'best' varieties are the ones with high yield in combination with 'good' peelability. Also, analysis of samples from the agronomic field trials testing different organic and conventional fertilisers (see page 16) found some differences in the kernel size distribution in the samples from trials with the various fertiliser types. Commercial volumes of the trialled varieties evaluated were also dehulled by Gilchesters Organics Ltd using a Buhler soda-stone dehulling mill. Good separation was achieved from all varieties but Oberkulmer Rotkorn performed particularly well with good wholegrain retention from the process. Aspiration was required for all spelt after dehulling, with additional secondary aspiration in combination with sieving for full husk removal for adequate cleanliness of samples prior to milling.

Milling and effects of temperature

For milling experiments, grain samples of spelt, emmer and einkorn were commercially purchased by **Stolzenberger Bakery** and supplied to **ILU** for milling (using impact milling with screen size 0.75 mm) and subsequent processing and evaluation. Processing by milling and sieving to recover fractions suitable for different food products leads to loss of antioxidative potential. The lowest value was measured in the bright endosperm powder fraction (<160 µm). In the fractions which became darker in their outer layer, a higher value was recorded in the flour fraction, following on the distribution of the main fractions. Concurrent milling evaluations at Gilchesters Organics Ltd of spelt and rye varieties from the field trials, alongside emmer and einkorn grains, confirmed the suitability of all varieties for stone milling processes. All produced flours of fine particulation which performed well from the full range of wholemeal (100%) through to fine white flours in their subsequent breads.

Toasting was used to model the high temperatures used in many food production processes. It was found that the antioxidant potential was also reduced by toasting. The higher the temperature, the more pronounced the reduction with longer application times (optimal temperature should be around 105°C and baking times between 40 and 110 minutes). The key intervention to overcome these deficits in antioxidative potential in the fractions was a short application time and lower temperature, leading to the lowest decrease in antioxidant potential.



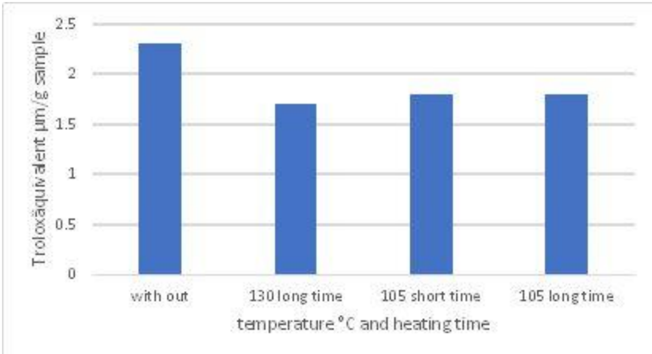


Figure 16. Changes in antioxidative potential in spelt due to milling and heat treatment

Baking quality

In order to exploit the maximum nutritional content of minor cereal ingredients for human consumption, which are predominantly localized in the surface layers, wholemeal flour must be used. To assess the baking behaviour of the selected cereals (spelt, emmer, einkorn), they were ground by impact grinding into wholemeal flour and then underwent a modified standard baking test originally developed for organic wholemeal spelt flour (whole-grain spelt baking trial with ascorbic acid) and a micro-baking test (developed by ILU). Even though the two types of baking tests are inherently different, both tests gave reproducible and important information about the baking characteristics of the examined samples (spelt, emmer, einkorn and common wheat).

Micro-baking test

The baking performance of the various samples has been primarily analyzed via micro-baking tests. For the micro-baking test, 50g flour was used and the further processing steps were standardized and made reproducible. By implementing the micro-baking test, ILU were thus able to determine the baking characteristics of each variety (40 varieties were tested in all), even with only small amounts of each sample.

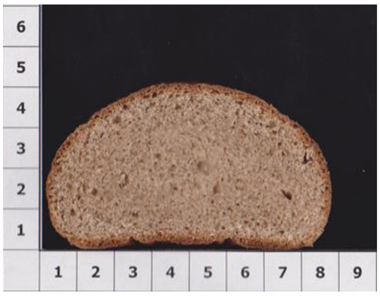


Figure 17. Emmer bread cross-section in micro-baking test

Modified standard baking test

The modified standard baking test was carried out as closely as possible to the baking test developed by the Max Rubner Institute for wholemeal spelt flour. The baking test was performed in loaf pans with a significantly greater amount of dough and using conventional technology (laboratory kneader and manual preparation). The advantage of this baking test is that it incorporates a greater quantity of water (flour-water ratio) in the recipe. Using an increased quantity of water benefits the quality of products made from emmer, spelt and einkorn and is common practice in bakeries.

Figure 18. Spelt bread cross-section in modified test

These tests were complemented by dough rheology experiments using the new Mixolab system, that can measure water absorption, dough development, start gelatinization and retrogradation, as well as the force required for kneading, all in one test.

Figure 19. Mixolab results predicting baking quality tests with three different emmer varieties

Process optimization for minor cereals

Based upon the outcomes of laboratory baking assays and rheological assessments, the main conclusion is that spelt, emmer and einkorn have a relatively challenging processability. Therefore, carefully chosen well-balanced mixtures (e.g. with common wheat) would have the greatest chances for being translated into their use in mass production, while still bringing to the end-consumers the same or improved nutritional features. Besides the possibility of respective flour patterns being mixed in different proportions and subsequently processed, some characteristics of minor cereals affecting baking quality were also improved by technological measures as well as implemented recipe-changes in practice, for example by using different types of sour dough and pre-dough, and using different sourdough starters (mild acidification / strong acidification). The quality of end products can be positively influenced by the kneading time and its intensity, by dough resting and fermentation parameters, and also by dough acidification agents (acerola cherry powder / ascorbic acid).

Figure 20. left to right: New baking products developed by SB; Sensory evaluation panel at SB; Showcasing new products by Reiner Stolzenberger at Bönnigheim trade fair

Table 1. Examples of improving the baking quality of minor cereals with challenging baking properties, through recipe-based and technological measures developed from lab-baking trials at ILU and IGV

ILU-D-028 / Zollernspelz (spelt)	**Implemented measures**
Generally a good baking quality was determined. However, during the fermentation process, stability deficits were noted.	• Use of sour dough with mild acidification to improve dough stability • Use of Acerola-Cherry-Powder • Dough resting periods were extended to increase firmness and dryness
IGV-D-030 / REPSA.18 (spelt)	**Implemented measures**
This spelt variety has a significantly reduced fermentation stability and gas holding capacity.	• Use of sour doughs with stronger acidification to improve dough stability • Dough resting periods shortened • Water content was reduced to increase firmness and dryness
EM-02 / Emmer unmixed	**Implemented measures**
For Emmer variety EM-02 a limited swelling capacity was determined. The doughs were wet and slackening.	• The dough resting periods were considerably extended to increase firmness and dryness • The initial baking temperatures were increased
EK-01 / Einkorn flour-commercial sample	**Implemented measures**
The einkorn variety has a reduced fermentation stability and gas-holding capacity. The doughs were wet and slackening.	• Use of sour dough with mild acidification to improve dough stability • The dough resting periods were slightly extended for improved firmness and dryness • Water content was slightly reduced
EK-02 / Einkorn unmixed	**Implemented measures**
The einkorn variety EK-02 showed generally a reduced volume of bread.	• Use of Acerola-Cherry-Powder or mild wheat sour to improve dough stability • The initial baking temperatures were increased

Industrial scale food processing of minor cereals

Technological transfer at the industrial scale has been performed for baked goods (breads) and extruded products (pastries, breakfast cereals), focusing on rye and spelt. Technological transfer has been performed at all stages, starting with raw material and then downstream through to intermediate product prototyping and all the way to end-products. In this way the desired improved processability of the minor cereals used in the finished products with higher quality - and the throughput safety features - have been demonstrated. Good Manufacturing Practice (GMP) requirements have been applied in the production trials commonly used for safety and quality integration. The baking and extrusion technological procedures have been assessed for this purpose.

