



C4F-  
REPORTS  
AND  
RESEARCH  
PORTRAITS

## C4F- Crops for the Future

*C4F has functioned as a research platform, which has been supporting a number of research projects that are connected to other large projects or programs funded by other funding agencies. During 2023, one project was finished and five new projects have been started, which will involve a PhD student or postdoc. The overall progress of the research within C4F has gone smoothly in 2023. A number of peer-reviewed articles of high quality have been published, while a number of manuscripts submitted or in the pipeline for publication. New PhD students or postdocs have been recruited. The annual C4F workshop was held on the 7th of Dec. in Lund.*

The post-docs have been recruited to the Green diapers superabsorbents project and the research work has started. Samples to be used for the superabsorbents were collected in the protein factory and lab-work has been intensely going on during the autumn 2023 to produce materials to evaluate. Functionalisation was started on sugar beet green juice and the influence of the parameters (centrifugation, autoclave time, cross-linking and acylation) was taken into account.

The work on plant protein fractionation has been ongoing and has resulted in three peer-reviewed publications in 2023. Novel experiments have also been started on gluten and potato proteins for evaluating water absorbance capacity of the proteins. For the green diapers project, the influence of the parameters was considered to increase water absorbance capacity of biomaterials.

A lot of results have been finalized on genetic background for quality traits in wheat during 2023, which have resulted in four peer-reviewed publications. The students have also been intensely analyzing samples during 2023 which has resulted in finalizing papers which are expected to be published in 2024. Additionally, results from previous projects have been finalized and published within 2023 (papers 2, 4, 5)

The overall outcome of the two projects, green and model protein systems and MAX IV techniques, indicated a positive progress and highlighted a new knowledge on the Cd and drought stress impact on wheat development,

micro-nutrient mapping in wheat using synchrotron imaging tools and on the characterization of the legume protein functional behavior in food systems. Three manuscripts are in the pipeline.

For the protein structures in mixtures of legumes and cereals project, a new PhD student has been recruited and started the project by generating new knowledge in the development of novel sustainable food. Our research activities have resulted in more contact with industry and society. We are generating new products from local plants using different processing techniques, and characterizing these process effects on texture, which would result in the presentation of novel tasty and safe foods.

For the medium chain fatty acid platform in Camelina, linder enzymes were characterized. Camelina was transformed with genes derived from Linder for medium chain fatty acid synthesis. Camelina PacBio long read genomic sequencing was performed. Camelina genes as targets for CRISPR/Cas9 modification for enhancement of medium chain fatty acids were identified.

The synthetic wax esters from plants project was started this year. We have conducted first steps necessary to achieve our goal of producing seed wax ester-depositing Camelina sativa lines, which would substitute fossil-reserves dependent feedstock of wax esters. Camelina PacBio long read genomic sequencing was done together with the MCFA Camelina C4F project.

The potato tuber, sink and starch development project was started in 2023. Lead genes to study tuberization as well as starch yield and quality in potato were defined. Editing of the selected genes was made. Generated potato plants edited in various genes will be the basis for further studies of the mechanisms of interest.

For trait improvement of oil crops, more CRISPS-edited mutation lines of rapeseed with the target genes for improving the seedcake quality have been generated and some of them have been grown in T2 generation and some chemical analyses have been performed in the lines. Manuscripts about some results are in pipeline for publication. For the high-throughput analysis, some induced mutation lines of rapeseed have been grown in greenhouse for phenotypical analysis and for collecting DNA for molecular analysis.

Regarding the autophagy project, we have made a discovery that opens up new possibilities for manipulating plant autophagy to improve crop fitness. We are continuing our collaboration with Dr. Kim Boutilier at Wageningen University, focusing on stress-induced microspore embryogenesis in Brassica napus. We are deploying our recently identified modulators of plant autophagy as potential tools to enhance the efficacy of embryogenesis.

In the efforts to develop new Timothy varieties with high-quality forage and minimal environmental impacts, field trials and chemical analyses for 15 forage quality parameters were carried out as planned.

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 has contributed to generation of novel plant materials for further breeding or direct uses in product quality research and future potential applications, applications of novel technologies such as CRISPR-mediated genome editing, next generation sequencing and MAXIV techniques and 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 new projects in 2023 and some of them are connected to the C4F projects.



Fig. 1. Bulk centrifugation of precipitate from leaf juice at about 300L/hr. Incoming juice with precipitate on the left and precipitate free "brown juice" on the right. (Photo by William Newson).

## Detailed research findings and progress

Additives can be used to modulate quality of materials produced with plant-based proteins, resulting in polymerization of the proteins and improved functionality (Fig. 1). The chemistry behind the protein polymerization differs depending on additive used but similar functionality can be obtained with greener solutions. Increased sustainability is not reached only by using plant-based solutions but processing conditions circulating chemicals are as well important.

Among wheat proteins, functionalized glutenin appears to have the most interesting water absorption capacity compared with gluten or gliadins. Its composition has been and continues to be studied to understand reactions and mechanisms. For potato proteins, we found that the sample with the best water absorption capacity was the one without acylation but with cross-linking without autoclaving. From then on, we tried to play on other parameters such as reaction time or pH and we observed a decrease in water absorption capacity.

