Slutredovisning projekt O-15-20-346

Titel: Ny havre för livsmedelsindustrin (new oat for the food industry)

Olof Olsson
CropTailor AB

1. Purpose and aims

The long-term goal of this project was to develop oat with new and unique properties useful for the food industry, i.e. grains with higher protein levels, higher betaglukan levels and lower contents of mycotoxins. Also oats with higher levels of avenanthramides, lipids and arabinoxylans, as well as grains with lower lignins in the husk has been targeted.

Our future customers are end-users of both whole oat grain and fractionated oat. Our gola is to, in collaboration with contract farmers to grow and deliver our oat directly to our customers after peeling and heat treatment.

To reach this goal, we have developed several world unique technology platforms on oat like a mutated population of the elite variety Belinda that has a very high genetic variability (Chawade et al, 2010), an arsenal of different molecular and biochemical screening and analytic methods, a unique data base of expressed oat RNA and protein sequences and as the first group int the world sequenced and aligned the entire oat Belinda genome (https://lantmannen.com/en/newsroom/press-releases/croptailor-cracks-the-oat-genetic-code/).

By taking advantage of these technologies we screened and identified oat lines from our mutated population with various different characters. Hundreds of these lines has been backcrossed to Belinda and we have lines that contain more than 20% protein, 7% betaglucan, 10% lipids, doubled avenanthramide content etc. when grown in the green house. We are now testing several of these lines in various field trials, and are also setting up systems for industrial trials in the ca 100 kg scale.

2. Background

Globally, approximately 25 million tons of oats are harvested every year. The commercial value of oat is derived both from a high quality grain and from superior break crop benefits. Oat grows especially well in Scandinavia, which produces the highest oat quality in the world. Since oat has a natural high disease tolerance and low nourishment requirements, oat fields have a comparatively low input demand of insecticides, fungicides and fertilizers. Crops growing in rotation after oat are less prone to infections, increasing the yield and further reducing the need for pesticides (Green, 1999). For environmental reasons it is therefore desirable to increase the proportion of oat in the Swedish cropping systems.

Oat has unique and well-documented cholesterol lowering effects, as a result of its soluble dietary fibers and high ß-glucan content. Oat and barley are the only cereal crops that carries a health claim in both the United States and in EU. According to the European Food Safety Authority (EFSA) Panel's conclusions, there is a cause and effect relationship between the consumption of at least 3 grams per day of ß-glucans and the reduction of blood cholesterol concentrations. The ß-glucan can be supplied from whole oats, oat bran or from mixtures of non-processed or minimally processed ß-glucans without loosing the effect (EFSA Journal 2010, 2011).

In addition, an oat rich diet greatly improves the well-being of persons with celiac disease and reduces the risk of other diet-related diseases. Oat kernels are rich in natural phenolic antioxidants such as tocopherols and inositol phosphates. In addition a unique class of phenolic compounds denoted avenanthramides (AVAs) are present. AVAs have both anti-inflammatory and anti-proliferative effects and prevent the development of cardiovascular disease and colon cancer. Furthermore, oat grains have a high of oil rich in unsaturated fatty acids and unique galactolipids, which can form very special micro-vesicles that have a potential use in drug delivery. Moreover, oat proteins have the highest proportion of globular proteins amongst any cereal starch.

However, despite the many good features of oat, total oat harvest has decreased during the last 15 years. The main reason for this decline is that oat feed to a large extent has been replaced by maize, wheat and soybean. On the positive side is that, during the same time period, human oat consumption has gone up. Since we are standing on the verge of a global obesity epidemic (Caballero et al, 2007) and need to initiate a systematic development of food products with higher levels of health promoting bioactive substances like antioxidants, fibers, proteins, starch, vitamins, sterols and unsaturated fatty acids, it is a very positive that people eat more oat. Given its favorable composition of many health promoting bioactive compounds, no doubt oat has a very high potential in the growing functional food area.

However, despite its many good properties, oats could be developed even further. Agricultural characteristics like disease resistance, especially to *Fusarium*, stress tolerance, nitrogen and phosphor uptake, less uptake of cadmium should be improved. Levels and quality of macromolecules like proteins, ß-glucan, avenanthramides, non-saturated fatty acids, non-non-polar or polar lipids could also be increased. New oat products, attractive to the modern consumer, like e.g. the Oatly oat drink, should be developed. Such a development would be very favorable for Swedish farmers, Swedish agriculture and Swedish economy.