Industrial scale milling

As the most nutritional content is located in the outer layers of minor cereal grains, product development used wholemeal flours for all new formulations. The quality characteristics for food grade cereals have to meet regulatory requirements for hygiene, residues of fertilizers, pesticides and heavy metals. Analysis in HealthyMinorCereals confirmed the existing known issues for rye (ergot) and oat (Mycotoxins T1; HT2).

Before we could start with product development for baking, extrusion and pasta production trials, we had to optimize the milling process. We assessed three different milling systems: roller milling (Bühler Mahlautomat), impact milling (Mühlomat) and friction milling (Zentrofan). These were tested with different kind of sieves and also different sieve sizes in order to find the appropriate setup for milling and reach high water-absorption and good dough rheological properties with high baking quality. One other characteristic studied was the flow index of the wholegrain flour, which is important for the continuous extrusion process and the throughput.

For spelt, stone mill flour was unsuitable for extrusion and pasta production processes, due to the coarse ratio (Figure 21). It can, however, be used for bread manufacturing, if the target product is a rustic-appearance and there are no high-quality demands regarding specific volumes. For extrusion processing, the wholemeal flour should not be too fine: only fine enough to pass through the die. Coarse bran cannot be recommended because the throughput was found lower than using finer bran. Furthermore, the appearance of the final product was positively influenced by using wholemeal flour with a different particle size of bran.

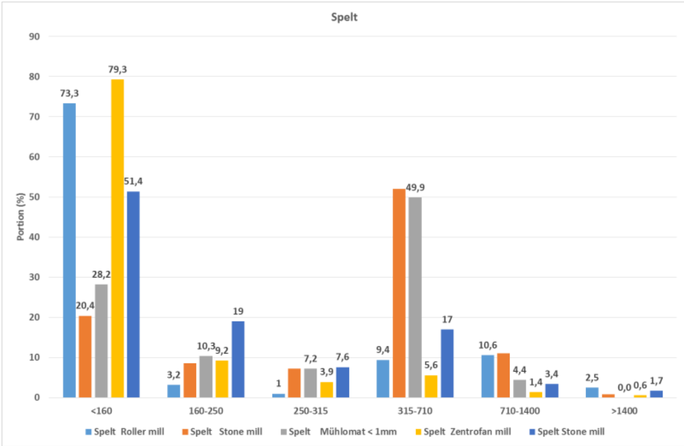


Figure 21. Spelt wholegrain flour particle size distribution from different mills

Following these trials, the use of impact mills are recommended for rye and spelt (and common wheat), since the process may be performed in one step. If roller mills are used the bran fractions become coarse, with a negative influence on the final products. However, for oat the milling process is more complicated. First, dehusking is required. The kernels were rich in fat (approx. 7%), and so the flowing-and-discharge process was difficult, and the flour was sticky, remaining on the surface of the rollers. Therefore, oat should be milled with impact mills.

Extrusion processing

After performing a comparison study on Key Performance Indicators between different types of conventional extrusion techniques (single- and twin-screw extrusion, available at BGK) and the emerging planetary roller extrusion, the latter was chosen due to several superior features. With this system, heat-sensitive materials (which must not exceed a certain temperature) can be gently treated and extremely well homogenized and dispersed. A very efficient extrusion can be realized due to the configuration of the planetary roller extruder, and in particular, the very-small-layer thickness of the raw materials which is possible in comparison to conventional extrusion. By combining with a heater, thermal energy, which is necessary for certain ingredient changes, can be effectively targeted in defined portions of the extrusion. Thus, the overall gentle character of the process is maintained and appropriate reactions can occur. Planetary roller extrusion (Figure 22) has been proven to have a 55% lower energy requirement, 12 times higher operation efficiency, and 7 times higher throughput in terms of volumes/time.

Based on a comparison of advantageous processing parameters, IGV performed extrusion trials at an industrial scale by using the planetary roller extruder so as to generate feasible samples for full characterisation. Samples of emmer, einkorn, spelt and rye (with corn as a control) have been extruded. Similarly, sourdough-facilitated, industrial, baking trials have been performed at the facilities of Stolzenberger Bakery, under real (HACCP validated) conditions.

Figure 22. Planetary roller extruder (left); central spindle (centre); extruded minor cereals (right)

Assessing nutritional content of minor cereals and their effects on human cell cultures

Leader: Sabanci University
Other partners involved: ILU

Analysis of micro and macro mineral nutrients, and bioactive compounds in minor cereals

The HealthyMinorCereals project has studied over 600 different genotypes of minor cereal species: rye (n = 54), oat (n = 200), spelt wheat (n = 200 from Austrian collection, n=100 Turkish spelts), and wild and primitive wheats including *Triticum monococcum, T. boeoticum* and several *Aegilops* species (n = 64).

For each genotype, information has been collected about their antioxidant capacity and the level of several bioactive compounds, such as β-glucan, total amount of phenolics, dietary fibre and alkylresorcinols, as well as mineral nutrients. For due comparison purposes, 12 widely-cultivated common wheat cultivars were also included in the analysis program.

The analysis has used established analytical approaches such as advanced mass spectrometry. However, for analysis of trace amounts of selenium and seleno-organic compounds, Sabanci University has received assistance from a collaborator working on specialised techniques able to detect trace quanties as described below.

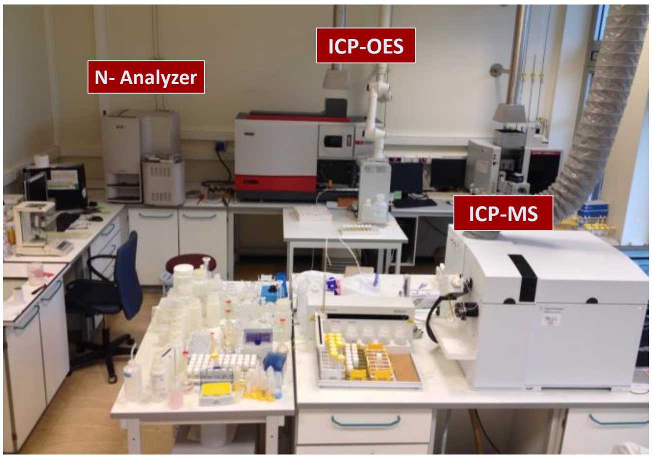


Figure 23. Equipment used for analysis of micro nutrients and N (protein) at Sabanci University

Rye and its antioxidant capacity

Rye genotypes were generally best in terms of their total antioxidant activity**.** The Trolox equivalent antioxidant assay was used to measure the total antioxidant activity of the grains, and the results obtained are presented in Table 2. Based on the average values, rye had the highest antioxidant activity while spelt from Austria and common wheat showed the lowest antioxidant activity. Higher antioxidant activity in spelt from Turkey compared to spelt from Austria indicates a role of growth conditions and environment in the levels of antioxidant potential in the seeds.

Table 2. Results of analysed concentrations of total antioxidants, phenolics, ß-glucan and dietary fibers

		Rye	Oat	Spelt (Austria)	Spelt (Turkey)	Wild wheat	Common wheat
Antioxidants (mmol/kg)	Max	23	27.8	14.3	25.4	26.5	6.45
	Min	11.1	0.89	0.63	0.8	2.95	3
	Average	**16.9**	**13.7**	**5.25**	13.2	**13**	**4.28**
Phenolics (mg/g)	Max	2.45	2.92	1.61	1.43	1.97	1.61
	Min	1.13	1.67	1.28	0.91	1.11	1.06
	Average	**1.63**	**2.01**	**1.41**	1.23	**1.36**	**1.22**
ß-Glucan (g/100 g)	Max	1.72	4.97	0.55	0.66	3.87	0.65
	Min	0.32	0.69	0.21	0.18	0.03	0.26
	Average	**0.97**	**3.2**	**0.39**	**0.35**	**1.4**	**0.45**
Dietary Fibers (w/w %)	Max	18.5	11.5	11.3		9.32	11.6
	Min	10.7	7.23	8.46		7.9	9.69
	Average	**16.3**	**8.94**	**9.34**		**8.65**	**10.8**

Rye is also rich in other highly bioactive compounds such as dietary fibre, phenolics, alkylresorcinols and phytosterols, in comparison to spelt wheat, oats and the wild and primitive wheats (Tables 2, 3, and 4).

