



Deliverable rapport from project no. 1

BIO-VALUE SPIR DELIVERABLE RAPORT

Project no. 1

Project title: Innovative biomass production systems, harvest and conservation technologies

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Executive summary

Innovative biomass production systems, harvest and conservation technologies.

The experimental work has focused on:

- Composition of agricultural systems and yield optimization
- Characterization and molecular mapping of cereal genotypes
- Development of machinery and modeling



Experimental activities planned in year 2014

- Experimental plan A - Biomass crops for biorefinery, AU
- Experimental plan B – Composition of agricultural systems, Agrotech
- Experimental plan C – Characterization of cereal genotypes, Sejet/KU
- Development of suitable machinery and modeling, Kongskilde/VFL

The project is well underway and the necessary staff has been recruited at universities. The individual sub-projects have all delivered as promised. A few activities have been delayed to a lesser extent.

The planned activities continue in 2015 without significant changes in relation to the project description.

Deliverable text

Description of the deliverable objective and the content

Expected deliverables in 2014 according to detailed project description:

Deliverables for KU's activities 2014:

- Field experiment with winter wheat is conducted in the growing season (field experiment embrace 14 different winter wheat varieties and one Triticale variety).
- Plant material sampled during the growing season for analysis and stored in frozen condition for further analysis: May-August 2014.
- Analyses of cell wall composition, concentration of other mineral elements and enzymatic saccharification.
- Final harvest of all plots and recording of yield and quality: August 2014

Deliverables for AU's activities 2014:

- Cropping systems with 16 different combinations are established at AU-Foulum and AU-Jyndevad.
- Crop growth is continuously followed by remote sensing registration of green leaf area development. May-December 2014.
- Soil carbon content of all experimental plots is determined.
- Nitrate leaching is measured in all plots establish a total N-balance of the different systems.
- Harvest and yield calculations for all plots: May-December 2014
- Sampling and storage of all biomasses in frozen conditions. May-? 2014
- Biomass samples for other project partners.

Deliverables for VFL's activities 2014:

- Note on current cropping systems for the selected crops: October 2014
- Recommendations for harvesting, transportation and storage methods for the selected crops: November 2014
- First version of excel model for cost calculations for harvest, transportation and storage: December 2014
- Desk study note on quality changes during storage: December 2014.

Deliverables for AgroTech's activities 2014:

- Delivery of tall fescue samples to KU for protein analysis: June 2014
- Delivery of strip harvested spring barley samples to KU for protein analysis: August-September 2014
- Delivery of Italian ryegrass catch crop samples to KU for protein analysis: October-November 2014
- Conference paper and/or peer-reviewed paper based on the trial with harvest and ensiling of mixtures of catch crops and straw (with Aalborg University): June 2014
- Peer-reviewed paper and/or magazine article based on the trial with ensiling of beet top and straw (with University of Southern Denmark): Autumn 2014
- Publication of results of the cereal-catch crop field trials with strip harvest of spring barley, in Oversigten over Landsforsøg 2014: December 2014

Deliverables for Kongskilde 2014: No deliverables for Kongskilde first year.

Deliverables for Sejet's activities 2014:

- Field trials with varieties of winter wheat established.
- Trial harvest of different winter wheat varieties: August 2014
- Sampling for straw yield and straw quality send for genotyping at KU: August 2014

Status for the deliverables is described for each partner in the following section.

The following deliverables and activities have been labeled according to this:

F: Fulfilled

P: In Progress

D: Delayed

A: Abandoned



Deliverables for KU's activities 2014

- Field experiment with winter wheat is conducted in the growing season (field experiment embrace 14 different winter wheat varieties and one Triticale variety). (F).
- Plant material sampled during the growing season for analysis and stored in frozen condition for further analysis: May-August 2014? (F).
- Analyses of cell wall composition, concentration of other mineral elements and enzymatic saccharification (P).
- Final harvest of all plots and recording of yield and quality: August 2014 (F).

