C4F-THEME REPORTS AND RESEARCH PORTRAITS

C4F- Crops for the Future

C4F program has been functioning as a research platform since its start, supporting a large number of projects, connected to other large projects. During 2022, about half of the projects were finished and new projects have been initiated, in which the involvement of young researchers (PhD students, postdoc and project leaders) has been further highlighted among the other important criteria. The overall progress of the research within C4F has been in principle as planned in 2022.

As a research platform, C4F has been supporting a number of research projects, which have connected to other large projects funded by other funding agencies. During phase 3 of the program, the update of projects have become more frequent, which facilitates implementation of timely projects dealing with current research of high interest for sustainable agriculture production.

During 2022, five projects were finished, of which one project has been prolonged for finishing PhD education. Five new projects have been initiated. All projects have involved young researchers (PhD student or postdoc/researcher) and two new project leaders with the docent competence have joined C4F. The overall progress of research within C4F has gone according to plan in 2022. A number of peer-reviewed articles of high quality have been published, while a number of other manuscripts have been submitted or in the pipeline for publication. Additionally, a number of new complementary research projects have been received from Nova Nordic Foundation, FORMAS, VR, etc. partially due to support from the C4F program. The annual C4F workshop was held on 29 Nov. in Malmö.

The plant model protein systems and the Cd stress projects progressed very well. New results on the protein rich systems have been generated and are in the pipeline to be complemented, finalized and submitted for publishing. We are soon about to finalize the Cd stress study which used advanced imaging to map plant stress, and a manuscript on the results will be submitted in coming weeks.

The plant protein fractionation project has progressed very well. We finalized and submitted the paper on greenhouse gas emissions from bio-based diapers during the spring 2022 and it was published in the very beginning of 2023. A range of new projects, based on activities related to this project or other work by the post-doc also started during 2022 (GreenLeaFood funded by Formas, Green2Feed funded by energimyndigheten, BSRC funded by energimyndigheten, Sensory project funded by Formas, HMMA project funded by Vinnova, a second C4F project, Mistra Food Future). As a result, the post-doc will leave this project and work in other projects, and we are currently recruiting a new post-doc for the present project.

The green diapers superabsorbents project was started in 2022 and it has progressed well since the start. A new post-doc (Anna-Lovisa Nynäs) was recruited during the spring 2022. She has focused on the fractionation procedure of the proteins from green biomass in order to get a higher protein yield that can then be used for absorbent materials. Therefore, protein fractionation has been carried out in the Plant Protein Factory, pulp has been collected and various fractionation methods are being evaluated in the lab to understand opportunities to increase protein extraction from the pulp. In parallel, a manuscript has been written, evaluating the path of the nitrogen in the green biomass and in what form it ends up in the various fractions.

For the sugar beet project, the lab work of the PhD-student has been finalized during 2022. Thus a study on understanding the physiological background of the use of biostimulants, a study on the effects of biostimulants combined with drought stress, and a study on effects of the use of biostimulants in field cultivation of sugar beet was carried out and finished. The student has started to compile the results into five manuscripts of which the first one was published in 2022. The student will defend his thesis in 2023.

For the legume project, papers on mixed faba bean gels, 3D-printing of faba bean-based materials and foaming properties of protein nanofibrils from mung bean has been published. A new PhD Student will start to work on this project in early 2023.

Perspectives of the genome analysis and evolution of oil accumulation in Cyperus tubers is in preparation for a manuscript. A manuscript is in preparation for genome edited potato with redirected carbon flow from modifying promoters of transcription factors. The sink involvement of plastid starch synthase has been investigated in potato, a previously unknown gene duplication was characterized and the involvement of the enzyme in starch reserve structure was for the first time determined. A manuscript is about to be submitted.

Fig. 1. (Left) Plant height and (right) root diameter of sugar beet treated with equal nitrogen content from PBB and NS, eight weeks after planting. (Figure By Okanlawon Lekan Jolayemi)

The results on wax esters in the pheromone production project was published and a novo application was granted to Kamil Demski. Integration of new precursors into oil has successfully been shown. A proof of concept for the technology was published. Work has now focused on gene mining of Lindera species (which store oil composed of medium chain fatty acids) with the perspective of gene editing in Camelina.

The efficient protoplast-based genome editing protocols for field cress and rapeseed established within the program have enabled generation of transgene-free mutants of the target crops by using CRISPR/Cas9. A large number of CRISPS-edited mutation lines of both species have been generated for improving the seedcake and oil qualities, and some of them have been evaluated and some are still under evaluation. Manuscripts about some of the results are under preparation and one manuscript with improved oil quality in field cress was accepted. The PhD student Sjur Sandgrind working on field cress within the program defended his thesis in the end of 2022.

After finishing her PhD defence in October 2021, Xue continued to work with the publication of her work in the beginning of 2022. PhD student Shishanthi has completed and published one review article and worked on revising one paper about starch branching in barley.

Regarding the autophagy project, genetic engineering, molecular and cell biology experiments were performed using the model plant Arabidopsis, by our team members in Uppsala campus SLU. Further elucidation of our findings and their transferability onto crop species will be carried out in synergistic collaboration with our colleagues at the Department of Plant Breeding in Alnarp.

Research outcomes and associated outreach activities deal with new knowledge and information on novel potential uses of plant oils, proteins, starches and other compounds from side streams, which can be used as food, feed and industrial applications. The program contributed to generation of novel plant materials for further breeding or direct uses in product quality research and future potential applications, emerging of new research areas such as bio-based composites for food and non-food uses, possibility of crop improvement by regulating autophagy process, and renewable sources of plant produced insect pheromones for pest management. SLU Grogrund has continued to support novel projects in 2022 and some of them are connected to the C4F projects.

Detailed research findings and progress

Protein-based biostimulants (PBBs) are derived from the hydrolysis of protein-rich raw materials of plant and/or animal origins, usually by-products or wastes from agro-industries. The active ingredients produced by hydrolysis have the capacity to influence physiological and metabolic processes in plants, leading to enhanced growth, nutrient and water-use efficiency, tolerance to abiotic and biotic stresses, and improved crop yield and quality. Two examples of PBBs (hydrolyzed wheat gluten and potato protein) were shown to have an effect on the early growth of three sugar beet varieties. Both PBBs had a significant stimulating effect on early sugar beet growth and development (Fig.1). The PBBs can be further developed into superabsorbent polymers (SAPs). To conclude, PBBs/SAPs developed from agro-industrial wastes have the potential for sustainably supplying water and nutrients in agricultural systems and for enhancing plant growth and development over a substantial period.