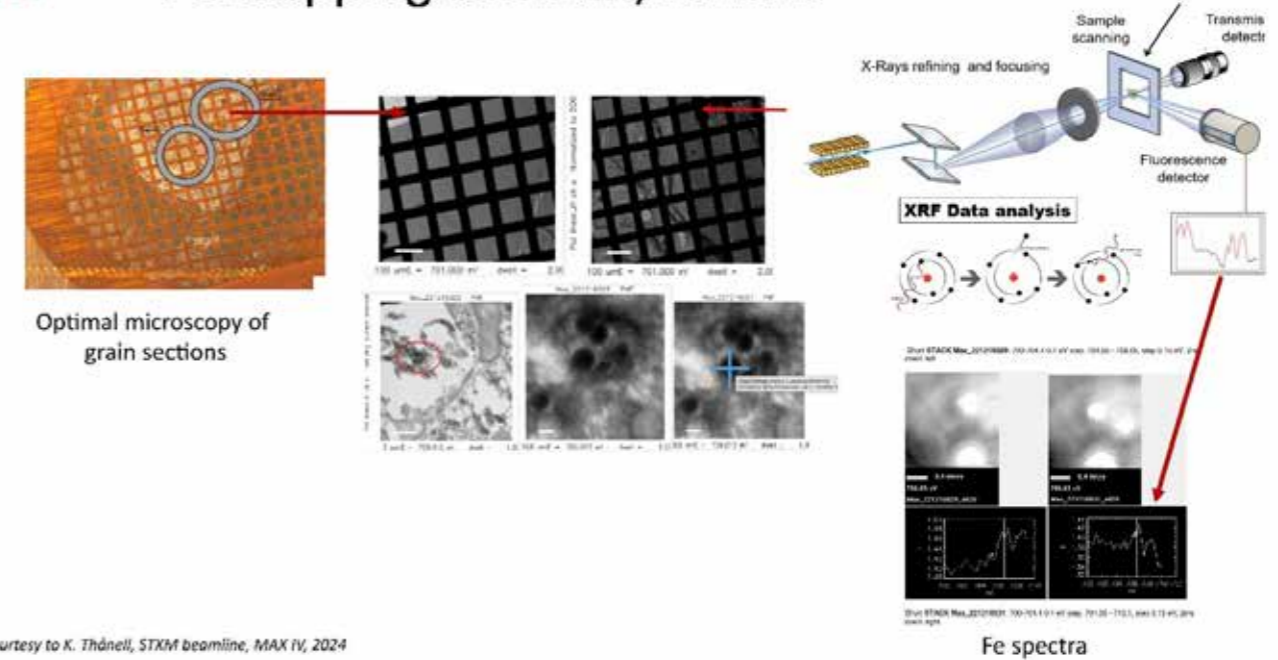


Fig.2 Wheat plants grown in biotron for evaluating drought resistance. Left: effect of early drought, applied 30 d after sowing by withholding water for 28 d. Right: effect of late drought, applies 60 d after sowing and lasted for 14 d. For both figures, left: control and right: drought. Compared to early drought treatment, late drought induced a larger reduction on all the yield components e.g., grain number, grain weight and spike length. (Figure by Yuzhou Lan)

Our studies have shown so far that rye chromosome 3 is extremely interesting to continue evaluating since it seems to contribute drought tolerance (Fig. 2), end-use quality and nutritional quality simultaneously as it does not hamper the yield. The major findings in the Cd-drought stress study on durum wheat highlighted the greatest abiotic stress impact on wheat development induced by a combined Cd-drought stresses and the high Cd concentration negative impact. The same study also shows for the first time a variation in the development of wheat roots under Cd stress in two growth media as investigated by neutron imaging and highlights a variation in the roots morphology. In the grain imaging study, the results indicated Fe localization in the wheat grain using the latest spectroscopy based approach at SoftiMAX beamline at MAX IV synchrotron (Fig. 3), as a new approach to probe micronutrients distribution in the grain. In the legume-fat emulsion functionality study we found a variety determined foam formation, which is of high importance in development of legume based foods and drinks.



## Fe mapping at STXM, MAX IV



Courtesy to K. Thånell, STXM beamline, MAX IV, 2024

Fig. 3. Fe mapping in grains at STXM, MAX IV (Figure by K. Thånell).

Screening of various microorganisms, including *Rhizopus oligosporus*, *Mucor circinelloides*, *Mucor plumbeus*, *Rhodotorula toruloides*, and *R. babjevae*, using novel cultivation media derived from faba bean starch and protein, resulted in identification of a consortium with the best potential for co-fermentation in solid-state conditions for a new product (Fig. 4).

Tempeh prototypes were developed using faba beans and oat kernels, separately and in different

combinations, through solid-state fermentation (Fig. 5). This process significantly improved the sensory qualities of faba beans, rendering the final product highly appealing in terms of texture, appearance, and flavour. Combining oat kernels with faba beans has the potential to enhance the nutritional profile by increasing the diversity and quality of amino acids and fiber content (Fig. 6). However, tempeh made solely from oat kernels lacks a solid and compact structure.



Fig.4 Microorganism Screening and Co-Fermentation, from faba bean agar plates to solid-state fermentation with faba beans. (Figure by Alejandra Fernandez Castaneda).



Fig. 5. Tempeh prototype with 75% faba bean and 15% oat kernels. (Figure by Alejandra Fernandez Castaneda).

It should be highlighted that the quality of the final product is affected by pre-treatment conditions and cooking time. Further sensory analysis is required to determine the optimal combination. The work on gel formation of faba bean protein and mixed gels from faba bean protein, starch and fibre has resulted in two publications. The gel formation of the two main protein fractions of faba bean has been characterized and the effect of NaCl elucidated. Furthermore, the texture and microstructure of mixed gels from faba bean protein and starch has been investigated, highlighting how the gel textural properties depend on the starch/protein ratio.



Fig. 7. Camelina plants grown in field (Photo by Per Hofvander).



The MCFA project and postdoc started in 2023, while most work related to the project subject has been conducted by a former postdoc (now early Researcher) and an MSc student. Several enzymes corresponding to genes of importance for medium chain fatty acid (MCFA) release and triacylglycerol assembly in *Lindera* have been functionally defined and selected for further characterization in a *Camelina* seed environment (Fig. 7).

For improving oil and seed qualities of oil crops, the efficient protoplast regeneration protocols have been developed or further optimized for several oil crop species, respectively, including rapeseed, *Lepidium*, *carinata* and *camelina*. Such protocols enable DNA-free CRISPR gene editing for direct production of transgene free edited lines with improved traits. Using these protocols, we have generated a large number of edited lines for rapeseed, *Lepidium* and *carinata* with target genes for improving the oil, seedcake and protein quality. Some of these lines are grown in biotron for obtaining homozygous lines (Fig. 8), while some of the lines were chemically analyzed showing improved target traits. Screening for homozygous lines with mutations in different target genes is still ongoing.



Fig. 6. Summary of experiments and food prototypes of faba bean and oat kernels tempeh. (Figure by Alejandra Fernandez Castaneda).

Furthermore, over 200 lines of the EMS-induced mutation population were grown in greenhouse and phenotyping and chemical analysis were

performed, showing variations in some important traits. The leaf samples were collected for molecular analysis.