Deliverables for Sejet's activities 2014

- Field trials with varieties of winter wheat established. (F)
- Trial harvest of different winter wheat varieties: August 2014(F)
- Sampling for straw yield and straw quality (F). Seed for genotyping at KU (This is conducted by Sejet): August 2014 (P)

Description of conducted deliverables for KU and Sejet 2014

The field experiment with winter wheat at KU (14 different winter wheat varieties and one Triticale variety) and at Sejet (36- & 96- different winter wheat varieties) are conducted, grown, and harvested (Final harvest). Sampling of straw samples, during final harvest, for conduction of straw yield and quality is made for all experiments (KU and Sejet) and the cultivars at Sejet are expected to be genotyped before Christmas 2014.

Plant material from the KU experiment was sampled during growth season and is stored in a frozen condition and the further analysis of the samples is ongoing.

All straw samples for both KU and Sejet experiments are being fine milled during November 2014, and will afterward be analyzed for mineral element concentration (January 2015), total C- and N- content (January 2015), and for cell wall composition analysis (February 2015). The enzymatic saccharification analysis is ongoing and a result is expected before Christmas 2014. Sejet have also measured morphological traits (grain and straw yield, straw height, lodging degree, and septoria degree) for samples established from both experiments at Sejet.

The new growing season for 2014/2015 is established both at KU and at Sejet and we expect to fulfill the same deliverables at KU and at Sejet as for this year regarding the growing season 2014/2015 field experiments.

Expected Deliverables for KU's activities 2015:

- Field experiment with winter wheat is conducted in the growing season (field experiment embrace 14 different winter wheat varieties and one Triticale variety).
- Plant material sampled during the growing season for analysis and stored in frozen condition for further analysis: May-August 2015
- Analyses of cell wall composition, concentration of other mineral elements and enzymatic saccharification.
- Final harvest of all plots and recording of yield and quality: August 2015
- Making the first part of the genome wide association assay with data from harvest 2014

Description of the deliverable objective and the content

Identification of the different N pools in wheat straw. The location and composition of residual N which remains in the straw at harvest will be identified.

Field trial in Tåstrup will be continued. Two additional N fertilization amounts will be added (60, 280 kg/ha) resulting in 60, 100, 160, 220 and 280 kg/ha N. Plants will be harvested at 3 time points, stored frozen and subsequently analysed. After harvesting, the straw structure of some samples will be examined microscopically. The analyses include extraction and measurement of N containing substances (N audit). Non protein N substances, soluble amino acids and proteins will be analysed.

The major objective is to identify biomarkers for selection of winter wheat cultivars with an improved straw quality and quantity for the use in biorefining processes. We aim to find markers that can select for cultivars with an improved sugar composition (cellulose, hemicellulose, and lignin) that can be used for instance by platform 5 or 6.

A deeper understanding of the role of nitrogen for straw productivity and structure, including the identification of nitrogen pools at different growth stages will be elucidated. A deeper understanding of the cell wall structure as affected by straw N status will be gained.

Expected Deliverables for Sejet's activities 2015:

- Field trials with varieties of winter wheat established including crossing population.
- Trial harvest of different winter wheat varieties: August 2015
- Sampling for straw yield and straw quality. Seed for genotyping at Sejet August 2015



Deliverables for AU's activities 2014

7 deliverables are stipulated, and in addition a significant method development on crop fractionation into easily extractable and fiber bound protein has been initiated in lab scale as part of a PhD study initiated in June. The method is soon expected to be developed for use on the actual crop samples.

- 1. Cropping systems with 16 different combinations are established at AU-Foulum and AU-Jyndevad (F).** Additionally, a slightly modified cropping experiment is under establishment at a clay soil at Flakkebjerg, and will be fully implemented during spring 2015.
- 2. Crop growth is continuously followed by remote sensing registration of green leaf area development. May-December 2014 (F).**
- 3. Soil carbon content of all experimental plots is determined (P).** Soil samples have been taken in 3 depths in all plots before experimental treatments were initiated, dried, and put on archive. After a few years new sampling will be done, and initial and new samples analysed simultaneously in order to investigate the development.
- 4. Nitrate leaching is measured in all plots in order to establish a total N-balance of the different systems (F).** For economic reasons a few treatments are not measured. Total N-balance will be established for the first years, when crop N-content, water balance and total leaching pr. Ha has been calculated.
- 5. Harvest and yield calculations for all plots: May-December 2014 (F).** By now, only the sugar beets are not yet harvested.
- 6. Sampling and storage of all biomasses in frozen conditions. May-? 2014 (F).** We do not have freezer capacity for several years storage, so we have thrown away samples from 2013, as there was no demand from other partners, and will have to do the same in 2015 if no demand.
- 7. Biomass samples for other project partners (F).** We have delivered samples for different partners but most often not from the main experiment. Rather there has been a demand for 1-2 specific biomass types in a short time, and with a specific pretreatment (not frozen) which have been taken from other experiments at Foulum. It would be interesting to have a better flow of well-defined biomass from our field trials through later pretreatments and conversion tests, so that final whole chain evaluation of environment and economy can be based on a broad (many different crops) data set covering the whole chain.