The possible uses of green biomass as a source for local production of protein suitable for human food and animal feed has received increased attention lately. Amino acids are the dominating type of nitrogen in all the fractions. Levels of nitrate and nitrite were generally low but differed between biomass sources and their fractions with the highest levels of nitrate in hemp biomass and fractions. Both RuBisCO and other types of proteins present in the green biomass sources were found in all fractions of all biomass sources, which was initially washed using the facility in the Plant Protein Factory in Alnarp (Fig. 2). Essential and non-essential amino acids correlated strongly with the total nitrogen content indicating an equal share of those to the total nitrogen in all fractions. The highest nitrogen content among fractions was found for the green protein fraction and the white protein fraction, although the lack of correlation with the protein content determined by HPLC, indicate a break-down of proteins during the fractionation process. The pulp fraction was found suitable as feed for ruminants, green protein and green juice as feed for pigs, green protein as feed for chicken and white protein as food for humans as related to their amino acid composition. However, some biomass sources e.g. hemp, showed high levels of nitrate that are not suitabe to be useful as feed and food. To conclude, the majority of the biomass sources evaluated here, has the potential in a biorefinery concept to be used as feed and food, although, content of antinutritional components, feasibility of the process, and uses of the brown juice have to be further evaluated before final industrial applications are possible.

Fig.2 The first part of the process in the pilot facility, where the material is fed into a washer before being further processed. (Photo by Anna-Lovisa Nynäs).

Superabsorbents contribute the highest CO_2 emission share in a biobased diaper. Recycling of reagents for production of biobased superabsorbents reduces CO_2 emission by 50%. Only a few biobased diapers resulted in lower CO_2 emissions than fossil-based ones. Biodegradable diapers are superior from a circular perspective. Biobased superabsorbents are currently on a low TRL and need to be developed.

Faba bean based edible inks for food 3D printing have been successfully produced. The effect of starch/protein and fiber/protein ratio on texture and microstructure of mixed faba bean gels has been investigated. At high starch concentrations, replacing starch with protein resulted in lower viscosity and weaker gels. However, at high protein concentrations, replacing protein with starch/fiber can create inhomogeneities in the protein network resulting in weaker gels (Fig. 3).

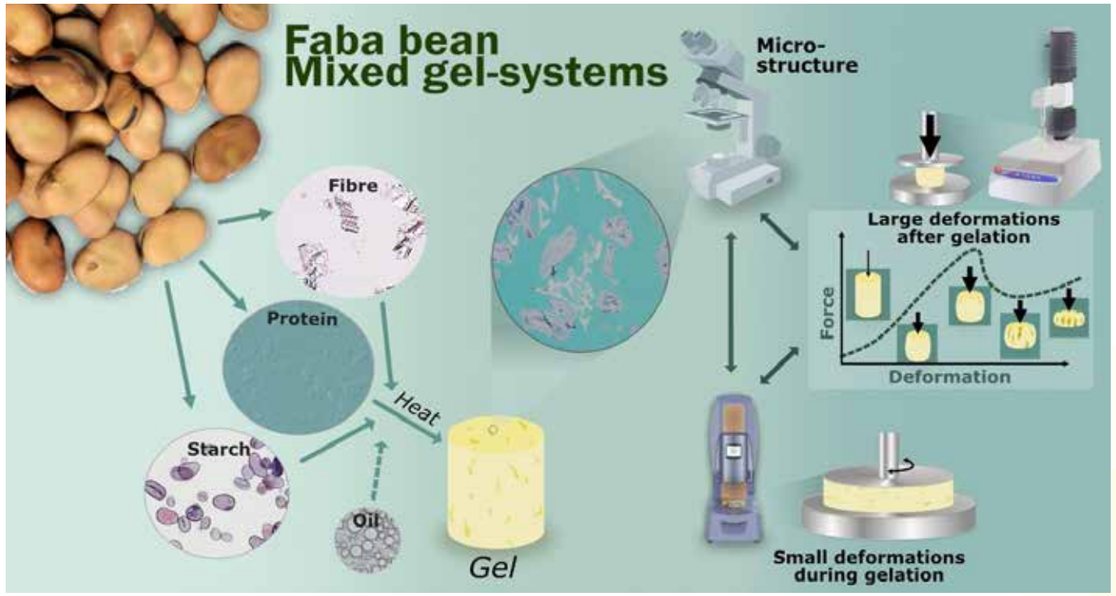

Fig. 3. Visual overview of studies performed on faba bean mixed gel systems. Starting from the left with the extraction of fractions from faba beans before combining with water in different ratios and heating to create gels that are characterized by microscopy and large and small deformations. (Figure by Mathias Johansson)

Improved protocol of how to produce protein nanofibrils (PNFs) from mung bean, how they are affected by food-related pH and how they can improve foam stability when compared to non-fibrillated protein from the same source was published. The result showed that when adding salt during protein extraction a more pure protein isolate could be produced that formed longer PNFs than observed before when using a protein isolate extracted with only isoelectric precipitation. The curved morphology of the PNFs was affected by increased pH, the morphology remained at pH 7 but the PNFs were a bit shorter. Curved PNFs from mung bean form more stable foams that have non-fibrillated protein even at low concentrations as 1 mg/mL.

The major progress in the plant model protein systems studied towards industrial end-uses included testing of the innovative processing of two protein rich systems and comparing pre-processed materials versus control ones. We observed clear differences between the variants differing in genetical make-up which showed different functional properties of the materials. New results obtained in the area of yet unexplored diverse genetic background having legumes, which might be very helpful in designing new protein-rich foods (Fig. 4). We also obtained new results on industrial wheat that was exposed to drought and Cd stresses in controlled conditions and we were able for the first time in non-destructive manner monitor the stress impact on plant roots.

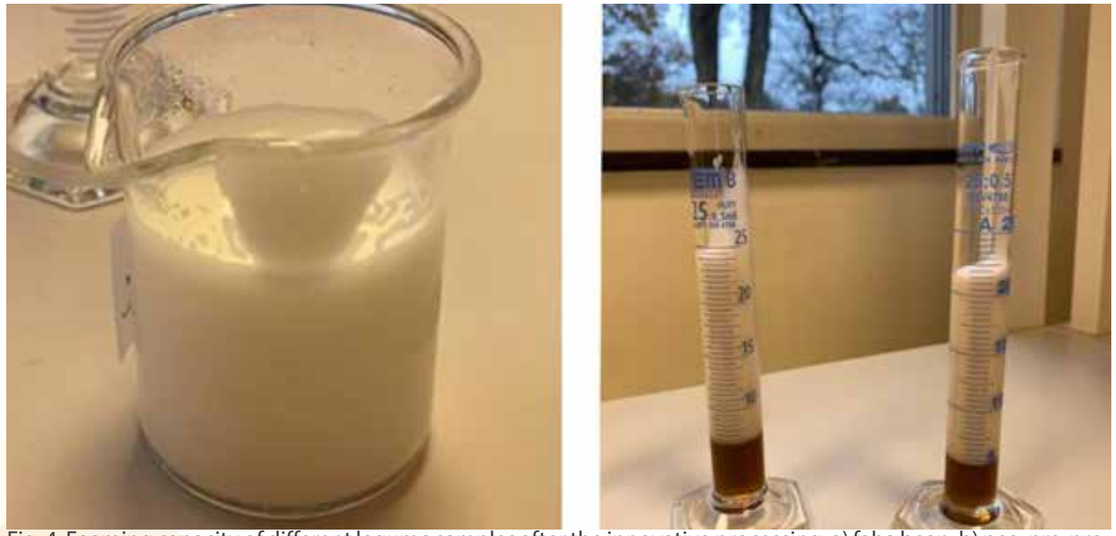

Fig. 4. Foaming capacity of different legume samples after the innovative processing; a) faba bean, b) pea, pre-processed (left) and not processed (right). (Photos by Faraz Muneer).