Fig. 8. Different developmental stages of CRISPR-edited lines of rapeseed grown in biotron (Photo by Li-Hua Zhu)

The wax ester (WE) project was started this year. We constructed 25 Level 1 single-gene Goldengate plant transformation vectors and 22 multi-gene vectors to be used for transformation of *Camelina sativa*, *Arabidopsis thaliana* and *Thlaspi arvense*. The vectors harbor genes associated with synthesis of medium-chain fatty acids, fatty acyl reductases, wax ester synthases, fatty alcohol oxidases, fatty aldehyde dehydrogenases, various lipid body formation-associated genes and selectable marker genes (Fig. 9). Most genes have been expressed under unique seed-specific or cotyledon-specific promoters. We also started developing transgenic control lines for producing spermaceti-like WE, without the added bottleneck-alleviating features.

The potato sink development project was initiated this year. The project aims at addressing competitive potato cultivation on northern latitudes with long days and shorter permissive growing season, and finding means of controlling sink strength for starch quantity and starch structure in starch potato cultivars. Potatoes have been edited in key starch synthesis genes to characterize their roles for starch quality and granule formation. This has led to one publication so far, studying a starch phosphorylase which was found to have a role in starch granule structure and amylose synthesis. The first edits have been made to induce mutations in the identified lead genes in potato to study early tuberization, improved tuber sink development and faster maturation. A study to alter starch loading in potato sink tissue has been initiated, using targeted promoter insertions to enhance starch synthesis.

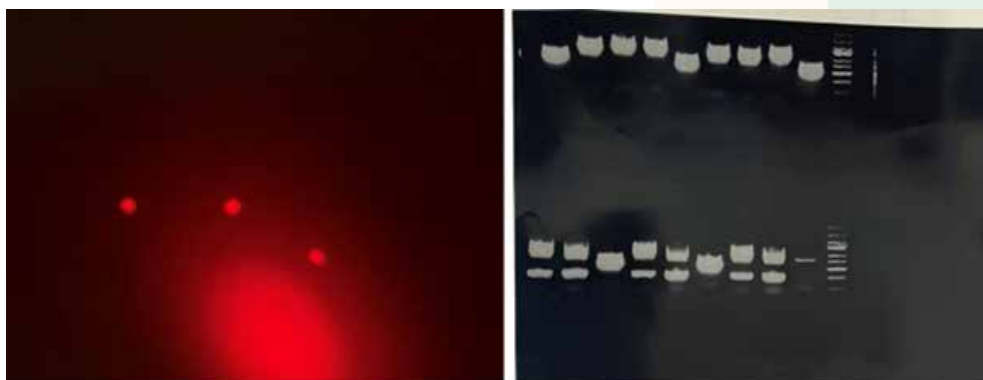


Fig. 9. Transgenic lines of *Camelina* were obtained, showing red color in seeds, indicating expression of the selective marker gene DsRed (left) and PCR results, indicating transgene integration (right). (Figure by Kamil Demski).

Autophagy plays an important role in plant growth and development (Fig. 10). We have uncovered a plant-specific aspect of the autophagic molecular machinery, strongly indicating evolutionary diversification of plant autophagosome maturation from similar processes in animals and fungi. This discovery holds promising potential for manipulating plant autophagy to enhance crop fitness, as summarized

in the manuscript currently undergoing revision in the *Nature Communications* journal. Additionally, we have published SPIRO, one of the three tools developed by our team to facilitate autophagy research in plants. Furthermore, our findings on the protein interactors of autophagy-related protein 5 (ATG5) have been published, revealing new potential roles of this protein in plant morphogenesis and stress response.