In this project the goal was therefore to develop new, more healthy oat than present varieties. We do this by combing new molecular tools with knowledge of the molecular mechanisms behind the new traits. We do genetic mapping and whole genome sequencing of oat lines identified from mutagenized population. A successful outcome of this work will lead to new oat varieties with a high market value and a high export potential for the benefit of both the Swedish farmers and the local environment.

3. Materials and methods and research program

3.1. Mutant-screening

A mutagenized oat population, developed from the elite spring variety Belinda was used (Chawade et al, 2010). Previously we demonstrated the very high variation in this population and and the possibility to identify lines in this population with virtually any character, including high ß-glucan and lignin (Sikora et al, 2011; 2013). In this project the screening has continued with a special focus on identifying and characterizing lines with kernels high in ß-glucan and protein and/or with lower *Fusarium* mycotoxin levels.

3.2. Plant cultivation

Lines identified as high ß-glucan, high protein and *Fusarium* tolerance have been propagated in climate chambers, greenhouses and in various field experiments. In all cultivation, non mutated Belinda has been included for comparison. Plants have routinely been tested for general growth behavior, time to heading and harvest, morphological characters like plant height, length, number of panicles and other phenotypes of interest. Constant growth conditions of 250 µmol photons m^{-2} s^{-1} and 25°C using a 18h light/6h dark cycle has been used in climate chambers and green houses. Field conditions have been varying a lot over the years, ranging from excess rain to extrem drought. In the field grown material plant architecture, vigor, development time and seed quality have been monitored. All atypical morphological characters have been documented. In addition, total protein and ß-glucan contents in the seeds has been measured. Yield trial have also been performed.

Most field trials have been done at the Lönnstorp field station outside Lomma, and in Svalöv. Plot sized have varied from single rows to >50m^2. Several thousands of lines have been tested during the three years this project has lasted.

3.3 Half seed technology

In order to be able to select individual seeds with the highest protein- and betaglukan values,, we developed a half seed technology for oat. The oat seed is divided in two halves, where the embryo plus endosperm is in one half and endosperm only in the other. Betaglucan and protein levels are then measured in the endosperm half and the seeds with the highest values are rescued

by planting the other half, containing the embryo (Fig. 1). If care is taken in the initial stages of half seed germination, seedling growth can be rescued to 100%. All plants coming up from a half seed thus have a single seed descent.

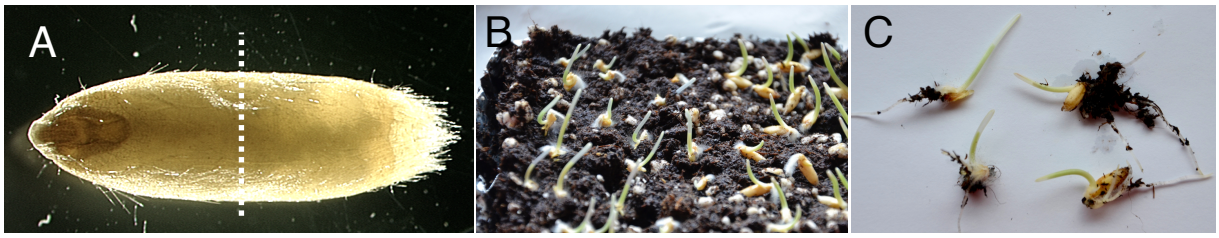


Figure 1. "Half-seed" method to isolate genetically clean lines

A. An oat kernel. The embryo is to the left. Dotted lines indicate where the cut is made.
B. Seedlings coming up from half seeds (left) and intakt seeds (right)
C. 2 days old seedlings. Note good seed development from both seeds and half seeds

3.4 Protein and ß-glucan analysis

Total kernel content of ß-glucan and protein has been analysed in several thousand lines grown in the green house or in the field. All assays were scaled down to only a few mg of flour, i.e what is normally obtained from a half seed and the methods were optimized for these small amounts. Protein measurements were done by a modified, BCA method from Sigma (https://www.sigmaaldrich.com/catalog/product/sigma/bca1?lang=en&region=SE). The method to microtiter plates, which means that 96 simultaneous measurements can be done. In addition, total protein in the seed was measured with the GrainSense NIT device (www.grainsense.com). In this instrument, intact kernels can be assayed for total protein content in a non-destructive way. We found a good agreement with the GrainSense readouts and the BCA values if at least 70 kernels were included in the NIT measurement.