Activity description.

All the crops from the biomass field experiment will be characterized for the quantity and quality of protein they contain. The characteristics of forages which relate most closely to digestibility and intake are defined to be protein, lignin and fibre, and these may also reflect extractability in a biorefinery. An accurate dry matter determination has a critical role in effective representation of the mentioned components.

Total crude protein is determined using Dumas method. However, a simple total crude protein determination is not adequate to delineate protein quality. In order to describe the degree of protein quality with regard to its contribution to un-degraded intake of protein and bacterial crude protein, Cornell Net Carbohydrate and Protein System (CNCPS) is introduced. Using this system, total crude nitrogen is fractionated based on solubility in protein precipitant agents, buffer and detergent solutions. According to CNCPS, the total crude protein is fractionated into three different fractions based on nutritional availability.

Fraction A is non-protein nitrogen (NPN). Fraction B is sub-fractionated into B1, B2 and B3 based on the rate of ruminal degradability. Fraction B1 is rapidly degraded in the rumen. Fraction B2 is fermented in the rumen and some escapes to the lower gut depending on the relative rate of digestion and passage. Fraction B3 is associated with the cell wall and as a result is a slowly degraded fraction. Fraction C is unavailable or bound protein which is associated with lignin, tannin-protein complexes, and Maillard products that are highly resistant to microbial and mammalian enzymes. Fraction C cannot be degraded by ruminal bacteria and does not provide amino acids post-rationally.

Near Infrared Reflectance (NIR) spectroscopy will be utilized to assess the quality parameters of the forages such as protein, fibre and lignin. In this context, the effectiveness of NIR spectroscopy to predict the chemical characteristics of biomass will be studied. Finally, the amino acid composition of the crude and different protein fractions will be analysed and the results will be discussed with regard to the type and amount of essential amino acid content in different protein fractions and their possible extraction (WP2).

Deliverables for KCA

Note on current cropping systems for the selected crops: October 2014

Note on cropping system for grain, rape, beet, maize, and grass with focus on current harvest methods, capacity and cost is written. The report further suggests topics for optimization in crop production and point out constraints. The report is currently under review and will be released in 2014 (F).

Recommendations for harvesting, transportation and storage methods for the selected crops: November 2014 To be finalized in 2015 (P).

First version of excel model for cost calculations for harvest, transportation and storage: December 2014

First version of model for cost calculations for harvest, transportation, and storage is ready for wheat straw, maize straw, rape straw, beets and meadow grass. Production of grass still awaits final modelling. Modelling of grass for production will be finalized in December. (F).

**Desk study note on quality changes during storage:** December 2014.

A desk study on quality changes during storage is being performed. A note is under development.

Recommendations on harvesting, transport and storage can be performed using the model. A note describing the model will be written in January 2015. (P).

Comments on developments of suitable machinery and modelling

The current cropping system is described and a survey on recommended harvesting method, transportation, and storage has been performed. A first version of an excel model for the selected biomasses is almost ready and will be finished during 2014. During 2015 the model will be further developed and the outcomes of the model will be compared to real life scenarios. A calculation of expected revenue of each type of biomass cannot be performed before end-use of the biomasses is known. In order to perform the calculations, we need suggestions of end use and pretreatment cost from other work packages. The price at the biorefinery gate depends on supply and demand as well as expected revenue at the biorefinery. The expected cost of production, however, can be performed for each biomass in the model. Storage possibilities and quality changes are under investigation as a desk study.