Regarding the total amount of dietary fibres, based on the average values, oat, spelt wheat, wild wheat and common wheat had very similar concentration of dietary fibres, but rye has higher average fibre content than the other species. The genetic variation for dietary fibres was minimal within each cereal species.

Analysis of alkylresorcinols and phytosterols has been realized in collaboration with ILU.

The highest total amount of alkylresorcinols was found in rye, followed by common wheat (Table 3). Spelt wheat and wild wheat had similar total amount of alkylresorcinols. Alkylresorcinols could not be detected in the tested oat genotypes.

Selected genotypes were also studied for the concentrations of phytosterols (Table 4). Wild wheat and rye genotypes exhibited higher phytosterol concentrations than the other cereal species. Oat had the lowest phytosterol concentrations**.**

The specific nutritional relevance of these compounds will be discussed in the forthcoming publications.

Table 3. Alkylresorcinols analysis of the minor cereal species and common wheat

		Rye	Oat	Spelt (Austria)	Wild wheat	Common wheat
Campesterol (mg/100 g)	Max	4.34	0.57	2.38	4.46	4.17
	Min	3.38	0.38	2.23	3.08	1.92
	Average	**4.03**	**0.51**	**2.29**	**4.09**	**2.71**
Stigmasterol (mg/100 g)	Max	1.15	0.34	0.51	1.3	0.62
	Min	0.89	0.19	0.41	0.25	0.32
	Average	**1.03**	**0.26**	**0.46**	**0.53**	**0.48**
ß-sitosterol (mg/100 g)	Max	11	4.21	7.13	13.1	13.2
	Min	8.35	2.66	6.94	12.1	6.5
	Average	**9.81**	**3.46**	**7.03**	**12.7**	**8.42**
Total (mg/100 g)	Max	16.3	5.11	9.88	17.9	17.9
	Min	12.9	3.23	9.58	16.7	8.74
	Average	**14.9**	**4.23**	**9.79**	**17.3**	**11.6**

Table 4. Phytosterols analysis of the minor cereals species and common wheat

		Rye	Spelt (Austria)	Wild wheat	Common wheat
C15:0 (mg/100 g)	Max	0.47	0.07	0.08	0.08
	Min	0.36	0.05	0.01	0.04
	Average	**0.43**	**0.06**	**0.03**	**0.06**
C17:0 (mg/100 g)	Max	6.43	1.15	3.85	1.61
	Min	5.57	0.94	0.07	1.29
	Average	**6.21**	**1.03**	**1.04**	**1.46**
C19:0 (mg/100 g)	Max	8.41	7.03	9.96	9.04
	Min	7.34	6.31	1.82	7.87
	Average	**7.78**	**6.68**	**4.13**	**8.52**
C21:0 (mg/100 g)	Max	6.94	11.9	9.35	12.6
	Min	5.16	10.2	8.16	11.5
	Average	**5.72**	**11.3**	**8.88**	**12.1**
C23:0 (mg/100 g)	Max	3.24	3.36	6.78	3.53
	Min	2.41	2.68	1.74	3.07
	Average	**2.74**	**2.89**	**5.43**	**3.27**
C25:0 (mg/100 g)	Max	3.25	1.41	3.37	1.4
	Min	2.26	0.91	0.65	1.06
	Average	**2.84**	**1.09**	**2.55**	**1.26**
Total (mg/100 g)	Max	35.2	25.7	26.3	28.7
	Min	30.5	23.3	21.9	26.1
	Average	**32.2**	**24.2**	**23.6**	**27.8**

Grain zinc (Zn), iron (Fe) and protein

Among all tested cereal species, spelt from Turkey had the highest average grain Zn concentration (58.2 mg kg^{-1}), while rye contained the lowest amount of Zn (25.3 mg kg^{-1}) (Table 5). Common wheat had also low grain Zn concentration (30.3 mg kg^{-1}). Oat, wild wheat and spelt wheat from Austria were similar in average grain Zn concentrations (i.e., around 38 mg kg^{-1}). Among all cereal species studied, common wheat showed much less genotypic variation for grain Zn compared to other species, probably due to lower number of genotypes studied. The largest genetic variation for grain Zn (almost 3-fold) was found in oat and wild wheat. Similar to Zn, rye had also the lowest Fe concentrations based on the average, max and min values. The highest Fe concentrations were found in wild wheat and spelt wheat from Turkey. The largest genotypic variation for grain Fe (about 3-fold) was found in spelt wheat from Turkey. Rye and common wheat had similar lower average values of Fe.

Spelt wheats from Austria and Turkey and wild wheat had higher average protein concentrations than common wheat, oat and rye. Rye was the cereal species having the lowest protein concentrations. However, rye together with wild wheat showed the largest variation in grain protein concentration (over 2-fold). There were several rye genotypes containing below 10 % of protein in grain, while a large number of genotypes of spelt and wild wheat were found to contain more than 20% protein.

Table 5. Zinc, iron, selenium and protein concentrations of minor cereal species and common wheat

		Rye	Oat	Spelt (Austria)	Spelt (Turkey)	Wild wheat	Common wheat
Zn (mg kg^{-1})	Max	35.4	63	49	82.4	63.2	34.9
	Min	19.5	22.2	28.8	37.4	23.8	27.2
	Average	**25.3**	**37.5**	**38.1**	**58.2**	**37.8**	**30.3**
Fe (mg kg^{-1})	Max	50.2	87.5	67.7	94.6	93.4	65.1
	Min	24.9	40	32.5	30.5	37.9	31.2
	Average	**34.3**	**56.2**	**44.9**	**56.1**	**57.3**	**38.1**
Protein (%)	Max	16.2	21.1	21	24.9	28.9	15.3
	Min	7.6	12.6	14.5	14.9	12.8	11.1
	Average	**11.7**	**15.8**	**17.7**	**19.4**	**19.3**	**13.7**
Se (µg kg^{-1})*	Max	66.5	30	81.2	93.9	87.9	161.1
	Min	≤10	≤10	≤10	≤10	≤10	16.9
	Average	**33.2**	**14.4**	**35.9**	**39.1**	**38.5**	**52.7**
SeM (µg kg^{-1})**	Max	43	29.9	1.83	nd	61	56.8
	Min	15	25.4	18	nd	20	22.4
	Average	**27.2**	**27.2**	**25.6**	**nd**	**30.8**	**39.3**

*measured by ICP-MS; **measured by ICP-MS-HPLC

Selenium (Se) is too low in cereals

As shown in Table 5**,** one of the interesting results of the HealthyMinorCereals project is the extremely low concentrations of selenium (Se) found in almost all samples analyzed by using ICP-MS (Inductively Coupled Plasma – Mass Spectrometer). The average Se values were between 30 to 40 µg kg^{-1} in seeds of spelt wheat, rye, and wild and primitive wheats, and about 15 µg kg^{-1} in oats. These values are too low and below the critical lowest optimum Se concentrations (100 µg kg^{-1}). In USA and Canada, average wheat seed Se concentrations are between 370 and 760 µg kg^{-1}, respectively (*Broadley et al., 2006, Proc. Nutr. Soc. 65: 169-181*). Probably, these low levels of Se in the seeds reflect the low levels of available Se in the European soils where these plants were grown.

Measurements of the major selenoprotein of cereal grains, selenomethionine (SeMet), were done by combining ICP-MS with High Performance Liquid Chromatography in collaboration with Pau University in France. Similarly, selenomethionine (SeMet), was also also found to be very low. It is clear that the minor cereal species, as well as the common wheat genotypes, tested contain very low amounts of total Se or SeMet, that are insufficient for human nutrition.