ß-glucan was measured by the Megazyme method or alternatively by a Congo red based method, which we have developed as a high trough-put assay. We can measure ß-glucan in a singel seeds by both methods. The Congo red method is based on the principle that a colored compound (Congo red) specifically binds to ß-glucan. The resulting ß-glucan/Congo red complex is then precipitated by adding dextran and the dextran/ß-glucan/Congo red complex is concentrated through centrifugation. The size of the pellet is proportional to the amount of ß-glucan originally present in the sample. The bigger the pellet, the less Congo red remains in the supernantant. Thus the less absorbance the more ß-glucan in a quantitative measurement.

3.5 Isolation of Fusarium DNA from oat kernels

20 individual kernels from infected material was pooled to eliminate individual differences between kernels. All samples were taken in 5 repeats and photographed prior to processing to document the degree of visual infection. The kernels were grinded in a Pulverisette 23 (Fritsch) and 30 to 40 mg of the obtained flour was homogenized in 500 µl extraction buffer (200 mM Tris-HCl, 250 mM NaCl, 25 mM EDTA, 0.5% sodium dodecyl sulfate, pH 8.5) in a Precellys homogenisator (Bertin Instruments). After this 10 µl RNaseA (1 mg/ml) was added and the mixture was incubated at 65°C during 15 min. 270 µl of 3 M natriumacetat (pH 5.2) was added and the mixture was incubated 10 min at 20°C. After centrifugation (16.000 rpm, 10 min) the supernatant was transferred into a new tube and 1 volume of ice cold isopropanol was added. The precipitated DNA was pelleted at 13,000 rpm during 10 min. The pellet was washed twice with 70% etanol and the DNA finally dissolved in 10 mM Tris-EDTA buffer.

3.6 Fusarium analysis

In the *Fusarium* trials, total *Fusarium* was monitored from green house and field trials. To monitor specific *Fusarium* sp. a multiplex, TaqMan based Q-PCR method was specifically developed for this purpose. The final, optimized reaction mix was; 500nM of each primer, 150 nM each of the specific probes that will hybridize to the primary PCR product in a master mix according the the LGC Biosearch (KlearKal) protocol. Final reaction volume was 15 µl . The amplification was done by an initial heating at 95°C for 15 min, the 40 cycles at 95°C 15 sec, 60°C 15 sec and 72°C

30 sec. The signals from the activated chromofores were detected in a CFX 96 (Bio-Rad) machine, alternatively in a LightCycler 480 (Roche).

3.7. Preparation of high molecular weight oat genomic DNA

The following kits are used; E.Z.N.A.® SQ Plant DNA Kit for 10x gDNA; E-Z 96® Plant DNA DS Kit for 30kb Illumina gDNA; Qiagen Mag-attract HMW DNA kit (for 10x)

3.8 Gene libraries and DNA sequencing

The following libraries were constructed; One library with 470bp inserts obtained without PCR (PCR free), one with on average 700bp inserts, a 10x Chromium library containing very big fragments (50 kb) and three different Mate Pair libraries of 2-4kb, 5-7kb and 8-10kb.

DNA from the different libraries were sequenced with the Illumina HiSeq 2500 machine at a 60x coverage (12 lanes), HiSeqX at 30x coverage (4 lanes) and with HiC sequencing (Chromium libraries). The sequencing was done at the University of Illinois, USA, SciLifeLabs in Sweden

3.9 Plant crossings

To eliminate unknown mutations in individual CT-lines that could reduce their field performance, all selected CT-lines were back crossed to Belinda one (BC1), two (BC2) or three times (BC3). In BC2 plants, 75% of all mutations have been eliminated and in BC3 87,5%. Several different BC2 lines has also been crossed together (mutant stacking) either to strengthen the ß-glucan and protein characters or to combine two different characters (high protein and high ß-glucan).

