Breeding and Genetic Improvement for a Net-Zero Future

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Keynote Speaker

A brief history of forage breeding in Britain: Lessons from the past and challenges for the future

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The early history of grassland agriculture in Britain is inherently difficult to pin down. Late Paleolithic glacial retreat provides a convenient starting point for consideration. Archaeological evidence points to a gradual indigenous development of semi-natural grassland in the Mesolithic, through deliberate land clearance, although the full extent of this is unknown. There is a blurred distinction between hunter-gatherers following herd migrations and early transhumance, but the origin of a pastoral economy at scale can be traced to the importation of sheep and domesticated cattle by Neolithic farmers around six thousand years ago.

The deliberate selection and breeding of livestock is known from classical authors like Pliny and Columella. While it is also apparent they knew about forage legumes and their capacity to improve soil, there does not appear, in Britain, to have been any development beyond semi-natural pasture until the post-Medieval period. Indeed, improvement of grassland by reseeding was unknown until the seventeenth century. The arrival of the movable type printing press facilitated access to translations of these classical authors, stimulating an interest in agricultural improvement.

The superior nutritional qualities of perennial ryegrass were discovered in the early seventeenth century and several improved landrace varieties became available for farmers over the following centuries. However, a lack of seed certification meant that inferior seed was often passed off as named varieties. Clover was brought into Britain from Belgium and the mixed sward of perennial ryegrass with white clover became the dominant form of improved grassland, as it is today.

Britain had increasingly become reliant on food imports through the nineteenth century. The targeting of merchant shipping by German U-Boats in World War I left the country in a precarious position, leading to serious food shortages. In the post-war years, the strengthening of food security motivated the formation of several national agricultural institutes, including the Welsh Plant Breeding Station (WPBS), with the focus being on introducing improved varieties of crop species and the introduction of seed testing and certification and general agricultural practice.

Under the leadership of George Stapledon, a thorough survey of British grassland was carried out. Stapledon, along with colleagues T.J. Jenkin and R.D. Williams, collected plants from across England and Wales, noting variations in response to abiotic stress of different species.

They used local farmer knowledge as a basis on where to collect germplasm, for instance from fields that were most productive. Selection and hybridization of plants to produce varieties followed recently rediscovered Mendelian principles. Some of the highlights of early genetic studies carried out at the WPBS include a systematic study of the genetics of self-incompatibility, and of interspecific and intergeneric hybridization of grasses.

Varieties bred at WPBS were given numbers and referred to as S-strains. These proved to be revolutionary in British agriculture and S23 perennial ryegrass in particular, bred in the 1930s, can be credited with transforming the productivity of British grassland. S23 remained in commerce for nearly 60 years. S184 white clover, first marketed in 1936 is still on UK recommended lists.

The development of reasonably high-throughput forage quality led to a systematic study of carbohydrate and protein content in ryegrasses from the early 1960s. The recognition that higher digestibility had a marked effect on productivity, and that increasing carbohydrate availability to rumen microflora improved nitrogen use efficiency, led to the development of the Aber High Sugar ryegrasses. This program, now in its seventeenth generation of recurrent selection, continues to produce varieties with increased forage quality every year.

Anthropogenic climate change presents enormous challenges for grassland agriculture worldwide. These can be considered both in terms of our continued ability to farm productively and from a mitigation perspective. Despite ambitious attempts to limit greenhouse gas emissions, optimistic projections (RCP 4.5) predict a global warming of 2 to 3°C above pre-industrial levels by 2100. This global change is difficult to visualize on a local level, but a reasonable analogy for Europe is to imagine a 1,000 km northward shift of bioclimatic regions. This has major implications for the grassland species that can be successfully grown in a given region, and for the need for new sources of abiotic and biotic stress tolerance and resistance.

At the same time, grassland agriculture's contribution to emissions needs to be taken seriously. Currently, UK emissions solely from the production and application of nitrogen fertilizer to grassland is around two million tonnes of CO_2 eq per year. Better exploitation of biological nitrogen fixation has the potential to significantly reduce this. Improving nitrogen use efficiency in the rumen and reduction of nitrogenous greenhouse gas emissions can be achieved both through more appropriate nutrition, protein protection and through modulation of the rumen microbiome. The breeding of forage crops has the potential to provide solutions to all of these. Similarly, solutions exist for reducing methane flux, both by suppressing methanogenic activity in the rumen and by increasing productivity per unit of methane produced.