Association between zinc, iron and protein

The intensive chemical analysis on minor cereals indicated the existence of strong positive correlations between protein, Zn, and Fe content (usually over +0.5), and these very positive correlations were found consistently in rye, oat and spelt. From previous studies it is known that Zn, Fe and protein correlate with each other in different plant genotypes. High protein in seeds is believed to create a “sink” for Zn and Fe.

Human cell culture assays to investigate biological activities of minor cereals

Sabanci University has also studied minor cereal species (20 genotypes each) for their antioxidative effects in cell biological studies, and the effects were compared with the effects of 12 common wheat varieties. Ethanol soluble extracts of seeds were used and examined in human cancer-derived cell lines including HeLa and HCT116, by analyzing cellular levels of reactive oxygen species, mitochondrial health, DNA damage and cell survival. In the assays, N-acetylcysteine (NAC), a commonly used antioxidant molecule, was included as positive control and its value is given relative to 100 (the dashed red lines in Figure 24).

In good agreement with the total antioxidant results (Table 2), the seed extracts of the minor cereals exhibited superior antioxidant activity against oxidative stress in human epithelial cells, when compared to common wheat varieties. When compared to common wheat, almost all genotypes of the minor cereal species tested exhibited superior antioxidant activity in HeLa cells treated with hydrogen peroxide. Many genotypes of minor cereal species showed antioxidant capacity similar to NAC, while no common wheat variety was found having similar or better antioxidant activity. In the case of oat, 4-5 genotypes had poor antioxidant activity when compared to NAC. However, it is important to highlight that only 12 common wheat genotypes were used in these comparative studies. One plausible explanation might be the loss of alleles during intensive breeding that has affected the antioxidant traits in common wheat seeds. The dilution in common wheat of their antioxidant pools through their high grain yield capacity might be a further reason for their low antioxidant capacity. It is, however, important to mention that rye has also a high and very comparable grain yield to common wheats. Nevertheless, rye was the best in terms of total antioxidative capacity.

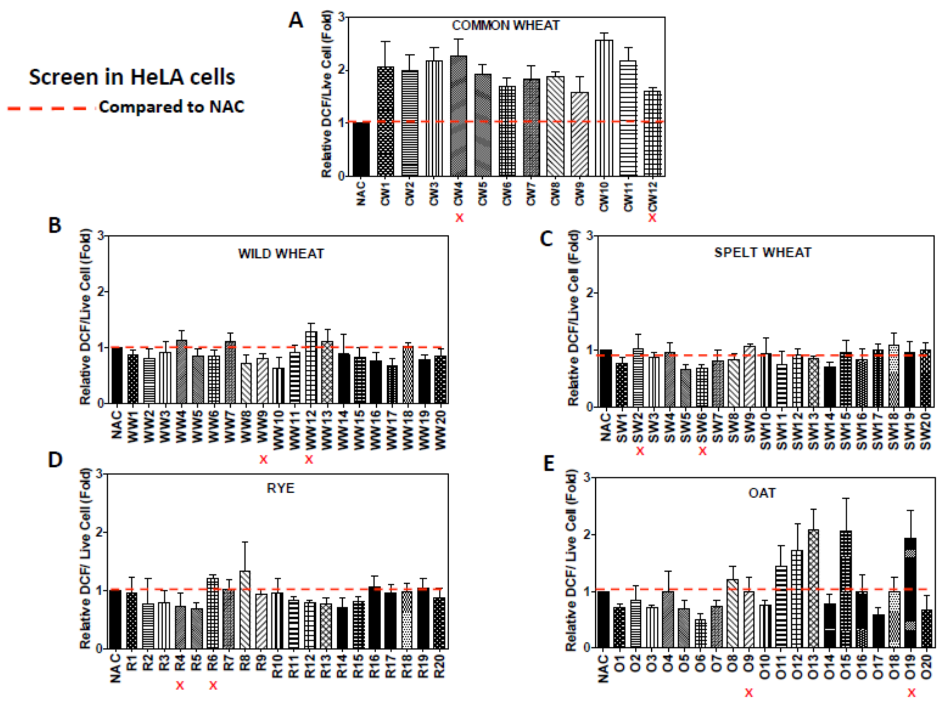


Figure 24. Effect of ethanolic seed extracts on generation of reactive oxygen species (ROS)

Enhancing the market prominence for minor cereals

Leader: Research Institute of Organic Agriculture (FiBL)
Other partners involved: ÖMKi, PRO-BIO, and all partners during consortium discussions

Literature review

In the first year of the project, FiBL studied the market potential of minor cereal crops and consumers trends, especially linked to cereals. The study included the analysis of national statistics on MC crop production, identification of market initiatives already existing for minor cereals and a literature review about consumer trends. It was found that the European cereal market is saturated and looks for innovation and market segmentation, and that there are some consumer trends in support of minor cereals, e.g. health, enjoyment, trust, environmental concerns and lifestyle. Some examples:

- Minor cereal products might be successful on the market when processed in whole grain quality and marketed as healthy and nutritional for consumers interested in issues as health and nutrition.
- Consumers might not be aware about the health benefits of minor cereals like rye or oats.
- European consumers link healthiness to naturalness and a low degree of processing.
- Taste and price are important factors that influence consumers' willingness to buy functional food. Health claims alone do not convince consumers.
- The demand for gluten-free products is increasing as the awareness of coeliac disease is rising.
- When minor cereal products are marketed by using designations of origin - linked to the region-trend - it is important to refer to a small, precisely defined regions.
- Tradition (history, folklore, region) might be a powerful attribute for spelt or emmer marketing.
- In the traditional food sector, consumers accept innovations only to a certain extent.

The report is published and available from the project website and the document is available via the FiBL repository organic e prints (www.orgeprints.org).

Case studies on best practice examples

A case study approach was then used to investigate examples of introduction of spelt, oat, rye but also einkorn or emmer and products thereof into the market. A case study questionnaire was developed covering the environmental, legal, technical, economic and social aspects of the selected cereal marketing initiatives.

The selected cases were

- Czech Republic: spelt and naked oat market, with a special focus on *PROBIO*, a trader of organic cereals, especially spelt and *Mix it company,* young entrepreneurs who run an online shop for muesli with speciality naked oats (*Avena nuda*)
- Switzerland: spelt and cereal market, with a special focus on IG Dinkel, the most important Swiss spelt trader and owner of the label PureSpelt (Urdinkel), and the cooperative Gran Alpin, a cereal supply chain from the Alpine region of Grisons.
- Hungary: Emmer and einkorn market with a special focus on *Naturgold farms*, a producer and trader of organic einkorn and emmer
- Estonia: oat and spelt market with a special focus on *Viru Wili*, a farmers cooperative and exporter of organic cereals, mainly oats

Figure 25. Minor cereals products from enterprises examined in the Swiss case studies

The market potential for minor cereals in Europe

The results from the case studies were compared in order to develop conclusions for the marketing of minor cereals. It was concluded that the main market potential was in the niche markets. Niche markets are built on long-term relationships, a special product quality and a strong consumer orientation. Niche marketing strategies do not only concentrate on local or short supply chains. A market niche can also be identified abroad, leading to the export of high-quality products.

From the work done during the HealthyMinorCereals project, the following factors in support of, or inhibiting of the market development of minor cereals and their products were identified:

- Factors supporting minor cereal market development
  - *Use of existing networks and links to different actors and stakeholders*
  - *Focus on niche markets*
  - *Skills for the market planning e.g. balance supply and demand*
  - *Good management skills e.g. transparency, communication, investment in trust and product quality*
  - *Combination of different fields of knowledge and expertise*
  - *Availability of adapted local structures to handle usually smaller quantities of produce*
  - *Shared values across supply chain partners*
  - *Integrate the old traditional knowledge with the new*
  - *Link up with well-established labels (e.g. organic, locally-produced, PDO-Protected Designation of Origin[1])*
- Factors inhibiting minor cereal market development
  - *Lack of knowledge concerning the benefits of minor cereals along the cereal supply chain and among consumers*
  - *Farmers unfamiliar with the crops and needing support linked to production*
  - *Lack of skills in special processing techniques, e.g. hulled wheat (spelt, einkorn, emmer) need de-husking technologies, and need for non-standard industrial processing, baking and brewing*
  - *Access denied to investments for developing the supply chain logistics and infrastructure*
  - *Lack of access to seeds*
  - *Lack of specific policy support for minor cereal production (but specific suport for other crops e.g maize for biofuel production)*

[1] Through the EU quality schemes, such as the PDO logo, consumers can easily recognise the regional origin of a product. Registration provides producers with legal protection against imitation or misuse of the product name. https://ec.europa.eu/agriculture/quality/schemes_en

Recommendations how to increase the market potential of minor cereals

By identifying successful market initiatives involved in the trading of minor cereal crops, we have developed recommendations for minor cereal marketing. The full report is published and available from the project website and the document is available via the FiBL repository organic e prints (www.orgeprints.org).