4. Results

4.1 Ongoing activities

In table 1 all activities that has been going on during the project time are listed. In the right column the present status of the work is indicated. As can be seen from the table, most of the items have been completed and we are well om the way to develop new, unique oat varieties.

4.2 Sequencing the oat genome

The Belinda genome was assembled Jan 2018. In table 2 the statistics are outlined. As can be seen, the assembly is impressive. We have almost 11Gb of DNA put together, which means thant>90% of all sequences have been placed in only 693 scaffolds. This is way ahead of any of our competitors and is by far the best oat assembly in the world.

This work has now continued by putting in the scaffolds into chromosomes. This work was done in collaboration with the Institute for Pflanzengenetik und Kulturpflanzenforschung (IPK) i Gatersleben in Germany. We have all 21 chromosomes identified based on which genome (A, C or D) and chromosome number the belong to. Genome annotation is now on the way. This work is done in collaboration with ScanOats.

4.3 RIL mutation mapping of sequenced CT-lines

Molecular mapping of specific mutations behind a quality character is crucial for a successful development of new tailor made oat cultivars, since this will facilitate the selection of chosen traits in backcrosses or crosses to other oat cultivars. For this reason, CT-lines selected as being especially interesting as breeding material has been further characterized with different molecular technologies both on the DNA structural level and on the gene expression level.

On the DNA level mutations in lines selected for specific phenotypic traits are being be mapped. One way to do this is to produce recombinant oat inbreed lines (RIL) between the mutated Belinda line and another oat line or variety that is genetically quite different from Belinda, like e.g. Matilda and Argentina. Such crosses will facilitate gene identification in the offspring. Presently we are at the F4 stage with the initial crosses. Using this offsping, advanced mapping to zoom in on the chromosome region carrying the mutation behind the trait can now be performed.

4.4 Verification of high ß-glucan- and protein lines in the green house and in the field

We have grown several 100 different CT-lines, sublimes, outcrossed lines originating from CT-lines originally identified as high levels of protein or ß-glucan in the kernels, as well as hundreds

Activity	2015-2019
Sequencing the oat genome	Done
Verification and stabilization of testing of high ß-glucan lines	Done
Physiological testing of high ß-glucan lines in the green house	Done
Physiological testing of high ß-glucan lines in the field	Done
Verification and stabilization of testing of high protein lines	Done
Physiological testing of high protein lines in the green house	Done
Physiological testing of high protein lines in the field	Done
Quantification of various macromolecules	Done
Development of a multiplex Q-PCR assay for Fusarium DNA quantification	Done
Mycotoxin measurements	Ongoing
Field test on Fusarium tolerant lines	Ongoing
RNA seq, proteomics and metabolomics data generation	Partly done
Development of molecular markers för high ß-glucan	Ongoing
Development of molecular markers för high protein	Ongoing
Development of molecular markers för Fusarium tolerance	To be done
Test of new unique oat in industrial processes	Ongoing
Outcrossing and trait stacking	Done
Publication of results in high impact journals	Ongoing

Table 1. Project activities
Original proposal of project activities between August 2015 to July 2018 and results up to date

Sequence details	Size
Total scaffolds	523,398
Assembly size	10,959,045,696 bp
Gaps size	673,880,634 bp
Gaps %	6.1491
N50	17,728,407 bp
N50 #sequences	150
N90	2,801,443 bp
N90 #sequences	693
Maximun scaffold size	113,812,991 bp