Invited Talk

Climate-resilient grasslands and the net zero agricultural transition

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Abstract: Land is a multi-functional finite resource, with requirements for food, feed, fibre and fuel, as well as for nature conservation and climate protection. At the same time for grassland systems, it is important to optimise the sustainability of livestock production whilst also exploring new uses for the existing grassland species, as well as the opportunities presented by incorporating new species. At IBERS, we are exploring all three of these options from the resilience of grassland mixtures in different environments and under different managements, through the biorefining of forage grasses and clovers to protein and other products, through to the development of new grasses for a range of downstream uses from feed to bedding and fibre. For example, biomass from Miscanthus is expected to play a role in future energy systems, particularly through the combination of bioenergy with carbon capture and storage (BECCS). However, such changes in land use need demonstrating at scale, and also an understanding of the environmental and social impacts as well as associated extension activities to enable adoption. Data and thinking from recent projects, such as the Perennial Biomass Crops for Green House Gas Removal (PBC4GGR), Biomass Connect, and the Land Use for Net Zero (LUNZ) Hub, which have sought to do this will be presented.

Session 1: Diversifying breeding populations (back to the wild)

Harnessing untapped diversity in white clover: core collections and landscape genomics

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Forage legumes such as white clover (*Trifolium repens*) are key components of temperate agriculture, but face challenges maintaining production in response to climate change. Developing new cultivars targeted for environmental resilience relies on discovery and utilisation of natural genetic variation for these traits. Germplasm centres, such as the Margot Forde Genebank (MFG) located at BSI-AgResearch Grasslands (New Zealand), are repositories of plant genetic variation that can be difficult to harness due to the large number of accessions – over 3,000 white clover populations in the MFG alone. One approach is to identify a reduced set that is a genetically representative subsample of the collection. This core then provides a practicable-sized set for trait characterisation.

To develop a white clover core collection, a set of 689 accessions from 60 countries representing the natural geographic range of white clover across western Europe/North Africa to Central Asia, and elsewhere around the globe, was sourced from the MFG. Additionally, 92 cultivars from across years and sources were included in the analysis. The populations were characterised genetically using pooled genotyping-by-sequencing with 30 individuals per accession. Approximately 31K SNPs were identified and discriminant analysis of principal components (DAPC) was used to determine population structure. Twelve populations were identified; all located within Europe/North Africa to Central Asia. Accessions from the Americas, Australasia and sub-Saharan Africa and the cultivars were members of three western European populations. This highlights that white clover cultivars are derived from a narrow genetic base and that there is much untapped genetic diversity across a wide range of ecogeographical zones.

Using two methodologies (k-medoids and Core Hunter), a set of 138 accessions were identified from across the 682 populations that represent a genetically representative subsample. This core collection provides a basis for developing diverse pre-breeding populations that are being assessed for environmental traits.

Focusing on accessions from the natural geographic range, a landscape genomics approach was applied to identify signatures of adaptation. Using a redundancy analysis (RDA)-based ordination method, 165 outlier SNPs strongly associated with environmental variation were identified. The environmental drivers shaping the populations were altitude, summer precipitation and mean temperature in the driest quarter – which suggests these outlier SNPs are involved in adaptation to heat and drought and will be investigated further for frequency in stress tolerant germplasm.

Combined with the insights from the landscape genomics, the core collection will support development of new cultivars providing greater climate resilience and improved environmental performance from NZ and other temperate pasture systems.

Linking genome diversity to root architecture and growth dynamics in perennial ryegrass

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Keywords
F2 segregating populations, Transcriptomics, Genomics, Perennial Ryegrass, Root architecture, Advanced Imaging Techniques

In temperate climates, global warming is projected to increase both the frequency and severity of summer droughts, and excessive rainfall and flooding events during winter and early spring months. Existing plants can be adapted to improve their resilience to such future climates through the enhancement of existing characteristics, such as root architecture. This project will investigate the genes responsible for regulating key perennial ryegrass (PRG) root architecture traits such as root length, while identifying the genetic loci responsible. A diverse set of PRG genotypes were compared from an initial screen of 200, which possessed varied root phenotypes. Rhizoboxes and RNA sequencing will be used to analyse phenotypic and transcriptome data. Phenotyping methods include 3D structural analysis using CT scanning, and photographic analysis using a range of 2D imaging software. F2 segregating populations are being developed to isolate and study the activation or suppression of genes that influence PRG root architecture traits of interest. Future work will identify the presence of single nucleotide polymorphisms (SNPs) through whole genome sequencing. This will be correlated with transcriptomic data, correlating SNPs presence in certain genes to variations in their transcription levels and root phenotypes across PRG lines. This study will also analyse the impact of the rhizosphere on root architecture, crop resistance to drought and excessive water exposure, biochemical cycling, and nutrient movement around root structures. We will attempt to identify adaptions in deep-rooting PRG lines by tracking the movement of key elements and nutrients along deeper rooting structures while also analysing both the rhizosphere and plant transcriptome at these lower soil depths. Understanding the underpinning gene networks responsible for variations in both abiotic resistance and root architecture will allow for the identification of genetic markers which can be targeted to inform future breeding strategies.