This is a promising approach to better understand the climate and Cd stress impact on wheat development and finding strategies to deal with the current climate change and Cd accumulation problems.

The work on CRISPR/Cas9-edited starch in potato has resulted in one manuscript that was recently accepted for publication. An important finding was that the enthalpy of gelatinisation and retrogradation was favored by amylopectin branching density. PhD student Shishanthi is compiling data for another paper about potato starch with simultaneous mutations in *SBE* and *GBSS* genes. We are also planning the last part of her thesis work that will focus on material properties that will be carried out in collaboration with a group at KTH. Shishanthi has completed and published a review article about starch-based blends and composites for bioplastics applications to obtain a good basis for the design of the material characterizations.

In carbon allocation, two articles were published. One article deals with proteomics in relation to oil in Cyperus that showed yellow nutsedge tubers to group with oilseeds and another is about oil induction in wheat endosperm with seed X-ray imaging. A manuscript on *WRINKLED1* overexpression in rice was submitted. Five additional manuscripts are in various stages of preparation, on evolution of oil accumulation in Cyperus and on heterologous characterization of transcription factor interactions promoting oil accumulation.

In pheromones three manuscripts were published. One manuscript validated the whole production process of pheromone precursors (Fig. 5) from field trial as oil constituents including their processing and evidenced application in pest control.

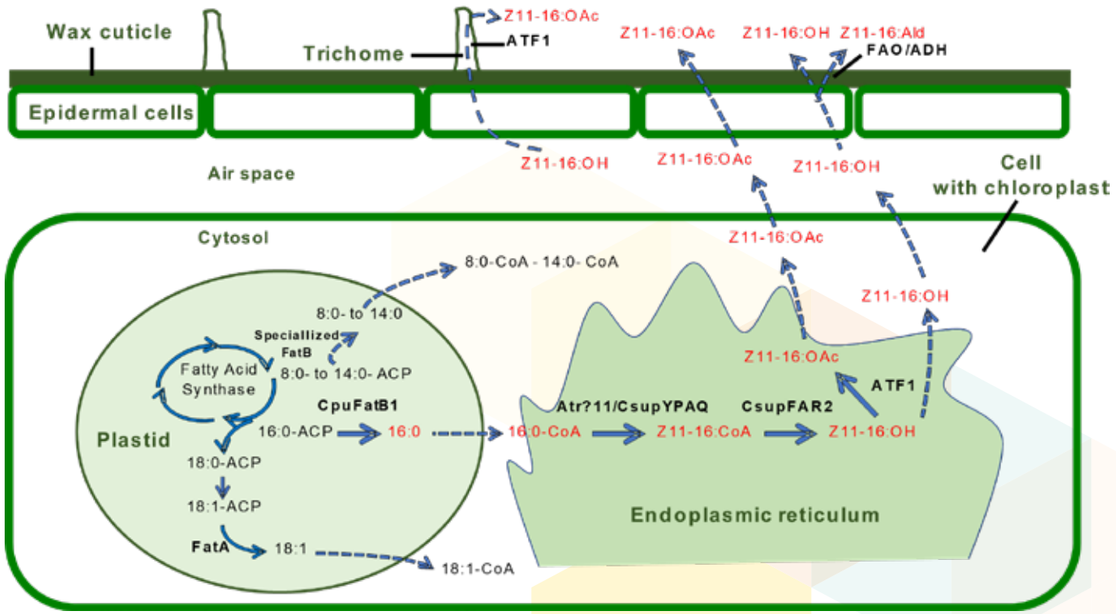

Fig. 5. Rapid assembly of pheromone biosynthetic pathway in Nicotiana benthamiana for the production and release of moth sex pheromone components. The Cauliflower mosaic virus 35S promoter (p35S) and Octopine Synthase gene terminator (tOCS) have been used to regulate gene expression in plants. ATF1 has also been controlled by trichome specific promoter pCYP71D16. (a) Step-wise metabolic engineering strategy for leaf-based pheromone production of (Z)-11-hexadecenol, (Z)-11-hexadecenal and (Z)-11-hexadecenyl acetate. (Figure by Yihan Xia)

Efficient protoplast regeneration protocols developed by us for genome editing by CRISPR/Cas9 for field cress and rapeseed are routinely used in our lab for generating transgene-free edited lines on the target genes. We have now generated more edited lines of rapeseed in order to have more of the 12 transporter (*GTR*) genes mutated simultaneously in the edited lines for effectively reducing the seed glucosinolate (GSL) level and have further evaluated some of the edited *GTR* lines in both species. Due to the difference in ploidy level, it is much easier to get homozygous lines for field cress than rapeseed. Screening for homozygous lines of rapeseed with editions in different target genes is still ongoing (Fig. 6).

The results on field cress showed that knocking out of *GTR*1 or *GTR*2 gene resulted in significantly reduced GSL contents in the seed of the mutants and preliminary results showed a stunted phenotype with a reduced seed yield. A number of mutation lines targeting on the genes regulating the phytic acid content in rapeseed have been obtained which are grown in biotron for obtaining homozygous lines. Knocking out of important genes affecting oil fatty acid profile in field cress resulted in significant increase in oleic acid level, but significantly reduced erucic acid level and the manuscript on this work was accepted for publishing.

Fig. 6. CRISPR-edited mutation lines of rapeseed grown in biotron (Photo by Li-Hua Zhu)

Timothy material was collected from a large-scale field trial (Fig. 7), started 2020, with 266 varieties to assess variation in forage quality parameters within and across seasons and geographic locations (Svalöv, Lövsta and Rödbäcksdalen). The experiments have been harvested three times (early, mid and late summer) and all harvested material have been collected, dried and shipped to Lantmännen Lantbruk AB. The samples have been ground and prepared for analyses by Lantmännen. At the moment, the first batch of samples are subjected near-infrared reflectance spectroscopy (NIRS) analysis by Lantmännen.
The results will tentatively be available later on regarding forage quality parameters - crude protein and fat content, total fibre content, cellulose and lignin content, sugar content, digestibility of organic matter, metabolic energy and net energy for lactation.