Table 2. Belinda genom sequence assembly statistics

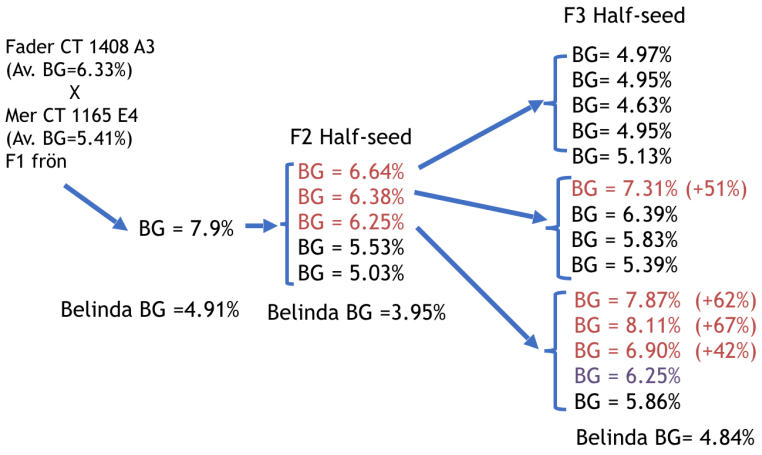


Figure 2. Procedure for genetically clean up lines through half seed technology
A number of individual seeds were selected from a high ß-glucan CT-line after a backcross to Belinda. Seeds were divided in two parts, and ß-glucan was measured in one half, while the other half was planted. The procedure was repeated until most seeds selected for the measurement show high levels of ß-glucan with minimum variation between individual seeds. Red text indicate seeds that were chosen from each generation.

of RIL offspring lines in the green house and in the field.

In Fig. 2 our scheme to produce sub-lines with single seed descent and still high in the wanted character, in this case ß-glucan, is shown. As can be be seen, in every step individual lines with higher and higher levels can be identified. Thus, we now have back-crossed CT-lines with ß-glucan levels close to 8% growing in the green house. They will be tested in the field during 2019.

CT-line	Protein level	SD (n=16)	% relative Belinda	% protein in highest seeds
1200	16,3	2,6	138	21,1 (Lönnstorp 2017)
1260	16,3	2,5	142	21,8 (Lönnstorp 2017)
1410	22,1	1,1	187	23,8 (greenhouse Lund)
2688	21,3	1,9	181	24,1 (greenhouse Lund)
2826	17,6	1,3	149	19,7 (greenhouse Lund)
Belinda	11,8	1,1	100	13,4 (greenhouse Lund)

Table 3. High protein lines clean up through half-seed technology
Three different CT-lines plus the Belinda control are shown. As can be seen, individual seeds can be identified from all three CT-lines that have close to or above 20% total protein (non-peeled material). Belinda is around 12% although individual kernels above 13% can be found.

In Table 3, typical results from in similar way genetically cleaned up high protein lines are shown.

4.5 Optimization of Q-PCR methods to detect and quantify Fusarium DNA

We optimized Q-PCR based Cyber green and TaqMan techniques to quantify *Fusarium* DNA in complex mixtures of plant and microbial DNA. The advantage with the TaqMan technique is that it is highly sensitive and very specific. With TaqMan one can also perform multiplex PCR i.e. to assay several different reactions in the same tube. The draw back with TaqMan is that it is expensive and complicated to optimize.

We tested parameters like DNA extraktion, reaction solutions, DNA concentrations, primer combinations, number of cycles and detection and verification of the synthesized PCR products (Figure 3). As can be sen from the figure, we obtained a good correlation between added *Fusarium* DNA and number of cycles required to reach the exponential phase of the amplification. By taking advantages of different Chromophores, several different Q-PCR TaqMan reactions could be run simultaneously in the same tube (Figure 4). The left part of the