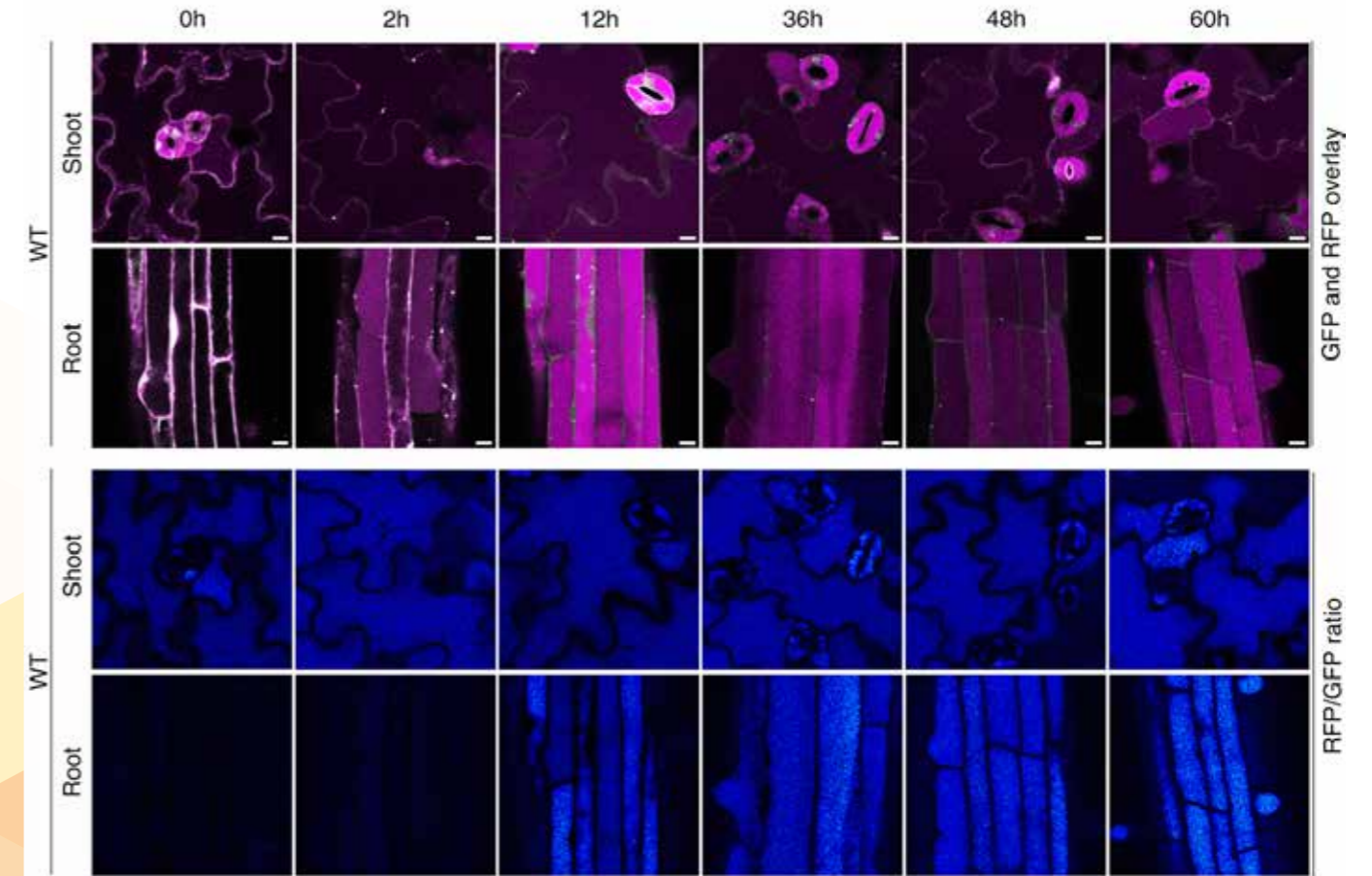


Fig. 10. Plant roots and shoots show different dynamics of autophagic activity under the same conditions. Note: *Arabidopsis thaliana* seedlings expressing the fluorescent autophagy reporter mRFP-GFP-ATG8a (pHusion-ATG8) were treated with AZD8055 to inhibit TORC1 activity and thereby induce autophagic activity. The seedlings were treated for 60 hours and imaged at specified time points using a confocal microscope. Autophagic activity leads to the translocation of pHusion-ATG8 from the cytoplasm to the vacuole of cells, resulting in the accumulation of the red fluorescent signal and a decrease in the green fluorescent signal. The top panels depict an overlay of the green (green) and red (magenta) fluorescent channels, while the bottom panels show the red-to-green intensity ratio for each pixel corresponding to the top panels. Scale bar, 10  $\mu$ m. (Figure by Alyona Minina)

To develop new timothy varieties with high-quality forage and minimal environmental impacts, around 15 standard forage quality parameters were measured using near infrared reflectance (NIR) spectroscopy, in samples collected from multi-locations Timothy field trial encompassing 264 genotypes in collaboration with the Association of German Agricultural Analytic and Research Institutes (VDLUFA), Germany. The quality parameters measured include digestibility (ME), net energy for lactation (NEL), neutral detergent fiber (NDF – hemicellulose), acid detergent fiber (ADF – cellulose and lignin), crude fat (XL), crude protein (XP), crude fiber, carbohydrates, ash and gas production. Based on the preliminary

analysis, forage quality parameters highly vary among Timothy genotypes within and across all three geographical locations (Svalöv, Uppsala and Röbbäcksdalen), indicating the presence of genotype differences and the impact of environmental conditions on Timothy forage quality. Similarly, forage quality parameters strongly vary among the three cuttings both within and among the three locations, suggesting the influence of Timothy growth/regrowth and physiological stages on forage nutritive value. Comparisons showing the variation in two selected important forage quality parameters, NDF and ADF, among genotypes, within and among the three locations and among cuttings are summarized in Fig. 11.

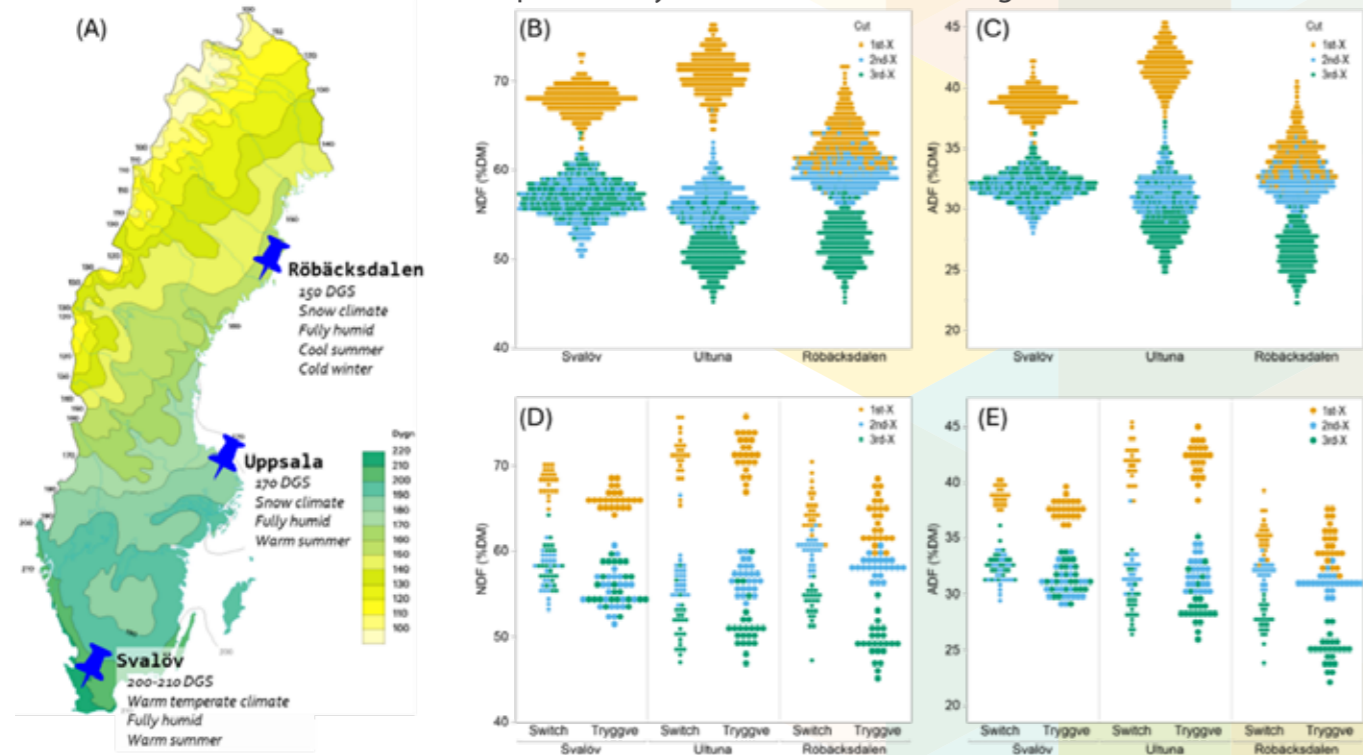


Fig. 11. A map showing the three geographical locations of Timothy field trial (A) and summary of the distributions of the two selected forage quality parameters (NDF and ADF) across the three locations and among the three cuttings (B-E). The NDF (B and D) and ADF (C and D) distributions for all genotypes (B-C) and for the two standard cultivars (D-E). Figure by Girma Bedada Chala, based on <https://www.smhi.se/data/meteorologi/kartor/normal/veg-langd>