In the autophagy project,

1) we made a massive progress in revealing time-resolved organ-specific dynamics of plant autophagy under three different types of stress (autophagy-inducing drug treatment, mimicking amino acid starvation; depletion of nitrogen or carbon).
2) We demonstrated that indeed plant autophagy is fine tuned to physiology of individual organs.

3) We demonstrated that autophagic activity previously considered as non-selective (i.e. "bulk") has selectivity that allows protection of essential cellular components even under severe stress-inducing conditions. This finding will be crucial to further elucidate previously hidden functions of a number of cellular components.

4) We have established a state of the art method that allowed us to isolate intact plant vacuoles together with trapped cargo delivered there by autophagy for degradation. We secured funding at the EPIC-XS facility (European facility for proteomics) to perform quantitative LC-MS analysis of the proteins contained within the purified autophagic cargo. Our samples are currently being processed at the facility in Ghent, Belgium.

5) We have discovered one of the core regulatory steps being significantly different in plant autophagy when compared to animal and fungal autophagy. This finding opens a new perspective on how plant autophagy might have co-evolved with plant endomembrane trafficking system.

6) We have established transgenic material and initial protocols required for further elucidation of how plant autophagy regulates population of organelles controlling response to oxidative stress, peroxisomes (Fig. 8). This project is run by Florentine Ballhaus, who was hired as a PhD student in our group on the April 1st, 2022.

7) We have also initiated the part of the project related to the potential role of autophagy in microspore embryogenesis. In our previous study we identified new small organic molecules specifically regulating plant autophagy and now have established a plan on testing those compounds for their capacity to enhance efficiency of microspore embryogenesis in calcitrant cultivars of *Brassica napus*. This work is planned in collaboration with our colleagues at the Department of Plant Breeding in Alnarp and from other European institutions.

Fig. 7. Field trial of timothy (Photo by Pär Ingvarsson)

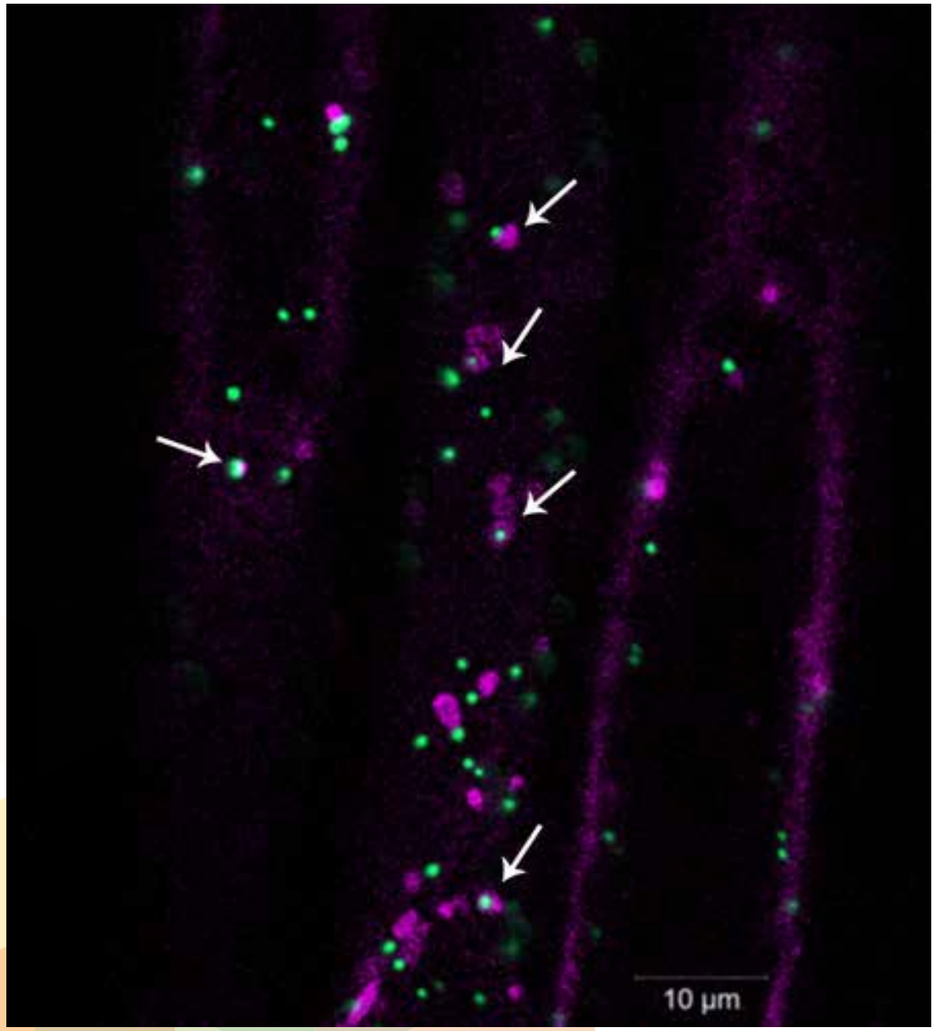

Fig. 8. Pexophagy, selective degradation of peroxisomes by autophagy. Confocal microscopy image of epidermal root cells expressing marker for autophagic structures (magenta) and marker for peroxisomes (green). Arabidopsis plants were subjected to salt stress that triggered proliferation of peroxisomes, organelles helping plants to cope with various types of abiotic stresses, including high salt. After the stress trigger was removed, plant cells decreased the population of peroxisomes by degrading the surplus of these organelles via selective autophagy, i.e pexophagy. White arrows point to autophagic structures engulfing overabundant peroxisomes to deliver them for degradation. Understanding how plant cells maintain the population of peroxisomes in various cell types will be an essential step in developing plants more tolerant to high soil salinity, elevated air temperatures and increased sun light.

In what way the research has contributed to social benefit

Within C4F program, some projects are closely connected to or have been transferred to UDIs or EIPs, one way to transfer TC4F knowledge into product-based projects, thus benefiting to the society. Whereas some other projects have potential significant social benefits, thus benefiting the society in long-run.

Opportunities to produce proteins for food, feed and material locally from green biomass will have a direct and strong social impact.

The use of biostimulants is seen as a novel and creative way to promote plant growth and replace part of the chemical input to agriculture. If successful biostimulants are produced and used in agriculture, this will contribute to novel job opportunities and also reduce the risks with using chemical inputs.

The research produced for the innovatively processed protein systems is contributing to society in different new ways to improve legume use in new protein-rich food applications. A popular science report about the research on Cd stress impact on the Swedish wheat has been available on-line for public.

The starch research aims to improve product quality in food as well as non-food applications. Our research on retrogradation can in the long run reduce food waste by prolonging product shelf life.

Good knowledge about the relationships between genetics, structure and properties is also vital for designing functional and sustainable materials that may be used in future food packages.