Wild *Vicia* species: genetic resources for agroecology and ecosystem restoration

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The genus *Vicia* comprises between 150 and 210 species worldwide, with approximately 55 species occurring in Europe (Javadi et al., 2022). The flora of the Czech Republic includes 36 *Vicia* species (Pladias, 2025). Wild vetch species are valuable genetic resources for modern agriculture. Beyond their traditional use in forage and cover cropping, they offer adaptations to abiotic and biotic stresses, contribute to soil improvement and hold potential for breeding new cultivars aimed at landscape restoration.

This study examines the variability of seven selected wild *Vicia* species, collected from diverse locations, across key phenotypic traits (morphological, phenological and physiological) and biomass production. The species (*V. cracca*, *V. sativa*, *V. angustifolia*, *V. tetrasperma*, *V. villosa*, *V. pisiformis*, *V. hirsuta*) were evaluated over two consecutive years at three experimental sites (Troubsko, Olomouc, Šumperk), each characterized by distinct climatic and soil conditions.

Studying phenotypic traits is crucial in botany, agriculture and breeding, as it provides insight into genetic and environmental influences and supports the selection of parental lines for breeding focused on biodiversity conservation and ecological stability. Given the increasing drought in the Czech Republic, breeding vetch varieties adapted to these conditions will be crucial for ensuring sustainable forage production, effective cover cropping and overall agricultural system resilience.

The analysis of seven *Vicia* species revealed significant variability in biomass composition, especially in carbohydrate and nitrogenous matter (NM), associated with both species and site differences. *V. tetrasperma* exhibited the highest carbohydrate and the lowest NM content; *V. villosa* had the lowest carbohydrate and the highest fibre content; *V. angustifolia* stood out for its the highest NM content. Each site exhibited a distinct soil property profile (pH, P, K, Mg and Cox content) and significant differences in biomass composition (sugar, NM and fibre content) was observed among the sites. A key finding is the statistically significant effect of soil factors on the nitrogenous substance and fibre content in the biomass of *Vicia* plants, irrespective of species. In contrast, carbohydrate content did not show a significant dependence on the studied soil parameters.

Interestingly, the Olomouc site recorded the highest average NM content (21.52% DM) despite having the highest pH value (6.80), suggesting other local factors can override general soil-biomass composition trends. The results highlight the complex interplay between the genetic predispositions of individual *Vicia* species and the specific soil conditions at a given site. This interaction shapes particularly the content of nitrogenous substances and fibre in the biomass. Furthermore, the findings indicate that, while general trends between soil and biomass composition exist, local conditions can cause notable deviations from these patterns.