### 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. Some important contributions of the C4F research to social benefit could be described as follows:

- Increased opportunities to use plant-based sustainable solutions within the materials field (absorbent materials). Transfer of suitable genes from rye chromosome 3 might contribute climate tolerant wheat.
- Abiotic stress impact in wheat contributing to new variety selection and production in Sweden (prediction of wheat under abiotic stresses).
- Fe localization in the unique wheat breeding material using the latest synchrotron imaging methods, contributing with new info for breeding of multipurpose wheat (disease resistant and nutritious).
- Uses of Swedish legumes in foods or drinks.
- Fermented foods are widely consumed because of their sensory and nutritional characteristics as well as health benefits. Carefully designed fermented foods from plant sources with optimal sensory quality and health benefits will facilitate a shift from animal-based to plant-based diets, which is a key in transition towards a more sustainable global food system.
- Attainment of commercial levels of medium chain fatty acid as part of oil in Camelina would be a platform for a non-tropical source of lauric, myristic and palmitic fatty acids, which is in line with the suggested SDN-1 framework for NGT plants in EU.

- Improved oil qualities or wax ester production in oil crops would partially replace fossil fuels in the market sector on one hand, and potentially enrich the market on the other hand.
- Improving starch yield and quality would enable to tailor starch quality for various industrial applications.
- Novel CRISPR-edited lines or chemically induced mutation lines of oilseed crops with improved oil and protein or seedcake qualities would in long-run contribute to increased plant oil production and making the seedcake be a source of high value protein for food and feed, and thus consequently reducing the fossil use and benefiting the human health and environment.
- Study on post-translational modifications regulating the essential autophagy-related protein will pave the way for new research avenues focused on regulating this pathway in plants to enhance plant productivity.

At least one example on how C4F takes basic research to application to be used

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:

- Better understanding green protein properties such as water absorbance capacity would facilitate development of green protein diapers.
- Further evaluations on rye chromosome 3 may contribute to drought tolerance in wheat, thus improving end-use quality and nutritional quality without penalty in yield.
- Basic research outcomes on legume

protein colloidal and emulsion behavior can benefit food industry in designing protein-rich drinks.

- Previous findings from basic research facilitate characterization of proteins and starch properties as well as physicochemical properties of faba beans and other local plants for developing innovative products for consumers and the food industry.

- 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.

- The characterization of the transcriptional network and enzymatic characterization for medium chain fatty acid synthesis in *Lindera* is of basic research in nature where findings are transferred in two steps into *Camelina*, first by regular transformation and secondly by CRISPR/Cas editing to comply with EU SDN-1 standards. The oil could then provide a temperate cultivation alternative to tropical deforestation for medium chain fatty acids.

- Studies on biochemical and biophysical limitations of a crop plant system for producing wax esters would facilitate development of a potential production strategy for a sought-after industrial product (spermaceti oil-like, wax ester-rich plant oil) in the target plant species.

- The novel CRISPR genome editing principle along with optimized DNA-free CRISPR editing methods are used for trait improvement of the target crops, resulting in transgene-free "Category 1 plants", as suggested in a current legislative proposal on NGT plants from the EU commission.

- CRISPR/Cas9 edited plant material was studied for bio-material applications

- We are endeavoring to enhance the efficiency of microspore embryogenesis for rapeseed. Improving important traits of the crop through microspore embryogenesis would speed up the breeding process and enhance the efficacy of rapeseed utilization in agriculture.



Vice program leader Li-Hua Zhu



## Girma Bedada

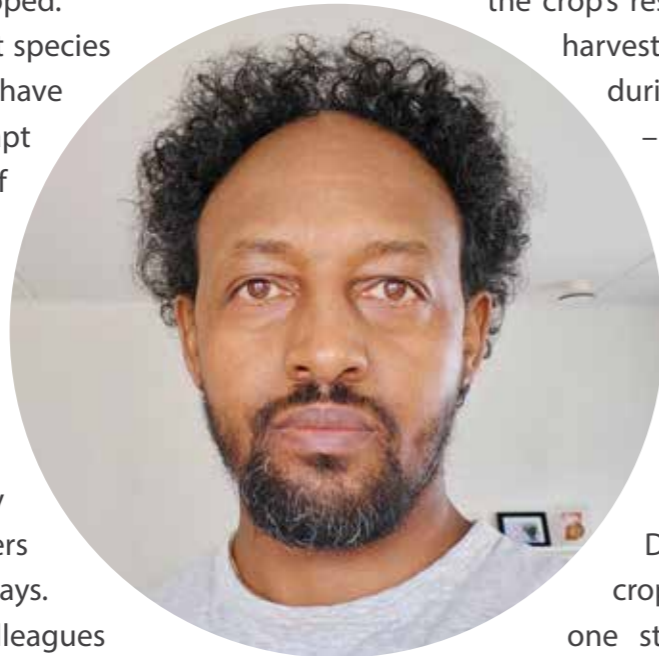
# Robust forage crops for the future climate

*Girma Bedada is one of our researchers working with the forage crop timothy. He enjoys being involved in all steps of the research, from the greenhouse experiments to the bioinformatics.*

The aim of Girma Bedada's work is to develop novel genomic resources for timothy grasses breeding and research. With knowledge about the genome architectures and the genetic diversity of the species, new robust varieties of this important forage crop can be developed.