Insect pheromones for pest management is non-toxic and produced from a renewable resource contrasting to pesticides. Camelina derived pheromone blends have been proven to work in the field.

Faba beans, oat, peas, rapeseed, field cress, potato are Swedish crops with a good nutritional profile, which can be a good plant based protein alternative to soy-products and gluten. Legumes and field cress can help with nitrogen fixation or prevent from nutrient leaching when incorporated into an intercropping system, which will thus enrich the soil and reduce over fertilization.

Novel CRISPR-edited lines of oilseed crops with improved oil and protein or seedcake qualities would in long-run contribute to increased plant oil production and improved seedcake quality as a source of high value protein for food, feed and industrial uses, and consequently reducing the fossil use and benefiting the human health and environment.

Diapers has a high carbon foot-print and if more sustainable alternatives can be developed, this will have a huge impact on the society.

How does C4F take basic research to application

The majority of the C4F projects are more orientated in applied research, in which we make our great efforts on transferring the knowledge obtained from basic research in oil, protein and starch as well as material science into potential food, feed and industrial applications in one way or another. Some examples are as followings:

1. The work on biostimulant uses on sugarbeet span from basic research on mechanisms in plants to application in novel biostimulants being used in agriculture.
2. Understanding the chemical background of superabsorbent capacity of functionalized proteins require basic research while the development of diapers for children or old people takes the project out to the reality.
3. We try to understand underlying mechanisms that determines protein extractability in green leaf, which is basic science. The results will though be used for production of local plant protein for food and feed which is a direct application of the research.
4. Wheat gluten quality for breeding climate stable wheat
5. Cd stress in wheat- management strategies
6. By understanding the structure and properties of the different fractions; protein , starch and fibre new foods can be developed and optimised. Mixtures will also be valuable to show how pure fractions is needed. Previous knowledge on the structure and behaviour of the faba bean fractions was used in the bio-ink development in the 3D-printing project.
7. Starch is a useful part in a composite material where it can provide oxygen barrier functionality and something else gives strength and water resistance. Starch with increased amylose content has nutritional benefits since it has more slow carbohydrates that potentially can decrease our insulin response and thereby reduce the risk to develop type II diabetes.
8. Pheromones from a plant production source for pest management is a good example of going from basic science on specific pheromone compounds and their genetic background in e.g. moths to applications in production and pest management. In 2022 the whole chain from biosynthesis to production via extraction and final pest interruption was shown under field conditions and published in Nature Sustainability.
9. Identified target genes in model species or the same species from basic research have been used in improving target traits in oil crops by CRISPR/Cas9.
10. CRISPR/Cas9 edited plant material was studied for bio-material applications

Vice program leader Li-Hua Zhu

Faraz Muneer

A protein researcher contributing to the Swedish food transition

Already as a master student Faraz Muneer got interested in how left over materials from plants can be used instead of simply ending up as waste and also industrial by-products of plant processing industries. Today he is doing research on the properties of locally produced proteins sources to produce highly nutritious food products.

In his research Faraz explores the plant materials that are wasted in agricultural systems, for example, when broccoli is harvested, around 70 percent of the plant is normally not used. He and his colleagues take these materials and extract proteins from it. They also get fibers and so-called brown juice as side products in the process. This brown juice can be turned into food, feed, bioplastics, biogas and fertilizers, Faraz Muneer explains.

While different proteins can be used for different things, it is also possible to modify the properties of proteins, either by processing them after the harvest or by doing plant breeding to make the plants produce the desired proteins. The focus of Faraz' team is to improve the processing of proteins, to get more proteins out, improve the functionality of the proteins and the efficiency of the whole process. They can for example add mechanical energy to break the proteins apart and make them more useable. This research is important since the more proteins we can get from our own fields in Sweden, the less we must import from countries far away.

More than 50 percent of the food consumed here is imported. The war in Ukraine and COVID-19 have affected the food supply chain, the food prices are increasing, and we need a long-term strategy for our food production. This has triggered Faraz to look at new alternatives. How can the local production of food in Sweden be increased, and how can research contribute to that.

Developing new plant-based materials instead of producing synthetic materials is also a step towards a more sustainable production. For example, by developing super absorbing materials from plants the synthetic materials that are used for example in diapers can be replaced.

In recent years Faraz Muneer has experienced a personal development as a researcher, going from mostly being interested in the basics of protein research to become more interested in the bigger picture. Looking more broadly at science, questions that interest him now are:

"How can we improve humanity and the problems we are facing now? What can be solved by research? How can we integrate different factors when we are doing research? "

Facts

Faraz Muneer came from Pakistan to Sweden in 2009 to study at SLU. As a PhD student here he used plant proteins to make bioplastics. As a researcher he explores how proteins from different plants can be used as food and animal feed. He also study how plants can be used to produce absorbent materials for the medical sector or hygiene products. He finds out how the proteins function and how their structure impact the properties of food or materials. He is into sports and spend a lot of his spare time playing cricket in a club in Malmö.

Author: Lisa Beste

Image Credits: Faraz Muneer and artverau via pixabay

C4F - Crops for the Future

Scientific publications

1. Andreasson E, Kieu N, Zahid MA, Carlsen F, Lenman M, **Sandgrind S**, et al. Invited Mini-Review Research Topic: Utilization of Protoplasts to Facilitate Gene Editing in Plants: Schemes for In Vitro Shoot Regeneration From Tissues and Protoplasts of Potato and Rapeseed: Implications of Bioengineering Such as Gene Editing of Broad-Leaved Plants. Frontiers in Genome Editing. 2022;4:780004.

2. Bettelli MA, Capezza AJ, Nilsson F, **Johansson E**, Olsson RT, Hedenqvist MS. Sustainable Wheat Protein Biofoams: Dry Upscalable Extrusion at Low Temperature. Biomacromolecules. 2022;23(12):5116-26.

3. **Demski K**, Ding B-J, Wang H-L, Tran TNT, Durrett TP, **Lager I**, **et al.** Manufacturing specialized wax esters in plants. Metabolic Engineering. 2022;72:391-402.

4. Fei M, Jin Y, Hu J, Dotsenko G, Ruan Y, Liu C, **et al.** Achieving of high-diet-fiber barley via managing fructan hydrolysis. Scientific Reports. 2022;12(1):19151.

5. **Jayarathna S**, **Andersson M,** Andersson R. Recent Advances in Starch-Based Blends and Composites for Bioplastics Applications. Polymers [Internet]. 2022; 14(21).

6. **Johansson M**, Johansson D, Ström A, Rydén J, **Nilsson K**, Karlsson J, et al. Effect of starch and fibre on faba bean protein gel characteristics. Food Hydrocolloids. 2022;131:107741.

7. **Johansson M**, **Nilsson K**, Knab F, **Langton M**. Faba Bean Fractions for 3D Printing of Protein-, Starch- and Fibre-Rich Foods. Processes [Internet]. 2022; 10(3).