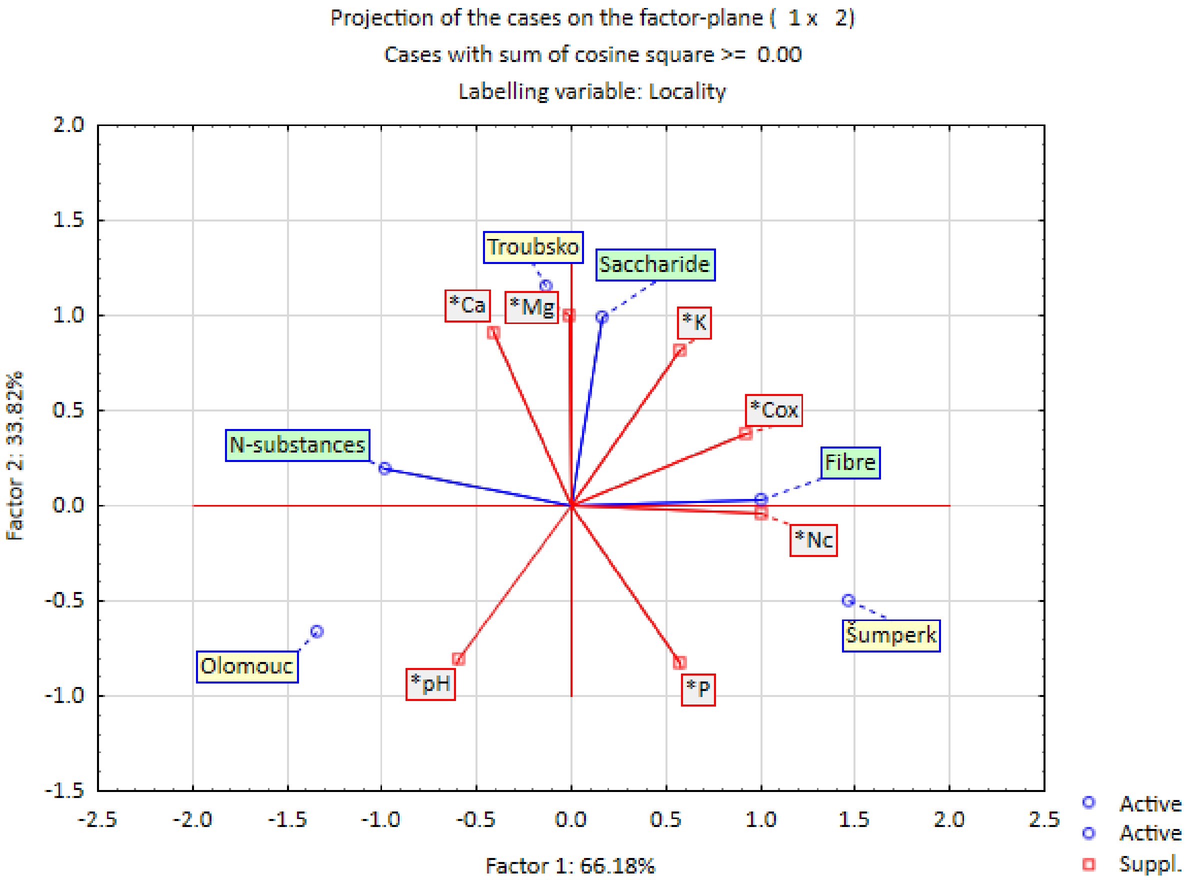

Figure 1. The figure illustrates the overall relationship between plant biomass parameters and soil characteristics across the experimental sites. The analysis identified two principal factors differentiating the sites. Factor 1 links plant nitrogenous compounds (N-substances) and fibre content with soil total nitrogen and organic carbon, and is primarily associated with the Šumperk and Olomouc sites. Factor 2 associates plant carbohydrate content with soil concentrations of K, Mg, P, Ca, and pH, and is mainly linked to the Troubsko site. According to the figure, plants at Troubsko exhibited the highest carbohydrate content, correlating with the highest soil levels of Ca, Mg, and K. The highest plant nitrogenenous compound content was recorded in Olomouc, which also had the highest soil pH, with elevated levels also observed in Troubsko. The greatest plant fibre content was found in Šumperk, corresponding to peak soil levels of P, total nitrogen, and organic carbon.

References

Javadi, H., Shanjani, P. S., Hoseini, L. F., Yeganeh, M. R., (2022) Evaluation of morphological and yield traits in the populations of Vicia spp. *SciELO, vol.13 no.4.* on-line ISSN 2448-6698. https://doi.org/10.22319/rmcp.v13i4.6125. Accessed 2025 April 12.

Pladias – Database of the Czech Flora and Vegetation. (2025). https://pladias.cz/taxon/overview/Vicia. Accessed 2025 April 2.

New forage species for a changing climate

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In Canada, over 13 million hectares of land are dedicated to forage production, accounting for approximately 65% of the country's agricultural land. Theses forages are essential to the beef and dairy sectors, which are major pillars of Canadian agriculture.

In recent years, fodder shortages have become an increasing concern, largely driving by the impacts of climate change, including more frequent droughts, extreme weather events, and shifting growing conditions. Therefore, to ensure sustainable livestock production and support the economic resilience of Canadian farmers, it is critical to explore and adopt new, climate-resilient forage species. In response, forage breeders across Canada have been actively evaluating three promising fodder crops for their adaptability to a range of Canadian environmental conditions. Plantain (*Plantago lanceolata*), chicory, (*Cichorium intybus*), and teff (*Eragrostis tef*) are all potentially suitable forage species for certain regions of Canada, especially as part of climate-resilient and diverse forage systems.