To further investigate the market potential of minor cereals, an online consumer survey was conducted in Germany, UK, Estonia, Czech Republic and Hungary. The report is shared with the partners in the project and, after publication, will be uploaded on orgeprints.org

The survey confirmed some previous findings, e.g. from a set of eleven potential drivers for willingness to pay more, 'healthier' is the most important one in all five countries. In addition, 'more fibre', 'better taste', 'food diversity' and 'fewer synthetic additives' count among the most important drivers in all five countries. One driver that is only important in the Czech Republic and Hungary is 'easier to digest'. Another driver that is only important in Germany, Estonia and the UK is 'support of smallholder farmers', while 'handmade' and 'biodiversity' are only important in Germany. Besides the topic 'support of smallholder farmers', this all confirms the trends identified already in the literature survey and case studies.

For the marketing, health attributes of rye, oats or spelt could be relevant. Regarding health, the following health claims linked to rye and oats are approved based on EU 432/2012:

- EU EFSA 2009; 7(9):1254; 2011; 9(6):2207 - Cholesterol: "Oat beta-glucan has been shown to lower/reduce blood cholesterol. Blood cholesterol lowering may reduce the risk of heart disease."
- EU EFSA 2011; 9(6):2207 - Blood glucose: "Consumption of beta-glucans from oats contributes to the reduction of the glucose rise after a meal."
- EU EFSA 2011; 9(6):2249 - Fibre: "Oat grain fibre contributes to an increase in faecal bulk."
- EU EFSA Journal 2011; 9(6): - Fibre: "Rye fibre contributes to normal bowel function"

However, health and nutritional claims on products should be used carefully and in accordance with European Union recommendations. In our research, regional or local production was identified as a consumer trend. The survey showed, that "domestic production" is not really important for consumers in UK, Germany, Czech Republic, and Hungary, but is important for Estonia.

Generally, we found no (or just a small) willingness to pay for products made of spelt, rye and oats. From a set of seven potential barriers for willingness to pay more, 'no interest' is among the most important ones in all five countries, 'lack of knowledge' is relevant in Czech Republic Estonia, Hungary and the UK, and 'no added value' is relevant in Germany, Estonia and the UK. Generally, in was found that Estonia and the UK follow a somewhat different pattern than Czech Republic, Germany and Hungary regarding knowledge. 'Good taste' and 'fresh' are significantly important attributes in all five countries.

From the data, we can conclude that increasing awareness is a key requirement, potentially in combination with the promotion of alternative purchase channels for grain-based products like bakeries. If possible, arguments referring to *health, fibre content, taste, food diversity* and *no synthetic additives* should be used in the promotion of products from HealthyMinorCereals. In Estonia, Germany and the UK the *support of smallholder farmers* could also be an important aspect in the communication with consumers.

Potential impact, dissemination and exploitation

Much of the potential impact of HealthyMinorCereals will depend on further work by the partners and users of the results outside the consortium, with the benefits being realised as much as 10 years ahead.

Impacts for breeders and gene banks– better characterised breeding material

The genotypes studied in HealthyMinorCereals are mostly not suited to commercial cultivation. For example, many landraces experience a high rate of lodging due to their height, and would not be approved for official registration as commercial varieties today.

Figure 26. Lodging of spelt landraces (Reine Koppel, ETKI)

However, the results of the phenotypic analysis, and also the genetic analysis, will be used in breeding programmes for minor cereals that can incorporate useful traits from genotypes into commercial varieties. The main target so far for breeding activities are the disease resistance traits identified in specific genotypes.

The analysis of the genetic relationships of the genotypes of oat, rye and spelt wheat will help breeders to make decisions about where best to look for crossing partners, while the results for wheat wild relatives suggest which resources are more suitable for use in breeding. The results of the genetic characterisation obtained by University of Kassel for the spelt collection will also help breeders to select accessions to use as parents for crosses and to develop markers for the selection of the respective loci associated to disease resistance. Sequence analysis shows the development and diversity of these loci and thereby the respective resistances against rusts and powdery mildew in spelt wheat.

The nutritional analysis performed in HealthyMinorCereals also suggests that breeders should pay particular attention to the highly-positive association among Zn, Fe and protein in their selection and breeding studies. Consuming protein-enriched cereals might be associated with a high intake of Zn and Fe. Today, the 'hidden hunger' problem (i.e. a deficiency of micronutrients, including Zn and Fe) is a growing health concern, particularly in children in the developing world, but also in many parts of Europe. Biofortification of food crops with Zn and Fe through a strategy of plant breeding and fertilizers is a widely-accepted solution to the hidden hunger problem. This close association between protein, Zn and Fe should be considered and integrated into the on-going agronomic and genetic biofortification efforts. Furthermore, the results of the nutritional analysis highlighted in particular the low Se levels in the minor cereal grains, as found in other cereals grown in Europe where soils are deficient in Se compared to, for example North America. This finding highlights the importance of enrichment (biofortification) with Se for cereals grown in Europe. Enrichment of Se could either be through plant breeding or fertilizer strategies. However, published data indicates insufficient genetic variability in seed Se concentrations for a successful breeding program.

Several of the HealthyMinorCereals partners are actively involved in breeding and have already initiated the development of new varieties based on the results of the project. F1 and F2 generations have so far been obtained and over 400 crosses are currently being evaluated. The breeding process for cereals generally takes around 10-12 years, so we expect new varieties to be introduced to the market in the early 2030s. Other breeding companies will equally be able to access the results and benefit from the data generated by the project.

Figure 27. The creation of new variability is essential in the breeding of new varieties. This is achieved by crossing which starts with the emasculation (removal of anthers) of the seed parent (mother line) before flowering to prevent self-pollination (left). The emasculated spike is protected by a bag to avoid unwanted cross pollination. A few days after emasculation the spike of the seed parent is pollinated by approaching a flowering spike of the pollinator (father line) under the bag (centre). To provide enough pollen, more than one spike can be used for pollination. The pollinated spikes are labelled with the cross combination and date of pollination (right). About 7 to 10 days after pollination, grain development in successfully pollinated florets becomes apparent. Cross combinations which were not successful can be repeated.

Exploitation of results

- Phenotypic and genetic analysis data will be published and available for gene banks and breeders. Gene banks generally record details of publications related to their genotypes, and so the availability of the data will be clearly visible for anyone accessing the material.
- **GZPK** is using the project results in its spelt breeding programme, focusing mainly on improving disease resistance. However, they are also investigating the diversification of physical appearance, as the spelt genotypes studied in HealthyMinorCereals are more diverse in colour and general appearance than current commercial varieties. This could be an interesting approach for marketing of varieties in future.
- **Selgen** are working with the varieties Dumont and AC Preaknes, aiming to improve resistance of oats.
- **ETKI** has initiated the development of new rye crosses and has initiated spelt breeding in Estonia for the first time. Spelt has not been previously cultivated in Estonia.
- **CRI** is using the results to breed new lines of disease resistant spelt, and wild wheat relatives for future genetic studies.
- While several genotypes with interesting disease resistance traits were identified, there is still scope for screening further genotypes from around the world. Further studies could also extend the approach to other important crop diseases. For example, loose smut is an important crop disease affecting organic farming and there is no information on genetic diversity of resistance in spelt.