8. **Jolayemi OL**, Malik AH, Ekblad T, Fredlund K, **Olsson ME, Johansson E**. Protein-Based Biostimulants to Enhance Plant Growth—State-of-the-Art and Future Direction with Sugar Beet as an Example. Agronomy. 2022;12(12):3211.

9. **Lama S**, Vallenback P, Hall SA, Kuzmenkova M, **Kuktaite R**. Prolonged heat and drought versus cool climate on the Swedish spring wheat breeding lines: Impact on the gluten protein quality and grain microstructure. Food and Energy Security. 2022;11(2):e376.

10. **Lan Y**, Chawade A, **Kuktaite R**, Johansson E. Climate Change Impact on Wheat Performance—Effects on Vigour, Plant Traits and Yield from Early and Late Drought Stress in Diverse Lines. International Journal of Molecular Sciences. 2022;23(6):3333.

11. **Markgren J**, Rasheed F, Hedenqvist MS, Skepö M, **Johansson E**. Clustering and cross-linking of the wheat storage protein α-gliadin: A combined experimental and theoretical approach. International Journal of Biological Macromolecules. 2022;211:592-615.

12. **Muneer F**, Hedenqvist MS, Hall S, **Kuktaite R**. Innovative Green Way to Design Biobased Electrospun Fibers from Wheat Gluten and These Fibers' Potential as Absorbents of Biofluids. ACS Environmental Au. 2022;2(3):232-41.

13. Niemeyer PW, Irisarri I, Scholz P, Schmitt K, Valerius O, Braus GH, **et al**. A seed-like proteome in oil-rich tubers. The Plant Journal. 2022;112(2):518-34.

14. **Nilsson K**, Sandström C, Özeren HD, Vilaplana F, Hedenqvist M, **Langton M**. Physiochemical and thermal characterisation of faba bean starch. Journal of Food Measurement and Characterization. 2022;16(6):4470-85.

15. **Snell P**, Wilkinson M, Taylor GJ, Hall S, **Sharma S**, Sirijovski N, **et al**. Characterisation of Grains and Flour Fractions from Field Grown Transgenic Oil-Accumulating Wheat Expressing Oat WRI1. Plants. 2022;11(7):889.

16. Statkevičiūtė G, Liatukas Ž, Cesevičienė J, Jaškūnė K, Armonienė R, **Kuktaite R**, et al. Impact of Combined Drought and Heat Stress and Nitrogen on Winter Wheat Productivity and End-Use Quality. Agronomy. 2022;12(6):1452.

17. Wang H-L, Ding B-J, Dai J-Q, Nazarenus TJ, Borges R, Mafra-Neto A, **et al.** Insect pest management with sex pheromone precursors from engineered oilseed plants. Nature Sustainability. 2022;5(11):981-90.

18. Xia Y-H, Ding B-J, Dong S-L, Wang H-L, **Hofvander P**, Löfstedt C. Release of moth pheromone compounds from Nicotiana benthamiana upon transient expression of heterologous biosynthetic genes. BMC Biology. 2022;20(1):80.

Popular scientific publications (reports etc)

https://www.vinnova.se/globalassets/mikrosajter/storskalig-forskningsinfrastruktur/dokument/slutrapporter-ensidingar/2020-00830_slu-phd-lan-yuzhou-.docx.pdf

Interviews and presence in media

- Interview about Climate smart wheat in Livsmedel i focus (link is under request of the journalist).

- De tar genvägen via växter för att lura insekter (2022) https://sverigesradio.se/artikel/de-tar-genvagen-via-vaxter-for-att-lura-insekter

Scientific presentations

- Muneer F. 2022. Applications of plant proteins in materials and food. Oral presentation, C4F workshop, Malmö, Sweden, November 29.

- Johansson, M., Johansson, D., Ström, A., Rydén, J., Nilsson, K., Karlsson, J., ... & Langton, M. 2022, Effect of starch and fibre on faba bean protein gel characteristics. Poster presentation at the conference Healthy, Safe, and Sustainable Foods of the Future, DTU, Copenhagen, Denmark, October 13th.

- Johansson, M., Johansson, D., Ström, A., Rydén, J., Nilsson, K., Karlsson, J., ... & Langton, M. 2022, Effect of starch and fibre on faba bean protein gel characteristics. Poster at the conference Data Driven Food Research & Innovation (Food Science Sweden), online, March 15th.

- Johansson, M., Nilsson, K., Knab, F., & Langton, M. 2022. Faba Bean Fractions for 3D Printing of Protein-, Starch- and Fibre-Rich Foods. Presentation at the SLU lunch seminar series "Värt att veta", online February 3rd, 2022. Video recording available at: https://play.slu.se/media/Worth+KnowingA+Faba+bean+fractions+for+3D-printing+of+protein-+and+fibre-rich+foods/0_3721h6v1

- Holla S., Bozhkov P.V., Schumacher K, Minina E.A. 2022. "Plant-specific role of cysteine protease atg4 in autophagy". A talk at the 5th Plant Proteases Conference in Ljubljana, Slovenia September.

- Ballhaus F., Hicks G.H., Sabljić I., Bozhkov P.V., Dauphinee A.N. 2022 . "Unravelling plant autophagy mechanisms using chemical genetics". A speed talk and poster presentation at the Conference of the Scandinavian Plant Physiology Society in Longyearbyen, Svalbard in August/September.

- Sandgrind S., Li X., Ivarson E., Wang E-S., Guan R., Kanagarajan S., and Zhu L.-H. 2022. CRISPR/Cas9 gene editing of the novel oil and cover crop, Lepidium camestre, for improvement of oil quality. Oral presentation. 25th International Symposium on Plant Lipids. July 10th-15th, 2022, Grenoble, France.

- Moss O., Li X., Kanagarajan S., Guan R., Ivarson E. and Zhu L-H. 2022. Genome editing of rapeseed by CRISPR/Cas9 for reducing phytic acid content. Poster presentation. 25th International Symposium on Plant Lipids. July 10th-15th, 2022, Grenoble, France.

- Sandgrind S., Li X., Ivarson E., Wang E-S., Guan R., Kanagarajan S., and Zhu L.-H. 2022. Domestication of the potential novel oilseed crop Lepidium campestre for the Nordic climate by gene editing. Oral presentation. The SPPS conference 2022. 30th of August - 2nd of September. Longyearbyen, Svalbard, Norway.

- Moss O., Li X., Kanagarajan S., Guan R., Ivarson E. and Zhu L-H. 2022. Genome Editing of Rapeseed by CRISPR/Cas9 for Reducing Phytic Acid Content. Poster presentation. The SPPS conference 2022. 30th of August - 2nd of September. Longyearbyen, Svalbard, Norway.