– Fortunately, many plant species are very diverse and have had the ability to adapt to different kinds of environments during the evolution. Now, we are developing the reference genomes and analyze this diversity, and use it to improve the quality and adaptability of the timothy that farmers grow for animal feed, he says.

Girma Bedada and his colleagues analyses the timothy materials from the Nordic genetic resource center (NordGen) and Lantmännen.



They explore the genetic diversity among different timothy accessions, by growing them at different locations in Sweden.

They want to link the genetics of timothy to the quality of the crop produced, for example the crop's responses to being cut and harvested two or three times during the growth season.

– Looking for how the genetic diversity contribute to different forage quality traits, is a new, quite untouched research area. Due to the climate changes, this research is becoming very important.

During a growth season, crops are facing more than one stress factor. It could be drought in the beginning, then waterlogging and a cold period on top of that.

– We work towards the development of high-quality plant materials that can adapt to different kinds of stress, says Girma Bedada.

His research ranges from studies of the evolution of timothy grass to applied research with the goal to improve forage crop quality.

– Understanding how the timothy grasses evolved and adapted to diverse climates in Sweden definitely contribute to finding the best candidate plant materials to be used in the plant breeding and research.

Today, Girma Bedada is mostly focusing on the bioinformatics part of the research, although he thinks it is important to also spend some time in the lab, as well as in the greenhouse and the fields where the plants grow.

– To get the full picture of things, I want to be involved in all parts of our experiments, from studying plant traits, stress responses, sampling of DNA or RNA, to the bioinformatics part where we analyze the data.

Girma Bedada originates from Ethiopia. After getting a bachelor degree in plant science he took part in the establishment of a plant biotechnology program at the Ethiopian Institute of Agricultural Research. He did his master education in Germany, and came to Sweden in 2009 for a PhD position at SLU, at the department where he is now a researcher. Beside the timothy research, he is involved in analyzing what makes some grasses perennial, and the genomic background behind lower methane emissions from rice.

*Author: Lisa Beste*

*Photo: Private*



# C4F - Crops for the Future

## Scientific publications

1. Capezza AJ, Newson WR, Muneer F, Johansson E, Cui Y, Hedenqvist MS, et al. Greenhouse gas emissions of biobased diapers containing chemically modified protein superabsorbents. *Journal of Cleaner Production*. 2023;387:135830.
2. Elander PH, Holla S, Sabljic I, Gutierrez-Beltran E, Willems P, Bozhkov PV, et al. Interactome of Arabidopsis ATG5 Suggests Functions beyond Autophagy. *International Journal of Molecular Sciences* [Internet]. 2023; 24(15).
3. Gadaleta A, Marcotuli I, Arriagada O, Johansson E, Rahmatov M, Berger Ceresino E, et al. Use of genetic resources and prebreeding activities in order to improve nutritional and health-related properties of cereals and pseudocereals. 2023. p. 5-24.
4. Herneke A, Karkehabadi S, Lu J, Lendel C, Langton M. Protein nanofibrils from mung bean: The effect of pH on morphology and the ability to form and stabilise foams. *Food Hydrocolloids*. 2023;136:108315.
5. Johansson E, Kuktaite R, Labuschagne M, Lama S, Lan Y, Nakimbugwe D, et al. Adaptation to abiotic stress factors and their effects on cereal and pseudocereal grain quality. 2023. p. 339-58.
6. Johansson M, Karkehabadi S, Johansson DP, Langton M. Gelation behaviour and gel properties of the 7S and 11S globulin protein fractions from faba bean (*Vicia faba* var. minor) at different NaCl concentrations. *Food Hydrocolloids*. 2023;142:108789.
7. Jolayemi O, Malik A, Vetukuri R, Saripella GV, Kalyandurg P, Ekblad T, et al. Metabolic Processes and Biological Macromolecules Defined the Positive Effects of Protein-Rich Biostimulants on Sugar Beet Plant Development. 2023;24:9720.

8. Kondić-Špika A, Trkulja D, Brbaklić L, Mikić S, Glogovac S, Johansson E, et al. Marker assisted selection for the improvement of cereals and pseudocereals. In: Rakszegi M, Papageorgiou M, Rocha J, editors. *Developing Sustainable and Health Promoting Cereals and Pseudocereals* 2023. p. 253-83.
9. Lama S, Leiva F, Vallenback P, Chawade A, Kuktaite R. Impacts of heat, drought, and combined heat-drought stress on yield, phenotypic traits, and gluten protein traits: capturing stability of spring wheat in excessive environments. *Frontiers in Plant Science*. 2023;14.
10. Lan Y, Kuktaite R, Chawade A, Johansson E. Diverse wheat lines to mitigate the effect of drought on end-use quality. *Frontiers in Food Science and Technology*. 2023;3.
11. Newson WR, Capezza AJ, Kuktaite R, Hedenqvist MS, Johansson E. Green Chemistry to Modify Functional Properties of Crambe Protein Isolate-Based Thermally Formed Films. *ACS Omega*. 2023;8(23):20342-51.
12. Nilsson K, Johansson M, Sandström C, Eriksson Röhnisch H, Hedenqvist MS, Langton M. Pasting and gelation of faba bean starch-protein mixtures. *Food Hydrocolloids*. 2023;138:108494.
13. Nynäs A-L, Newson WR, Langton M, Wouters AGB, Johansson E. Applicability of leaf protein concentrates from various sources in food: Solubility at food-relevant pH values and air-water interfacial properties. *LWT*. 2023;184:114962.
14. Ohlsson JA, Leong JX, Elander PH, Ballhaus F, Holla S, Dauphinee AN, et al. SPIRO – the automated Petri plate imaging platform designed by biologists, for biologists. *The Plant Journal*. 2024;118(2):584-600.
15. Perez-Puyana V, Capezza AJ, Newson W, Bengoechea C, Johansson E, Guerrero A, et al. Functionalization Routes for Keratin from Poultry Industry Side-Streams—Towards Bio-Based Absorbent Polymers. *Polymers*. 2023;15:351.

16. Pop O, Suharoschi R, Socaci S, Berger, Ceresino E, Weber A, et al. Polyphenols-Ensured Accessibility from Food to the Human Metabolism by Chemical and Biotechnological Treatments. *Antioxidants*. 2023;12.