Impacts for farmers – benefit of new minor cereal varieties and agronomic practices

There is tremendous scope for HealthyMinorCereals to improve the selection of crop varieties available to farmers in Europe. The range of minor cereals varieties available to farmers has so far been poor, as little as one variety per species in some countries. Diversity of available varieties for any crop species is essential for farmers to adapt to changing climate and crop disease pressures, by switching to varieties that have higher resistance/tolerance to specific diseases. Eventually, exploitation of the results of HealthyMinorCereals by breeders (see above) will see a greater number of varieties available especially for spelt, oat and rye. Greater variety diversity also allows farmers to exploit different markets based on the grain quality (bread, other foods, brewing etc). Minor cereals are particularly suited to sustainable, low input agriculture, and for marketing purposes are mainly associated among consumers with organic production, local economies and regional identities.

Figure 28. HealthyMinor Cereals filming the cultivation of einkorn in the region of Kastamonu, Turkey

The results of HealthyMinorCereals will be of particular interest to organic farmers, and to farming enterprises located in remote and marginal lands, for example mountainous and arid regions. Rye, in particular is already reported in the literature to be well-suited for cultivation in marginal areas affected by heat stress, frost damage, and where soils are deficient in micronutrients such as zinc, or have concentrations of elements such as aluminium that normally affect crop productivity. Especially considering also its high nutritional value as confirmed in HealthyMinorCereals, rye could be an important crop in the future when facing global challenges of climate change and increasing demands for food production.

Farmers may also benefit from the marketing strategies analysed by HealthyMinorCereals, working with food producers in local networks and often engaged themselves in milling and food production. In principle, minor cereals could be an attractive business proposition for farmers, as an alternative to major cereal crops, depending on the overall conditions of the market and levels of demand. With the exception of Austria and Switzerland, there are no national policies or subsidies in EU countries to encourage the production of underutilized or heritage crop varieties. In fact some countries have stopped their previous incentives. A suitably designed policy could stimulate a positive feedback cycle between production, availability of minor cereals-based foods, and consumer demand.

HealthyMinorCereals has investigated several innovative agronomic strategies for improving the cultivation of minor cereals, generating data in contrasting climates and soil types. The results of these field trials illustrated the wide range of differences among minor cereals varieties, showing again the value of increasing variety diversity for farmers.

The most interesting results based on analysis of field trials completed to date concern the application of digestate from biogas production, which has been found to give similar, or in some trials even higher yields compared to conventional artificial nitrogen fertilizer. This result could lead to eventual economic benefits to farmers. Inadequate nutrient supply is a major factor contributing to the yield gap between conventional and organic crop production systems. This is largely attributed to a reduced N availability especially during the critical late seed/grain filling phase in organic systems. The synchronisation of nitrogen supply with crop demand is a major challenge particularly in organic production systems, which is compounded by the fact that modern cereal cultivars are bred and selected for in response to high quantities of mineral N supplied as artificial fertilizer (e.g. ammonium nitrate).

Figure 29 Biogas digestate application in HealthyMinorCereals field trials

Organic farmers could therefore use biogas digestate as a new source of nitrogen to increase crop growth and protein content to boost crop yield and quality, and hence their profits. They may apply for a derogation from their organic certification body, if use of biogas digestate is not already specifically permitted. Conventional farmers could switch part of their fertilizer inputs to biogas digestate without losing yield and quality, and this is usually cheaper than mineral N fertilizer. In principle, a greater use of digestate has environmental sustainability benefits, using waste-derived materials rather than natural gas-based artificial fertilizers. However, there remain some practical challenges for the use of digestate as fertilizer in general, in particular difficulties in transporting a high volume low nutrient content product over larger distances and variability in its quality based on the original feedstocks and processing conditions used.

In addition the results from studies of varietal mixtures confirm that this approach is relevant for minor cereals to produce a greater yield consistency and reduced levels of disease.

Exploitation of results

- Farmers will have access to new varieties developed by breeders under normal commercial conditions
- Results from the field trials will be published in peer-reviewed journals by Newcastle University. Data will be freely available for use by farmers and researchers, including yield, grain analysis and application protocols.
- HealthyMinorCereals partners PROBIO, Gilchesters and Geokomi are directly involved in farming and may in future benefit from these results in their farming operations.

Impacts for food producers – optimised food production and new recipes for minor cereals

While major food companies are selling already breakfast cereals or bread with a small percentage of spelt or other minor cereals, the majority of companies already active in the minor cereals food market are SMEs, because it is still largely a niche, specialist and low volume market. The results related to minor cereal food processing are therefore mainly of interest to SMEs in the baking, processed foods and specialist nutrition sectors, as well as millers selling minor cereal flours directly to consumers.

The partners involved in the food production-related research have generated new data and knowhow concerning how best to process minor cereals grains.

- Data has been obtained on the loss of nutritional quality during processing, with some knowhow developed on how to minimise such losses.
- Milling and extrusion processes have been optimised for minor cereals, of particular relevance for production of breakfast cereals, pasta and novel foods. Planetary roller extrusion has been demonstrated to significantly reduce energy requirements
- Specific modifications to sourdough production have been proposed to optimise baking quality for spelt, rye, einkorn and emmer, as well as optimisation of industrial-scale baking. These innovations are already being implemented by our partner Stolzenberger's Bakery.

Figure 29. New emmer-einkorn mixed products developed by Stolzenberger's Bakery

For exploitation beyond the consortium, partners ILU and IGV have planned to communicate the results to the food industry in Germany and surrounding countries and will transfer the knowhow to companies under commercial agreements.

Also, SMEs in the food industry can benefit from the recommendations to increase the market potential of minor cereal-based foods with the nutritional data being of particular value in marketing and labelling. During the project, Gilchesters Organics Ltd was able to evaluate the market for minor cereal grains, through their customer base of domestic and commercial customers. Small scale artisan bakeries which expressed an interest in spelt and rye at the beginning of the project have now established bread products from both wholemeal flours. Two medium scale London-based bakery customers have demonstrated significant increases in sales of rye products in the past few years. Gilchesters' sales of spelt and rye flour to domestic customers have also increased. The inclusion of nutritional data from the project in product information, particularly for rye, has been influential on customer choice. Use of emmer and einkorn flours has also increased in the past two years. These grains are little known in the UK, and are now being milled and sold by Gilchesters to both commercial bakeries and home bakers.

Exploitation of results

- Stolzenberger's Bakery is implementing new recipes for minor cereals bakery products in its range, including new products using spelt, rye, emmer and einkorn flours
- New products launched by Gilchesters Organics are wholegrains of spelt, rye, emmer and einkorn, whole rye flour, with a heritage flour blend of spelt, emmer and einkorn still to be launched.
- IGV and ILU will be organising workshops, baking trials and other engagement with SMEs to transfer knowhow to food industry SMEs beyond the consortium. They expect to involve at least 50-75 companies.
- IGV will exploit knowhow developed in HealthyMinorCereals in its own novel foods development programme.
- Use of nutritional data in marketing activities by food enterprises developing minor cereals products

Figure 30. The new range of spelt, emmer, einkorn and rye products available from Gilchesters Organics flour mill in the UK

Impacts for consumers – higher nutritional value of minor cereals

Results of the comprehensive analysis of nutritional content of minor cereals genotypes at Sabanci University have clearly demonstrated that:

- Minor cereals in general have higher levels of important biologically active compounds, compared to common wheat. Rye stands out as being consistently high in most nutrients studied. Further research is also required to investigate the physiological and genetic factors behind the differences in antioxidant capacity between common wheat and minor cereals. It is known that most of the antioxidants existing in cereal grains are concentrated in the coat and bran parts of seeds, so it could be inteersting to measure concentrations of antioxidants in the bran, embryo and endosperm parts.

- Biological human cell assays have demonstrated higher overall antioxidant activities of minor cereal seed extracts in comparison to common wheat. These results suggest that increasing consumption of minor cereals (particularly rye), could have beneficial effects for human health, though much more research is required to understand the potential benefits and mechanisms involved.