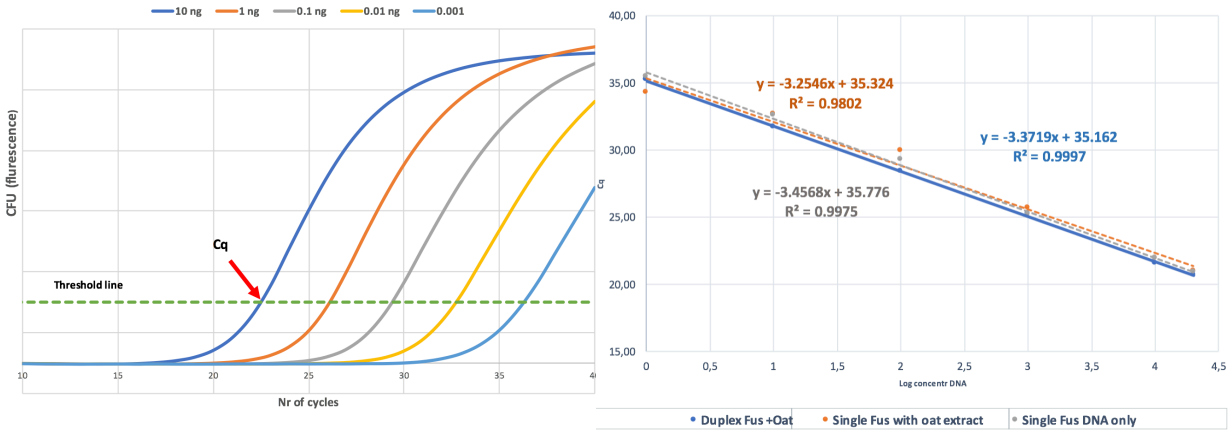
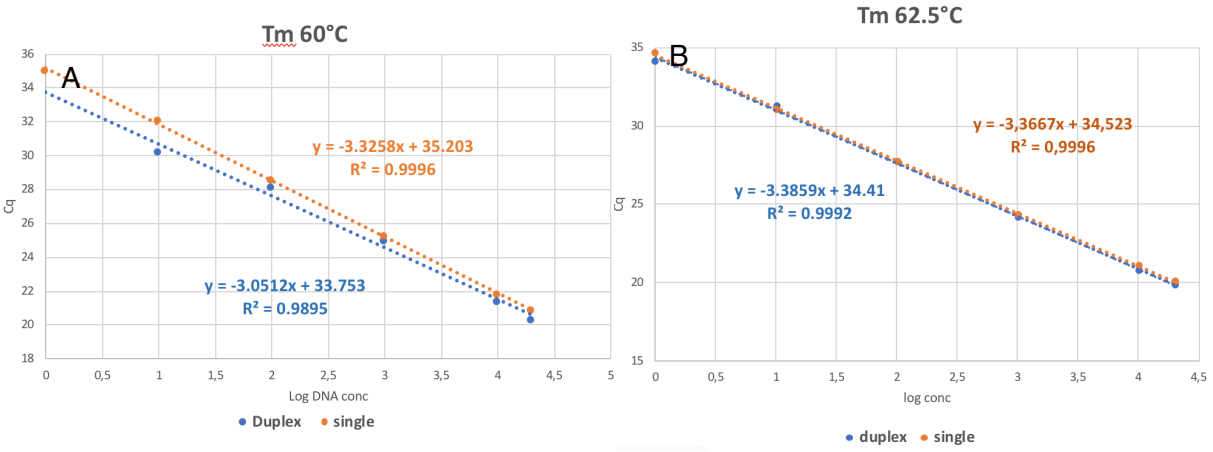


Figure 3. Optimizing the TaqMan reactions resultat of increasing specific DNA koncentration in the sample.

Left; correlation between amounts of DNA and amplification cycles needed to reach the exponential phase.

Right, the equation of the curve. Y-values around -3,3 are considered very good.

figure show a slope for a suboptimal (Y-value - 3,05). However, after optimization both primer pairs work well in the same tube (Figure 4B). This technology has made it possible for us to assay many different tubes for several different *Fusarium* sp.



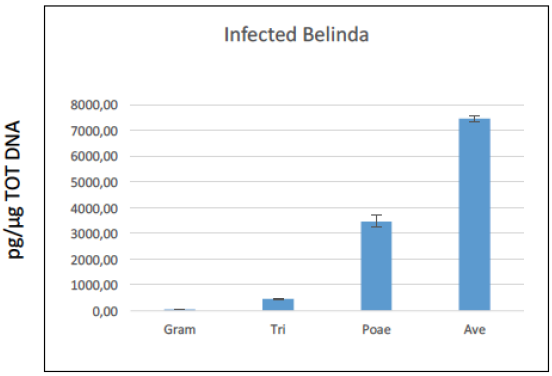
Figur 4. Multiplex PCR

A: Shows an example of a non-optimal reaction since the slope koefficient is under 3,3 (ca 3,0).

B: Shows reactions after optimization. Both slopes now have accepted values (ca 3,3).