## Popular scientific publications (reports etc)

- Li-Hua Zhu et al. Etablering av mutationspopulation av raps och fältkrassing. *LTV-Fakultetens Faktablad*, 2023:15.
- Publications on SLU homepage:
  - <https://www.slu.se/en/ew-news/2023/11/effost-advances-production-of-sustainable-healthy-food/>
  - Article in *Cerealier*: "En mångfacetterad böna" *cerealier-nr3-2023---tema-nnr2023.pdf* ([lantmannen.se](http://lantmannen.se))

## Interviews and presence in media

- Alejandra Castaneda, 2023. Interview at Annual HealthFerm Meeting, 5th-6th of September 2023. ETH Zurich. <https://www.youtube.com/watch?v=ityqNcd3roE&t=72s>
- Mathias Johansson, 2023. Video of PhD project published on SLU YouTube channel. <https://www.youtube.com/watch?v=Vx25eWQOE3M>
- Li-Hua Zhu, SVT nyheter Lokalt, Om rapsprojektet - Restprodukt från raps kan ersätta sojaprotein. 230626. <https://www.svt.se/nyheter/lokalt/skane/vaxtforskare-hoppas-pa-snara-besked-om-gensaxen>.

## Scientific presentations

- Demski K. 2023. Effective wax ester production in plant seeds and its applications. Presentation at the C4F Workshop 2023. 7th December, Lund.
- Diakité M. S., Capezza A. J., Muneer, F., Nynäs, A-L, Johansson, E. 2022. Plant protein fractionation, products thereof and their feasibility: Superabsorbents biomaterials . Oral presentation, C4F Workshop. 7th December, Lund, Sweden.

• Lama S., Kuzmenkova M., Kuktaite, R. 2023. Wheat gluten quality in a changing Swedish climate: Striving for stability in excessive environments. Oral presentation at XIV International Gluten Workshop, 19-21th June, Madrid, Spain.

• Lan Y., Kuktaite R., Chawade A., Johansson E. 2023. Environmental stress on bread making quality-drought on protein composition of diverse lines and relationship between protein and yield traits. Poster presentation at XIV International Gluten Workshop, 19-21th June, Madrid, Spain.

• Johansson, M. 2023. Texture and microstructure of mixed gels from faba bean protein, starch and fibre, oral presentation at Nordic rheology conference, 12-14th April, Aarhus, Denmark.

• Johansson, M. 2023. Legume based gels – texture and microstructure, oral presentation and poster at Food Science Sweden conference "Research and Innovation for Food Security", 15th March, Uppsala, Sweden.

• Castaneda A. 2023. Fermentation of plant material of European origin to produce Tempeh-like foods. Oral presentation at Annual HealthFerm Meeting, 5-6th of September. ETH Zurich.

• Moss O., 2023. Genome editing of rapeseed for improving seedcake quality. Oral presentation at C4F-Workshop, Elite Hotel Ideon, 7th of December, Lund.

• Wang, E. S., Fonskov, J., Shahriar, S., Nilsson E., Olsson O., Ceplitis A. and Zhu, L-H. 2023. EMS-induced mutation population for increasing genetic variation in rapeseed. Oral presentation at C4F-Workshop, Elite Hotel Ideon, 7th of Dec., Lund.

• Castaneda A. 2023. Screening of Microfungi for solid-state fermentation of faba bean Poster and abstract presentation at EFFoST conference, 6-8th of November, Valencia, Spain.

- Castaneda A. 2023. Screening of Microfungi for Solid-State Fermentation of Faba Bean. Oral presentation at C4F workshop, 7th of December 2023, Lund, Sweden.

- Demski K. 2023. Synthetic Wax Esters from Plants. Presentation at the Plant Biotechnology Division Meeting 2023, Department of Plant Breeding, SLU. Alnarp, December 12th.

### Collaboration with industry or other parts of society

- Lantmännen
- Gasum
- Oriflame
- Grönsaksmästarna
- Region Skåne
- Lilla Harrie Valskvarn
- Lyckeby Stärkelse AB
- Orkla
- Havredals Biodevelop AB
- RISE
- KTH
- Chalmers
- KI
- Sveriges Stärkelseproducenter Förening
- DLF Beet Seed AB
- Kalmar Ölands Trädgårdsprodukter
- Findus
- FoodHills
- ISCA Technologies
- Lund University
- Gunnarshögs Gård AB
- Syngenta
- Planta LLC
- SLU Grogrund (A number of research projects connected to the C4F program)
- Nelson Seed
- VVT
- ETH Zurich
- Helsinki University
- University of Turku

- Copenhagen University
- MAX Hamburgare
- ICA
- Bio Gaia
- Uppsala University
- Örebro University

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

- Anja Herneke received funding from the Lantmännens research foundation based on work made during her PhD that was partly funded by C4F.
- Co-funding are from SLU-Grogrund, Nordic Research Foundations, Formas, VR, Carl Tryggers Stiftelse and EPIC-XS, etc.

### Education

#### a) PhD theses, MSc theses, Bachelor theses

Jolayemi, Okanlawon Lekan. (Male). 2023. Doctoral thesis: Biostimulant potential of agro-industrial side-streams - sustainable sugar beet cultivation and drought tolerance in wheat. Lomma. Sveriges lantbruksuniversitet. Acta Universitatis Agriculturae, 2023:71. ISBN: 978-91-8046-194-8. Swedish University of Agricultural Sciences.

Lama, Satie. (Female). 2023. Doctoral thesis: Wheat quality under a climate spell. Lomma. Sveriges lantbruksuniversitet. Acta Universitatis Agriculturae, 2023:26. ISBN: 978-91-8046-104-7. Swedish University of Agricultural Sciences.

Nilsson, Klara. (Female). 2023. Doctoral thesis: Faba bean foods: Structure and texture. Uppsala. Sveriges Lantbruksuniversitet. Acta Universitatis Agriculturae, 2023:48. ISBN: 978-91-8046-148-1. Swedish University of Agricultural Sciences.

Holla, Sanjana. (Female). 2023. Doctoral thesis: Autophagy beyond convention: plant-specific mechanisms for cellular recycling. Uppsala: Sveriges lantbruksuniversitet. Acta Universitatis agriculturae Sueciae, 2023. 2023:91. ISBN: 978-91-8046-236-5.