- Moss O. 2022. Genome editing of rapeseed for reducing antinutritional compounds in the seed. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Sharma S. 2022. Carbon channelling of storage compounds in sink tissues. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Kuktaite R. 2022. Climate impact on the gluten polymers evaluated by a proteomic Approach. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Jolayemi O.L. 2022. Effect of equal N from PBB and chemical source on growth and physiology of sugar beet. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Jayarathna S. 2022. New starch for novel applications. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Lan Y. 2022. The impact of Cd and drought on wheat and study root architecture using neutron computed tomography. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Demskil K. 2022. Replacing Pesticides by Developing an Oilseed Platform for Insect Pheromone Production. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Klara Nilsson and Mathias Johansson have presented their work on 3D printing at the lunch seminar series "Värt att veta" at SLU https://play.slu.se/media/Worth+Knowing-A+Faba+bean+fractions+for+3D-printing+of+protein-+and+fibre-rich+foods/0_3721h6v1

- Jolayemi O.L. 2022 Comparative effect of equal N content from PBB and NS on agronomy, physiology and metabolism of sugar beet. Presentation at C4F annual workshop 29 November.

- Sandgrind S. 2022. Genome editing of Lepidium campestre using CRISPR/Cas9. Oral Presentation at C4F annual workshop 29 November

- Mathias Johansson. 2022. Legume based gels – texture and microstructure. Oral Presentation at C4F annual workshop 29 November

- Anna-Lovisa Nynäs. 2022. Presentation of the pilot facility at the PlantLink day in Alnarp, 5th of October.

- Anna-Lovisa Nynäs. 2022. Proteins from green biomass – Food, feed and superabsorbents. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Chala G.B. 2022. Assessing forage quality parameters in Timothy to enable breeding of high-quality forage with minimal environmental impact. Oral presentation, C4F workshop, November 29, Malmö, Sweden.

- Minina E. A. 2022. Recruiting plant autophagy for developing smart crops. . Oral presentation, C4F workshop, November 29, Malmö, Sweden.

Collaboration with industry or other parts of society

- Lantmännen
- Gasum
- Oriflame
- Grönsaksmästarna
- Region Skåne
- Lilla Harrie Valskvarn
- Ly Lyckeby Stärkelse AB
- Orkla
- Havredals Biodevelop AB
- RISE
- KTH
- Chalmers
- KI
- Sveriges Stärkelseproducenter Förening
- DLF Beet Seed AB
- Kalmar Ölands Trädgårdsprodukter
- Findus

- FoodHills and ISCA Technologies
- Lund University
- Gunnarshögs Gård AB
- Syngenta
- Planta LLC
- SLU Grogrund – a number of research projects connected to the C4F program
- Nelson Seed

Other funding that has been received partially or fully due to the TC4F research

- Novo Nordisk Foundation, Synthetic Wax Esters from Plants - A Green Source of the Pivotal Feedstock. 2.5 mil DKK, Kamil Demski main applicant

- Formas, Breeding oilseed rape (Brassica napus L.) for durable disease resistance and improved seed cake nutritional quality, 3 mil. SEK, Selvaraju Kanagarajan and Lihua Zhu, main and co-applicants.

- Formas, Unlocking plant protein and CRISPR potato to starch novel uses: Design of fibre-based absorbents with tailored functionalities. Ramune Kuktaite, main applicant.

- VR, Increasing food security and decreasing malnutrition via innovation agro-platforms, crop breeding, and novel food processing of common beans in Uganda. 2.99 mil. Ramune Kuktaite, main applicant.

- Formas, Nutritious, tasty and health-promoting novel wheat products in research infrastructure, 6 mil, Eva Johansson, main applicant.

- Formas, A strong pulse - resilient Swedish grain legume supply from field and factory to fork. 5.99 mil. Maud Langton, main applicant.

Education

a) PhD theses, MSc theses, Bachelor theses

Herneke, Anja. (Female) 2022. Doctoral thesis: Plant protein nanofibrils: characterising properties for future food. Uppsala: Sveriges lantbruksuniversitet. Acta Universitatis Agriculturae Sueciae, 2022:44. ISBN: 978-91-7760-963-6. Swedish University of Agricultural Sciences.

Sjur Sandgrind (Male) 2022. Doctoral thesis: Genome editing of oilseed species by CRISPR/Cas9 for trait improvement. Lomma: Sveriges lantbruksuniversitet. Acta Universitatis Agriculturae Sueciae, 2022:77. ISBN: 978-91-8046-030-9, eISBN: 978-91-8046-031-6. Swedish University of Agricultural Sciences.

Elander, Pernilla (Female) 2022. Doctoral thesis: Living through hard times: Dispose of or sequester? Plant subcellular strategies for stress resilience. Uppsala: Acta Universitatis Agriculturae Sueciae, 2022:60. ISBN: 978-91-7760-995-7, eISBN: 978-91-7760-996-4. SLU. (https://res.slu.se/id/publ/119473). Swedish University of Agricultural Sciences.

Evelyn Elizabeth Villanueva Gutierrez (Female). 2022. Doctoral thesis: Bolivian tomatoes: genetic diversity, quality traits and value chains. Lomma: Sveriges lantbruksuniversitet. Acta Universitatis Agriculturae Sueciae, 2022:62. ISBN: 978-91-8046-000-2, eISBN: 978-91-8046-001-9.

b) Supervision and teaching (include supervision of finished and on-going students, include teaching and organization of courses)

LMuneer, Faraz. Main supervisor for Intern-student Vandamme, Lilou. Title: Processing of faba bean and pea protein to improve their functional properties for food application. 31st Oct to 23rd December, 2022.

Kuktaite, Ramune. Grogrund Workshop organizer and teacher "Product quality in PLS0089 Workshop Series II – Methods in Modern Plant Breeding", Dec 2, 2022, Alnarp, Sweden.

Kuktaite, Ramune. Teaching at the course "Plant biology for breeding and protection (BI1296), Alnarp.

Herneke, Anja. Teaching at the course "Food Technology", (LV0112) 15 ECTS, Uppsala

Johansson, Mathias. Lab supervisor at the course Food Chemistry and Food Physics (LVO110), 15 ECTS, Uppsala

Langton, Maud Main supervisor for PhD-student Herneke, Anja. Title: Functionalization of nanofibers from plant based proteins

Langton, Maud Main supervisor for PhD-student Johansson, Mathias. Title: Legume based Gels – Microstructure and Texture

Hofvander, Per. Supervisor for PostDoc Shrikant Sharma

Hofvander, Per. Supervisor for PostDoc Kamil Demski

Zhu, Li-Hua. Supervisor for PhD candidate Sjur Sandgren. Tentative title: Genome editing of oil crops. Expected date for dissertation: 2022.

Zhu, Li-Hua. Supervisor for PhD candidate Oliver Moss. Tentative title: Improvement of seedcake quality of rapeseed for high quality food and food uses. Expected date for dissertation: 2025.