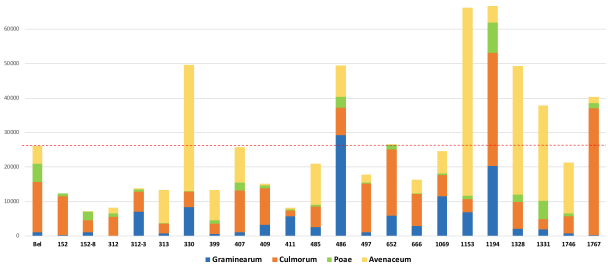
We tested the method using specific primers detecting *F. culmorum, F. graminearum, F. poae, F. sporotricioides, F. tricinctum, F. avenaceum* och *F. langsethiae*. We cultivated all these Fusarium sp in the laboratory, isolated DNA from them and optimized each of the reactions. In Figure 5, the results from a Belinda originally infected in the green house by 5 different *Fusarium sp* using by the same number of spores for each species is shown. Interestingly, after the growth season, when comparing which *Fusarium* sp that were present on the kernels *F. avenaceum* is the dominating *Fusarium*, demonstrating the importance of not only measure DON och test for *Fusarium*, but also to identify the different sub-species.

We applied our multiplex Q-PCR method on samples isolated from three different field experiments during 2017 and 2018. We also tested samples from six different green house experiments and on archive material obtained from official testing sites. We tested infection levels of both market varieties and CT-lines. The results varied a lot from year to year, both regarding infection levels, which plants that get infected and which *Fusarium* that was dominating. In figure 6 a typical result from all these experiments is shown. Here 60 different CT-linjer were tested together with three control plants (Belinda, Ivory och Kerstin) using a mix of 6 different



Figur 5. Belinda infected with 6 different *Fusarium* sp.
At the end of the trial, *F. poae, F. tricinctum* och *F. avenaceum* were identified, but at different levels. Traces of *F. graminearum* could also be seen.

Fusarium (*F. culmorum, F. graminearum, F. poae, F. sporotricioides, F. tricinctum, F. avenaceum*). At the end of the experiment all the different plants were separately harvested, thrashed, rinsed and DNA was isolated from random kernels from each plant.



Figur 6. Amount of Fusarium DNA (pg) per µg oat DNA (tubuline) in different CT-lines and in Belinda
The Y-axis shows the total DNA amount detected, i.e the sum of DNA from *F. graminearum, F. culmorum, F. poae* och*F. avenaceum.* The X-axis shows Belinda and 22 chosen CT-lines tested. The red dotted line indicates the amount of fungi DNA detected in Belinda. As can be seen, several of the CT-lines had lower fungi DNA levels than Belinda, i.e they have an increased tolerance for *Fusarium.*

By Q-PCR we could show that only 4 of the original 6 fungi could be detected at the end of the experiment. *F. avenaceum* was strongly dominating in some samples, but also *F. poae, F. culmorum* and *F. graminearum,* was present in signifikant amounts while *F. sporotricioides* and *F. tricinctum* were virtually absent (figure 6). As an internal DNA reference we used tubuline originating from the oat genom. The conclusion is that there is a fierce completion between different *Fusarium* sp. in real life conditions, where several are being outcompeted, although different one in different lines and localities. This shows the importance of monitoring the *Fungi* itself and not only a mycotoxin like e.g. DON, which is a metabolite from only a few *Fusarium* sp and will not give the whole picture.

Other important results from these experiments are that also in the CT-lines the *F. poae, F. tricinctum,* and *F. avenaceum* fungi was dominating, although several of the tested CT-lines showed an increased tolerance to one or several *Fusarium* compared to Belinda, Kerstin and Ivory (figur 6). Five CT-lines had lower DNA levels of all the tested *Fasurium sp.* compared to the control plants.