Quach, Judy. (Female) 2023. Master thesis: Unusual fatty acids in Lindera seed oil: the role of acyl-CoA:diacylglyceroltransferases (DGATs) on fatty acid composition of triacylglycerol. Alnarp: Sveriges lantbruksuniversitet.

Bogahawatta Anusha. (Female). 2023. Master thesis, 60 hp: Knockout of transcription factor MYB28 by CRISPR/Cas9 for reducing glucosinolate content in rapeseed (*Brassica napus* L.).

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

Kuktaite, Ramune. Co-supervisor for PhD-candidate Leiva, Fernanda. Title: Developing affordable high-throughput plant phenotyping methods for breeding of cereals and tuber crops. Dissertation date: May, 2023.

Kuktaite, Ramune. Co-supervisor for PhD-candidate Yuzhou, Lan. Thesis title: Exploring the genetic toolbox for climate-resilient spring wheat: Drought impact on yield, breadmaking quality, nutritional value and toxicity. Date for dissertation: 2024-05-30.

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

Minina, Alyona. Co-supervisor for Bachelor project student Sari, Volkan. Tentative title: Developing tools to study plant stress physiology. Expected date for dissertation: June, 2024.

Ballhaus, Florentine. Supervision of Master student Nicolò Brugnone. Tentative title: Targeting a new potential autophagy-related protein using CRISPR/Cas9. Date of the Master thesis defense: 28th of May 2024

Hofvander, Per. Supervisor for PostDoc Shrikant Sharma

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 Oliver Moss. Tentative title: Improvement of seedcake quality of rapeseed for high quality food and food uses. Expected date for dissertation: 2025.

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

Alejandra Castaneda. Lab supervisor in the course Food Chemistry and Food Physics (LVO110), 15 ECTS, Ultuna

Klara Nilsson, Lecturing in Food Chemistry and Food Physics (LVO110), 15 ECTS, Ultuna, Director of studies Food Science Program at SLU

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 membran-less organelles in plant stress response. Expected date for dissertation: September 2026.

Alyona Minina. Main supervisor for project student Kjelstrom, 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

### 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                                  |
| Volkmar Passoth       | 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                                  |
| Elaine Ceresino       | F, Researcher     | 5                                  |
| Mahbubjon Rahmatov    | M, Researcher     | 0                                  |
| Bill Newson           | M, 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% |
| Matías González           | M, Postdoc       | 30% |
| Faraz Muneer              | M, postdoc       | 50% |
| Kamil Demski              | M, Postdoc       | 30% |
| Shrikant Sharma           | M, Postdoc       | 0   |
| Neha Salaria              | F, Postdoc       | 30% |
| Maya-Setan Diakité        | F, Postdoc       | 50% |
| Adrian Dauphinee          | M, Postdoc       | 0   |
| Florentine Ballhaus       | F, PhD student   | 35% |
| Anna-Lovisa Nynäs         | F, PhD-student   | 50% |
| Oliver Moss               | M, PhD-student   | 50% |
| Alejandra Castaneda       | F, PhD-student   | 50% |
| Klara Nilsson             | F, PhD-student   | 50% |
| Olawale Olalekan          | M, PhD-student   | 0   |
| Sanjana Holla             | F, PhD-student   | 0   |
| Sbatie Lama               | F, PhD student   | 0   |
| Shishanthi Jayarathna     | F, PhD student   | 50% |
| Mathias Johansson         | M, PhD student   | 0   |
| Judy Quach                | F, MSc Student   | 0   |
| Lan Yuzhou                | M, PhD student   | 50% |
| Emelie Ivarson            | F, Research eng. | 50% |
| Linda Öhlund (Lantmännen) | F, Foragebreeder | 0   |
| Mirela Beganovic          | F, Resarch ass.  | 0   |
| Josefin Alverup           | F, Research ass. | 0   |
| Xueyuan Li                | M, Research ass. | 0   |

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

## TC4F Economy 2023

In 2023, TC4F received 29,1 mio SEK of funding which were distributed according to the budget of which 107% were used. The deficit was caused by spending money that had accumulated due to delays in recruitment due to the Covid-19 pandemic.

|  | SLU           | UmU          | Skogforsk    | Total         |
|--|---------------|--------------|--------------|---------------|
| <b>Distributed Funds (tkr)</b>           |               |              |              |               |
| Coordination                             | 3 164         |              |              | 3 164         |
| Plant Physiology (UMU)                   |               | 5100         |              | 5 100         |
| Forest Genetics and Plant Physiology     | 4 301*        |              |              | 4 301         |
| Southern Swedish Forest                  | 3 605         |              |              | 3 605         |
| Forest Ecology and Management            | 3 605         |              |              | 3 605         |
| Wildlife, Fish and Environmental Studies | 950*          |              |              | 950           |
| Skogforsk                                |               |              | 1 100        | 1 100         |
| C4F (LTV)                                | 7 275         |              |              | 7 275         |
| <b>TOTAL</b>                             | <b>22 900</b> | <b>5 100</b> | <b>1 100</b> | <b>29 100</b> |
| <b>Costs, spent funds (tkr)</b>          |               |              |              |               |
| Coordination                             | 1 893         |              |              | 1 893         |
| Plant Physiology (UMU)                   |               | 5100         |              | 5100          |
| Forest Genetics and Plant Physiology     | 6 181         |              |              | 6 181         |
| Southern Swedish Forest                  | 4 170         |              |              | 4 170         |
| Forest Ecology and Management            | 3 964         |              |              | 3 964         |
| Wildlife, Fish and Environmental Studies | 983           |              |              | 983           |
| Skogforsk                                |               |              | 1 100        | 1100          |
| C4F (LTV)                                | 7 833         |              |              | 7 833         |
| <b>Total</b>                             | <b>25 023</b> | <b>5100</b>  | <b>1 100</b> | <b>31 223</b> |
| <b>RESULT T4F</b>                        | <b>-1 566</b> | <b>0</b>     | <b>0</b>     |               |
| <b>RESULT C4F</b>                        | <b>-558</b>   |              |              |               |
| <b>Total RESULT</b>                      | <b>-2 123</b> | <b>0</b>     | <b>0</b>     | <b>-2 123</b> |

\*assigned postdoc grants