Roger Andersson. Main supervisor for PhD-candidate Shishanthi Jayarathna. Tentative title: New starch for novel applications. Expected date for dissertation: January, 2024.

Kanagarajan, Selvaraju. Co-supervisor for PhD candidate Sjur Sandgrind. Tentative title: Genome editing of oil crops. Expected date for dissertation: 2022.

Kanagarajan, Selvaraju. Supervisor for PhD candidate Oliver Moss. Tentative title: Improvement of seedcake quality of rapeseed for high quality food and food uses. Expected date for dissertation: 2025.

Kuktaite, Ramune. Main supervisor for PhD-candidate Sbatie, Lama. Tentative title: Wheat quality in a varying climate. Expected date for dissertation: June, 2023.

Minina, EA. Course organizer and teacher at the course "Real Time Quantitative PCR – theory, experimental design and data analysis", (PNS0215), 3.5 ECTS, SLU.

Roger Andersson. Course organizer and teaching at the course "Plant food science", (LV0113), 15 ECTS, SLU.

Hofvander, Per. Teaching at the course "Applied Plant Biotechnology" (BI1344), Alnarp.

Hofvander, Per. Teaching at the course "Sustainable plant production" (BI1295), Alnarp

Sharma, Shrikant. Teaching at the course "Växters kemi och biokemi" (KE0070), Alnarp.

Grimberg, Åsa. Teaching at the course "Växtförädling och växtfysiologi" (BI1367), Alnarp.

Grimberg, Åsa. Teaching at the course "Odling och kvalitet" (TD0010), Alnarp.

Grimberg, Åsa. Teaching at the course "Advanced plant breeding and genetic resources" (BI1345), Alnarp.

Grimberg, Åsa. Teaching at the course "Växters kemi och biokemi" (KE0070), Alnarp.

Andersson, Mariette. Teaching at the course "Advanced plant breeding and genetic resources" (BI1345), Alnarp.

Lager, Ida. Course leader and teaching at the course "Växters kemi och biokemi" (KE0070), Alnarp

Zhu, Li-Hua. Course organiser and teaching at the course "Advanced Plant Breeding and Genetic Resources" (BI1345), Alnarp

Zhu, Li-Hua. Course organiser and teaching at the course "Applied Plant Biotechnology" (BI1344), Alnarp.

Alyona Minina. Main supervisor for PhD-candidate Holla, Sanjana. Tentative title: Revealing the dynamics of plant autophagy. Expected date for dissertation: September 2023.

Alyona Minina. Main supervisor for PhD-candidate Ballhaus Florentine. Tentative title: Membrane-bound and membraneless organelles in plant stress response. Expected date for dissertation: September 2026.

Alyona Minina. Main supervisor for project student Kjellstrom, Jarl. Title: Use of Fungal bioluminescence pathway as a reporter for plant autophagy. 15 ECTS.

Alyona Minina. Lecture on advanced microscopy methods for the Masters degree course at Uppsala University "Genetic and Molecular Plant Science" (15 ECTS), September-October 2022

Alyona Minina. Organizer and teacher of the qPCR course (3.5 HEC, P000008 F0027) for the Organism Biology PhD School. SLU, Uppsala. November-December 2022

Florentine Ballhaus. Lab and seminar teacher for the Masters degree course at Uppsala University "Genetic and Molecular Plant Science" (15 ECTS), September-October 2022

Zhu, Li-Hua. Supervisor for PhD candidate Sjur Sandgren. Tentative title: Genome editing of oil crops. Expected date for dissertation: 2022.

Zhu, Li-Hua. Supervisor for PhD candidate Oliver Moss. Tentative title: Improvement of seedcake quality of rapeseed for high quality food and food uses. Expected date for dissertation: 2025.

C4F- Crops for the Future, Personnel

Name	Gender & Position	Part of full time financed by TC4F
Eva Johansson	F, Professor	15%
Li-Hua Zhu	F, Professor	10%
Maud Langton	F, Professor	0
Roger Andersson	M, Professor	0
Pär Ingvarsson	M, Professor	0
Anne-Maj Gustavsson	F, Docent	0
Anna Westerbergh	F, Docent	0
Thomas Prade	M, Docent	0
Galia Zamaratskaia	F, Ass. Prof.	0
Mariette Andersson	F, Researcher	0
Ramune Kuktaite	F, Researcher	0
Åsa Grimberg	F, Researcher	0
Ida Lager	F, Researcher	0
Selvaraju Kanagarajan	M, Researcher	8%
Alyona Minina	F, ass. Prof.	35%
Per Hofvander	M, Researcher	0
Girma Bedada Chala	M, Researcher	25
Sjur Sandgrind	M, PhD student	50%
Faraz Muneer	M, postdoc	100%
Kamil Demski	M, Postdoc	40%
Shrikant Sharma	M, Postdoc	40%
Adrian Dauphinee	M, Postdoc	0%
Florentine Ballhaus	F, PhD student	35%
Anna-Lovisa Nynäs	F, PhD student	50%
Oliver Moss	M, PhD student	50%
Anja Herneke	F, PhD student	0
Sanjana Holla	F, PhD student	0
Sbatie Lama	F, PhD student	0
Shishanthi Jayarathna	F, PhD student	30%
Mathias Johansson	M, PhD student	0
Lan Yuzhou	M, PhD student	25%
Lekan Jolayemi	M, PhD student	50%
Linda Öhlund (Lantmännen)	F, Foragebreeder	0
Xueyuan Li	M, Research assistant	0

*Researchers listed with 0% have received financing from TC4F earlier which resulted in projects with independent financing.

TC4F Economy 2022

In 2022, TC4F received 28,8 mio SEK of funding which were distributed according to the budget of which 88% were used. The surplus was caused by delays in recruitment due to the Covid-19 pandemic.

	SLU	UmU	Skogforsk	Total
Distributed Funds (tkr)				
Coordination	2969			2969
Plant Physiology (UMU)		5100		5 100
Forest Genetics and Plant Physiology	4 454*			4 454
Southern Swedish Forest	4 454*			4 454
Forest Ecology and Management	3 504			3 504
Wildlife, Fish and Environmental Studies	0*			0
Skogforsk			1 100	1 100
C4F (LTV)	7194			7194
TOTAL	22 575	5 100	1 100	28 775
Costs, spent funds (tkr)				
Coordination	846			846
Plant Physiology (UMU)		5100		5100
Forest Genetics and Plant Physiology	1 947			1 947
Southern Swedish Forest	5 065			5 046
Forest Ecology and Management	3 946			3 946
Wildlife, Fish and Environmental Studies	731			731
Skogforsk			1 100	1100
C4F (LTV)	6 709			6 709
Total	19 246	5100	1 100	25 449
PROFIT T4F	2 845	0	0	
PROFIT C4F	484			
Total PROFIT	3 329	0	0	3329

*assigned postdoc grants