European laws do not allow food producers to claim specific benefits to health from certain foods, for example a certain food product or ingredient may protect against heart disease or cancer. But we can certainly state that in comparison to common wheat, minor cereals often have a higher content of individual nutrients that are known to be important for health, such as iron, zinc, antioxidant compounds or beta-glucans.

Figure 31. Flours obtained from minor cereal grains (left to right: rye, spelt, emmer and einkorn)

Given the standout results from the analysis of rye, a clear message may be to "**eat more rye**". Considering its superior nutritional value and wide adaptation ability to diverse marginal soil and adverse climatic conditions it is recommended that rye cultivation and consumption should be extended significantly. Oat and spelt also have a higher content of specific nutrients and total antioxidant activities, compared with common wheat.

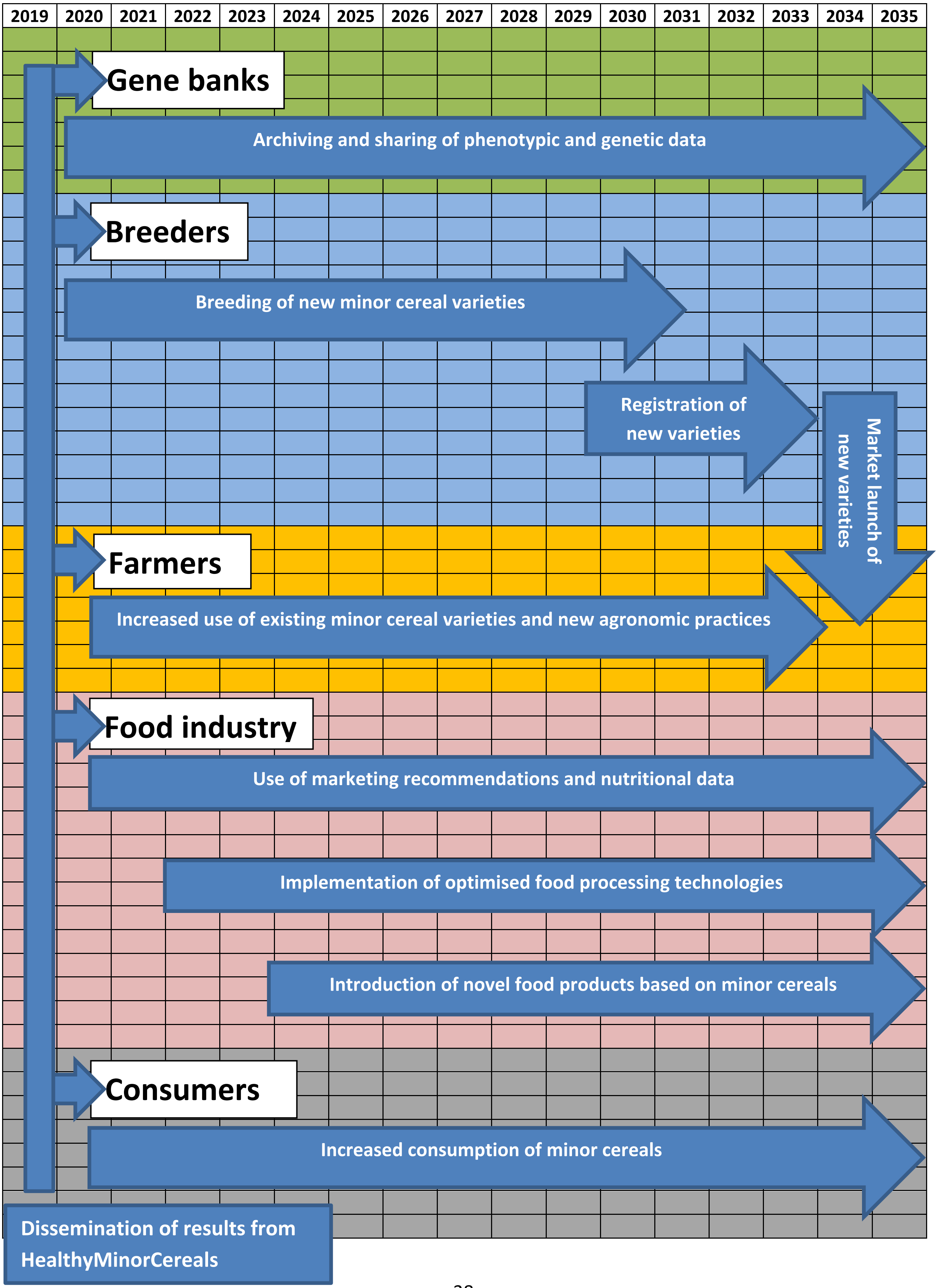
Depending on the country or region, several minor cereals are often readily available in the market, often being part of traditional diets, for example as ryebread or porridge oats, or as minor cereal flours for home baking. A greater interest among consumers for existing minor cereals food products can be generated by communicating information about the nutritional content as well as established evidence connecting the specific nutrients to health benefits. However, only a certain proportion of consumers may be reached in this way. These are consumer groups that may already be including minor cereals products in their diet, that already like these foods, and that usually have an existing strong interest in food that is healthy, regionally-sourced or organic. Other specialist consumer groups include those interested in vegan diets or sports nutrition.

A wider group of consumers are perhaps less interested or even not aware of minor cereals, and may face other barriers in the way of a purchasing decision, such as cost, or convenience. Here also, more information about the nutritional content and cultural aspect of minor cereals as "ancient" or "traditional" grains may encourage more consumer interest, but the barriers to purchase decisions also need to be overcome by developing new products, such as novel convenience foods. Blends of minor cereals with major cereals in baked goods, breakfast cereals, pasta and other foods can also be a way to increase minor cereals consumption in a way affordable for the majority of consumers.

Exploitation of results

- Research findings on the nutritional and antioxidant activities of minor cereals are being communicated to consumers and policymakers. These should encourage consumers to buy more minor-cereal based foods that are already on the market, or that will be introduced to the market in the coming years.
- Further research can investigate in more detail how bound nutrients in minor cereal grains may be released. It has been already suggested that cereals are an underestimated source of nutritional compounds, given that many nutrients exist in bound forms that are not often detected by current analytical methods, but that may still have a nutritional benefit in the human body. Research is also needed to understand better the effects of food processing on these bound forms of nutrients.

HealthyMinorCereals use of results



Impact for European cooperation and a multi-actor approach

HealthyMinorCereals has involved contributions from over 160 people, from 17 organisations located across 10 countries. Meetings of the consortium have been held in Prague, Budapest, Potsdam, Crete and Estonia.

The HealthyMinorCereals consortium has shown how a multi-actor approach can really benefit how research is conducted. As well as research organisations and universities, our consortium has involved:

- Integrated farming/food production enterprises (PRO-BIO, Gilchesters, Nikolaos Volkakis/Geokomi)
- An artisan bakery (Stolzenberger's Bakery)
- A breakfast cereals manufacturer (BGK)
- Breeders (Selgen, GZPK)

These industry partners (all small or medium-sized enterprises) have contributed significantly in the various parts of the project, for example working closely with academic partners to conduct experimental trials, develop new products and processes, and evaluating results. Also, our project meetings have allowed for interaction across the consortium, with contributions from all partners to the discussion of results. In particular, results from work by FiBL on enhancing the market potential have been discussed in detail in interactive sessions during project meetings, involving input from the different kinds of organisations represented in the project.

"HealthyMinorCereals is probably the most fruitful of the 3 EU projects that Gilchesters have participated in. We've developed new connections with breeders and processors working in minor cereals"

Andrew Wilkinson, Gilchesters Organics Ltd

The research has also involved cooperation with companies and farmers outside the consortium, for example through the farmer participatory field trials and the case studies of enterprises that have successfully introduced minor cereal products to the market.