Deliverables for Agrotech**Delivery of tall fescue samples to KU for protein analysis:** June 2014

Objective: The objective is to study the effect of nitrogen fertilization level on the protein profile of the perennial grass species tall fescue grown on wet, sandy soil.

Status: Samples have been harvested from two field trials with tall fescue, each with four different levels of nitrogen fertilization and frozen samples have been delivered to KU in June 2014. (F).

Delivery of strip harvested spring barley samples to KU for protein analysis: August-September 2014

Objective: The objective is to quantify the yield as well as the feed value and protein content of spring barley harvested with strip harvesting prior to maturity as compared to traditional combined harvesting of grain at maturity. Also, the aim is to study the effect of ensiling duration on the quality of strip harvested barley.

Status: Two field trials have been carried out in 2014, and samples of the grain fraction have been taken at the various harvest times and frozen immediately. From one of the trials, samples from various harvest times have been ensiled in vacuum bags and the ensiling process has been terminated after 2, 4, 8, and 16 weeks. (F).

Delivery of Italian ryegrass catch crop samples to KU for protein analysis: October-November 2014

Objective: The objective is to quantify the yield and protein content of Italian ryegrass when grown as undersown catch crop after strip harvested barley and depending on nitrogen fertilization of the catch crop.

Status: In the two field trials with strip harvest of barley (see above), Italian ryegrass has been harvested in October, and frozen samples from plots fertilized with 0 and 50 kg nitrogen per ha have been delivered to KU in October-November 2014. (F).

Conference paper and/or peer-reviewed paper based on the trial with harvest and ensiling of mixtures of catch crops and straw (with Aalborg University): June 2014

Objective: The objective is to publish the results from experimental work carried out autumn 2013. Within the Bio-Value project, a field trial was done with spring wheat with undersown perennial ryegrass as catch crop. Spring wheat was harvested at different stubble height in august, and the straw and catch crop was harvested together in October, and the effect of stubble height and catch crop on methane yield was analyzed.

Status: The results have been presented on The European Biomass Conference and Exhibition in Hamburg in June 2014, and the results have been published in the proceedings:

Molinuevo-Salces, B., Larsen, S.U., Ahrling, B.K. & Uellendahl, H. (2014). Biogas production from catch crops: A sustainable agricultural strategy to increase biomass yield by co-harvest of catch crops and straw. Proceedings from the 22nd European Biomass Conference and Exhibition, Hamburg, Tyskland, 23-26th June 2014. (F)

Peer-reviewed paper and/or magazine article based on the trial with ensiling of beet top and straw (with University of Southern Denmark): Autumn 2014

Objective: The objective is to publish results from experimental work carried out in November 2013 where various mixtures of beet top and straw were ensiled in vacuum bags. The aim is to study the potentially synergistic effect of ensiling straw with a wet biomass to achieve a higher methane yield from the straw compared to untreated straw.

Besides, the straw may absorb excess juice from the beet top and, hence, avoid seepage.

Status: Since there appears to be an effect of the duration of the ensiling process, samples have been analyzed for methane potential after two months and are currently being analyzed again after ten months of ensiling. Therefore, the full data set is not available yet, and publication of the results will be postponed until 2015. (P)

Publication of results of the cereal-catch crop field trials with strip harvest of spring barley, in Oversigten over Landsforsøg 2014: December 2014

Objective: The objective is to publish the results of the two field trials with strip harvest of spring barley followed by an Italian ryegrass catch crop (see above).

Status: Results of yield and feed value of strip harvested barley vs. combined harvested barley as well as the dry matter yield of the catch crop are currently being elaborated and are expected to be published as planned in Oversigten over Landsforsøg in December 2014. Analyses of the protein profile in strip harvested barley and in Italian ryegrass have not yet been carried out, and these results will be published later. (P)

**Delivery of:**

- tall fescue samples to KU for protein analysis: June 2014
- strip harvested spring barley samples to KU for protein analysis: August-September 2014
- Italian ryegrass catch crop samples to KU for protein analysis: October-November 2014