5. Discussion

We have developed a high quality assembled Belinda genome sequence, which gives us a world unique possibility to obtain full sequences of other oat genomes, like e.g. the mutated CT-lines, in a fraction of time and cost compared to the original Belinda sequencing. Genomic sequencing of the CT-lines can now be done with only ca 15 times redundancy (compared to 270 times for Belinda), greatly reducing the cost and complexity. We have isolated genomic DNA from 25 CT-lines selected on the basis of high betaglucan, protein or oil content or for other interesting characters, like dwarfness, naked oat, big seeds and *Fusarium* tolerance. The whole genome of these CT-lines are now in the process of being sequenced. The ca 1 million introduced EMS mutations in each line will be pinpointed by means of a special algorithm that we have developed. Once the EMS mutations have been identified and mapped to the original Belinda sequence, the

next step is to decide which of all these mutations is the one causing the altered trait that we selected for. From the mapping experiments, we can come down to a chromosomal location of ca ±5 million bp or better that carries the specific mutation that gives rise to the phenotype. From the RNA seq data we can then check for the RNA transcripts that differ in this region between Belinda and the CT-line. If there are differences, which would be expected, since the phenotype is different in the CT-line, we would only have to focus on a chromosomal region of 1 million bp or less. Since the mutation frequency in the CT line is about 1 mutation per 30000 bp, we can directly analyze the ca 30-40 mutations in the region we focus on. From the nature of the mutation (silent, missense, frameshift, in coding regions or promoter regions, etc), and from knowing which genes are mutated in that region and what they are coding for, we will be able to decide which specific mutation that gives rise to the phenotype.

From our mutated population we have screened out lines with seeds high in betaglucan and protein as well as lines mote tolerant to *Fusarium* infection than Belinda, our control variety. We have back crossed the best lines several tines and also crossed together (stacking) different CT-lines high in betaglucan. Several of these crosses gives lines exceeding 8% betaglucan. Thus we have many lines of high potential that now can be developed to new varieties.

Once a specific mutation from these supervenes is successfully linked to a specific phenotype, a molecular marker for the mutation can be developed. A PCR primer covering the mutation could then easily be designed. Marker assisted breeding (MAS) is a fast, cheap and efficient way to do breeding. MAS has a much higher precision than conventional visual or biochemical selection methods and the selection can be done already on young plants. The stacking of different traits also becomes a lot more feasible if MAS markers are available. Finally, the fact that a specific trait can be coupled to a specific genetic event will also make it possible to patent the trait itself, which gives a better protection that the breeders right which only protects the variety, not the trait.

6. Value for the end users

The final outcome from this project will be new oat varieties with unique properties. Thus, they will directly contribute to the end-users competitiveness either by making the industrial process more efficient, like a high ß-glucan oat in a process where ß-glucans are extracted, or in the final product, like high protein flakes or oat drinks with a higher protein content. All end-user will be also interested in oat with a low mycotoxin content. An assumed added value of 50 öre per kilo of the harvested oat would give an increased income of 2500-3500 kr/ha. Presently ca 150.000 ton oat is used in the production of food and food ingredients and the human oat consumption is presently in strong growth period. Oat export is also increasing. Thus, this project will result in increased areas of oat cultivation in Sweden. Since oat is a very good rotation crop with few diseases this is a positive development also from an environmental point of view. Since in general oat grows exceptionally well in Sweden and is of a very high quality, different more niched special oat varieties will strongly contribute to an increase Swedish export in the future.

7. The project group

Responsible for the project has been Olof Olssons at LTH, Lund University (LU). We have worked in close collaboration with CropTailor AB, who also has sponsored a major part of this project. From CropTailor we have access to the mutagenized oat population, the Belinda genome sequence and and various methods. Thanks to this project we have also managed to create ScanOats, an oat centre finance by the Strategic Foundation and localized at Lund University. We have long and well-established collaborations with industrial actors like Lantmännen Lantbruk, Swedish Oat Fibre (SOF), Oatly, Aventure, Glucanova. We cover the whole chain from advance molecular biology in the lab, oat cultivation and field trials to industrial applications.

8. Comments on the budget

We received in total 3,5 million SEK for this project, divided in four calendar years. To carry through the R&D plan we have used various researchers, postdocs and masters level students. In addition to salary costs, the rest of the project expenses has been analytical costs (i.e. instrument

time, repair and maintenance), costs for RNA and protein sequencing and costs for reagents. The other major part of the budget is dedicated to plant cultivation to cover green house costs, climate chambers and field trials. The project costs have been substantially more than 1 million SEK per year, but the rest of the costs has be covered by CropTailor AB.

However, without the support from SLF, we would not have had the resources to carry through the project in the way that we did, with a special highlight on being the first in the world to sequence and assemble the entire oat genome.

9. Key references

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