Figure 32. HealthyMinorCereals consortium at Silvas, Crete, May 2017

HealthyMinorCereals has built new lasting links between groups and companies working on minor cereals in Europe. Some additional research collaboration has already been organised, with a study on the use of NIR spectroscopy to analyse minor cereals involving SU and OMKi, while six HealthyMinorCereals partners are collaborating in ECOBREED, a new Horizon 2020 consortium working on breeding of major organic crops that is running 2018-2023. Some of the HealthyMinorCereals data will be useful also for organic wheat breeding.

Dissemination and communication activities

HealthyMinorCereals has organised an ambitious programme of dissemination and communication activities during its 5 years to raise awareness of the project and its results.

The **project website** is http://healthyminorcereals.eu and the **project twitter account** is @MinorCereals_eu. Both will be maintained for the next 5 years.

Newsletters have been prepared around once a year and are published on the project website.

The project launch in 2013 was **covered on Czech television**. Several videos have been produced during the project. Finally, a **30 minute documentary film** was commissioned in the last year of the project, featuring work at several partners around Europe. This will be first shown at a workshop on wheat in Prague in December 2018, and then available on the project website and YouTube.

The project has been promoted through annual **open days and field days** organised by various partners, interacting with breeders and seed companies, farmers, food companies and the general public. The project was also represented at **major exhibitions** such as Biofach by our industry partners.

HealthyMinorCereals has been featured in several international publications, with a series of articles in Open Government (particularly for policymakers), the Science Impact magazine, and the EU CORDIS website.

Figure 33. Open day organised by CRI

Research publications are in preparation for submission to peer reviewed journals. We eventually expect **at least 15-20 publications** in peer reviewed journals. Already published or in press are:

- Chrpová J, Dumalasová V, Hanzalová A, Janovská D. 2017. Resistance of spelt wheat against fungal diseases. Úroda 65(5): 23 – 26 (in Czech language)
- Chrpová J, Štěrbová L, Trávníčková M, Palicová, Janovská D. 2018. Resistance of spelt wheat against fungal Fusarium head blight. Úroda, in press (in Czech language)

Oral and poster presentations have also been given at several conferences, for example:

- Market potential of minor cereal crops and consumers perceptions about them in different European regions; presented by FIBL at the 20 IGV scientific conference on "Healthy grain for a healthy diet" in Potsdam, Germany, on April 23, 2015.
- First results on rye and oat fertilization input type and level; oral presentation by ETKI at the conference "Cereal varieties, agro-technology and fertilization", Põltsamaa, Estonia, 23 February 2016.
- Presentation on the nutritional value of minor cereals presented by Sabanci University at the 15th International Cereal and Bread Congress, Istanbul, Turkey, 18-21 April 2016.
- Dumalasová and Bartoš: Reaction of winter wheat and spelt wheat genotypes to common bunt and dwarf bunt, at the XIX International Workshop on Smuts and Bunts, Izmir, Turkey, 3-6 May 2016.
- Leišová-Svobodová et al.: Diversity of oat genetic resources, at the international conference Oats 2016, Russia, St. Petersburg, 11-15 July 2016.
- Lyubenova et al: Antioxidant capacity of selected European oat genotypes presented at the international conference Oats 2016, Russia, St. Petersburg, 11-15 July 2016.
- Poster presentation by Vavera, R., Káš, M., Eiseltová, M. & Janovská, D.: Effects of contrasting genotypes, legume intercrops and fertiliser input types on performance of oat in organic farming, 3rd International Congress Agrobiodiversity, Novi Sad, Serbia, 1-3 June 2017.
- Dumalasová, V., Hanzalová, A., Bartoš, P., Chrpová, J. 2017. Evaluation of resistance against common bunt in spelt wheat 67. Tagung BOKU-University of Natural Resources and Life Sciences, Vienna, 41 – 45.

In collaboration with several other related EU projects (LIVESEED, DIVERSIFy, ReMIX, Wheatamix, INSUSFAR and ECO-PB) HealthyMinorCereals organised an **international symposium** at the EUCARPIA conference on organic and low-input agriculture, University of Kassel, in February 2018. HealthyMinorCereals partners presented results from the genetic and phenotypic analyses and human cell antioxidant studies:

- D. Janovská, M. Hutař, V. Chour, *et al.* HealthyMinorCereals: An integrated approach to diversify the genetic base, improve stress resistance, agronomic management and nutritional / processing quality of minor cereal crops for human nutrition in Europe (oral presentation)
- Dumalasova V, Buerstmayr H, Cuendet C, Dell'Avo F, Eshonkulov B, Grausgruber H, Koppel R, Muellner AE, Weyermann V, Janovska D. Disease resistance of spelt wheat - results from the HealthyMinorCereals project (oral presentation)
- Akkoc Y, Lyubenova L, Cakmak I, Grausgruber H, Janovská D, Gozuacik D. Minor cereals exhibit superior antioxidative effect in human cancer cell lines compared to common wheat (poster presentation)
- Kreps V, Dennenmoser D, and Backes G. Genotypic characterisation of spelt (*Triticum spelta* L.) (poster presentation)

In 2018 HealthyMinorCereals was honoured to be invited to two events organised by the European Commission (see Figure 34).

Finally, a **dissemination pack** has been produced to summarise publishable results from each part of the project. This will be published in late 2018 on the project website.

Figure 34. Top left to right: partners ILU and Stolzenberger's Bakery attended *La Fête du pain* at Brussels in May 2018, where members of the public could try new minor cereals baked food products. Bottom left and centre: poster presentations at the EUCARPIA meeting in February 2018. Bottom right: HealthyMinorCereals represented by Stolzenberger's Bakery and PRO-BIO at the FOOD2030 Village event organised in June 2018 by the EU Bulgarian presidency at Plovdiv, Bulgaria.

HealthyMinorCereals partners

	Name of organisation	Short name	Country	Website	Main contact
1	Crop Research Institute (Coordinator)	CRI	Czech Republic	http://www.vurv.cz	Dr Dagmar Janovska
2	PRO-BIO Trading Company Ltd*	PRO-BIO	Czech Republic	http://www.probio.cz/	Mr Martin Hutař
3	Selgen A.S*	SELGEN	Czech Republic	http://www.selgen.cz	Mr Vlastimil Chour
4	Newcastle University	UNEW	United Kingdom	http://www.ncl.ac.uk	Dr Paul Bilsborrow
5	Gilchesters Organics Ltd*	GIL	United Kingdom	http://www.gilchesters.com	Mr Andrew Wilkinson
6	Sabanci University	SU	Turkey	http://www.sabanciuniv.edu	Prof Ismail Cakmak
7	Research Institute of Organic Agriculture	FiBL	Switzerland	http://www.fibl.org	Dr Heidrun Moschitz Dr Bernadette Oehen
8	Getreidezüchtung Peter Kunz*	GZPK	Switzerland	http://www.getreidezuechtung.ch/	Dipl. agr. Peter Kunz
9	Nikolaos Volkakis (Geokomi)*	GEO	Greece	http://www.geokomi.com	Dr Nikolaos Volkakis
10	Estonian Crop Research Institute	ETKI	Estonia	http://www.etki.ee	Dr Ilmar Tamm
11	University of Natural Resources and Life Sciences	BOKU	Austria	http://www.boku.ac.at	Dr Heinrich Grausgruber
12	Institut für Lebensmittel- und Umweltforschung e.V.	ILU	Germany	http://www.ilu-ev.eu	Dipl. Ing Rosemarie Schneeweiß
13	Stolzenberger's Bakery*	SB	Germany	http://www.baecker-stolzenberger.de	Mr Reiner Stolzenberger
14	University of Kassel	UNI KASSEL	Germany	http://www.uni-kassel.de	Prof. Dr. Gunter Backes
15	Grupa BGK Spółka z o.o*	BGK	Poland	Company is no longer operating	Mr Tomasz Lasocki
16	Hungarian Research Institute of Organic Agriculture	ÖMKi	Hungary	http://www.biokutatas.hu	Dr Dóra Drexler
17	IGV Institut für Getreideverarbeitung GmbH*	IGV	Germany	http://www.igv-gmbh.de/	Mr János Petrusán

*SMEs