Brief description of the state of the art

The field data that will be used for identification of potential genotypic differences, using SNP biomarkers, in straw yield and quality of winter wheat cultivars will primarily be from the 96 wheat cultivars experiment grown at Sejet for a 3 year period, where around 50% of the cultivars will be grown repeatedly during the 3 year period. The cultivars will be SNP genotyped and the following traits will be measured: grain and straw yield, plant height, lodging degree, septoria degree (all measured at Sejet), total content of carbon and nitrogen (measured by IR-MS), the straw sugar release ability (using a hydrothermal pretreatment followed by enzymatic hydrolysis (CellTec², for 72 h) and HPLC determination of sugars), a compositional analysis of the cell wall (qualitative measurement of lignin and quantitative measurements of cellulose and hemicellulose) and a compositional analysis of the complete sample (protein, cellulose, hemicellulose, lignin, and ash) will if needed be created for selected samples. The traits and the SNPs will then be investigated for any significant association using a genome wide association study (GWAS).

Data from the first generation, will besides of GWAS, be used to select interesting cultivars with a high and low straw yield, and create mapping populations for these, which then will be phenotyped for variation in the straw yield and with the identified SNP markers using QTL mapping or linkage studies. Furthermore, the wheat cultivars will also be used to test the effect of the pre-treatment in the sugar release assay.

Ample nitrogen is essential for the productivity of wheat plants in terms of grain and straw biomass. But on the other hand, too much nitrogen may have negative consequences for the physical strength of the straw which may lead to lodging. Very limited information is available on how nitrogen supply effects the chemical composition and the physical structure of cell walls in the straw. One important aspects of this are cell wall proteins. The relationship between cell wall proteins and straw nitrogen status is not known.

The location and composition of the residual N remaining in the straw at harvest is not known yet. Our N-Audit should elucidate this N pool. There is no comprehensive N audit of wheat published. The publications are limited to some of the following parameters: organs, growth stages, compositional analysis but not combining them in our targeted depth. Our N audit will contain different N containing substances, different organs as well as different harvest points, which can't be found in existing literature.

Deviation from objectives and corrective actions

In the objectives for 2014 it was stated that the seeds were send for genotyping at KU, but Sejet have consented to conduct this task instead of KU.

Agrotech plans new activities, not described in the detailed project description July 2014:

- Storage trials with pure chopped sugar beet root and mixtures of chopped sugar beet root and chopped winter wheat straw.
Objective: The objective is 1) to study the effect of co-ensiling of wheat straw with sugar beet root on the quality, including the effect of wheat proportion and the duration of ensiling, 2) to study the storage loss during ensiling of pure chopped sugar beet root and with sugar beet root mixed with straw.
Status: A lab-scale storage trial was initiated in November 2014. A series of vacuum bags have been made with various mixtures of chopped sugar beet root and chopped winter wheat straw (0, 7, 13 and 20 % straw by fresh matter weight) resulting in a range of dry matter contents (20.0, 24.1, 27.7 and 32.8 %). Each mixture will be ensiled for 0, 2, 4 or 6 months to study the effect of the ensiling duration. A pilot-scale storage was established end of October 2014 with four 2.6 m³ silos. Two silos were loaded with pure chopped sugar beet root and two silos were loaded with a mixture of 14 % (fresh matter) chopped winter wheat straw and 86 % chopped sugar beet root, resulting in a dry matter content of 30.3 %. The content of each silo was weighed at the start of the experiment and will be weighed again after approx. 6 months of ensiling. For the silos with mixtures of straw and beet, seepage will be measured during the trial period.

Innovation brought and technological progress

Not relevant yet.

Analysis of the results

Not relevant yet.

Impacts of the results

Not relevant yet.

Related IPR

Not relevant yet.



Publishable information

- Conference paper and/or peer-reviewed paper based on the trial with harvest and ensiling of mixtures of catch crops and straw (Agrotech with Aalborg University). June 2014
- Peer-reviewed paper and/or magazine article based on the trial with ensiling of beet top and straw (Agrotech with University of Southern Denmark). Autumn 2014
- Publication of results of the cereal-catch crop field trials with strip harvest of spring barley (Agrotech in Oversigten over Landsforsøg 2014). December 2014
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Conclusion

The project is well underway and the necessary staff has been recruited at universities. The individual sub-projects have all delivered as promised. A few activities have been delayed to a lesser extent.

The planned activities continues in 2015 without significant changes in relation to the project description.

Annex

Annex: Technical content or further references...