The present study was designed to characterized these three species for their biomass yield, persistence, and nutritive value. In 2023 and 2024, plants of each species were seeded at three Canadian sites: St-Augustin-de-Desmaures, QC (highest precipitation and snowfall and lowest growing degree days); Saskatoon, SK (lowest mean annual temperature); and Swift Current, SK (lowest precipitation and snowfall and highest growing degree days). Depending on the site, year, and species, plants were harvested one to four times per growing season.
Species survival and productivity varied markedly between the three study sites. Among the two perennial species tested, only chicory survived, and this occurred exclusively in Quebec. Its survival was likely due to the insulating effect of substantial snow cover in St-Augustin, which protected the plants from extreme winter temperatures.

Quebec also recorded the highest annual precipitation, which corresponded with the greatest biomass production across all species. Under these humid conditions, all three species achieved their highest productivity, with annual dry matter yields ranging from 4.4 to 6.7 tons per hectare (T DM/ha). Over the two-year period, average yields were 5.5 T DM/ha for both teff and plantain, and 5.2 T DM/ha for chicory.

In contrast, under the drier conditions of Saskatchewan, overall productivity was substantially lower. Teff was the only species to maintain relatively consistent yields, averaging 2.4 T/ha over the two-year period. Plantain and chicory exhibited much lower productivity, with average yields of 1.6 T/ha and 0.5 T/ha, respectively. Notably, chicory's limited survival and low biomass in Saskatchewan reflect its poor adaptability to the region's cold and dry conditions. These results underscore the importance of matching forage species to local environmental conditions.

Effects of nitrogen fertilization and harvest timing on forage quality of intermediate wheatgrass, Kernza®

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Keywords: Intermediate wheatgrass, perennial forage, harvest timing, nitrogen fertilization, Kernza, feed self-sufficiency

Perennial crops offer opportunities to mitigate environmental challenges associated with annual monocultures. Their deep roots and extended growing seasons provide ecological benefits including fixation of carbon, reduced input needs and enhanced resilience to drought. Kernza® (Intermediate wheatgrass, *Thinopyrum intermedium*), developed as a dual-purpose crop for forage and grain, has shown potential to serve as a sustainable fodder source for ruminants in northern Europe.

In Sweden, forage accounts often for over half of the total feed rations for ruminants. Enhancing the nutrient density of these forages through optimized management practices is crucial for achieving on-farm feed self-sufficiency and reducing dependency on costly concentrate-feeds. One key factor influencing forage quality is harvest timing, which is affected by the plant's maturity. In addition to harvest timing, the rate of nitrogen fertilization plays a significant role, affecting not only total yield but also, to some extent, the nutrient composition of the forage.

In 2024, field trials were conducted at three Swedish sites (Lönnstorp, Rådde, and Umeå), to cover a large gradient of latitudes and growing conditions, to assess the impact of harvest timing and nitrogen (N) fertilization on the forage quality of Kernza. Plots received four N treatments (0, 70, 105, and 140 kg N/ha) and were harvested at three timings: late May (early), early June (mid), and late June (late). The first results from one of the locations (Rådde), indicate a clear interaction between N rate and harvest timing. The highest forage nutrient content was observed at the early harvest with 140 kg N/ha (table 1).

These findings support the use of Kernza as a climate-resilient forage option for Nordic conditions and emphasize the importance of tailored fertilization and harvest strategies to maximize its nutritive value.

Table 1. Chemical composition and digestibility (% DM) of Kernza harvested at different times and subjected to four different N-treatments in Rådde, Sweden.

Harvest time	N rate (kg N/ha)	CP*	NDF	WSC	Digestibility
Early	0	9.55	53.1	13.6	86.9
Early	70 + 70	13.75	52.0	10.3	85.1
Early	105 + 105	16.11	50.7	9.87	84.4
Early	140 + 140	17.5	49.4	9.90	85.0
Mid	0	6.91	64.3	7.91	78.0
Mid	70 + 70	10.4	61.8	7.00	75.2
Mid	105 + 105	12.79	60.9	6.32	75.8
Mid	140 + 140	13.6	59.9	6.21	75.6
Late	0	5.89	61.2	11.9	73.8
Late	70 + 70	6.97	63.0	13.0	73.0
Late	105 + 105	8.26	61.5	13.0	71.1
Late	140 + 140	9.05	61.5	12.5	73.0
Significances					
Harvest time		<0.0001	<0.0001	<0.0001	<0.0001
N rate		<0.0001	ns	0.138	0.001
HxN		<0.0001	0.576	0.007	0.370

*CP = crude protein, NDF = neutral detergent fibre, WSC = water soluble carbohydrates

Monitoring genetic diversity: unveiling species and cultivar dynamics in temporary grassland

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Temporary grasslands are an essential component for sustainable ruminant production and integrated crop-livestock systems. Grass-legume seed mixtures for temporary grasslands typically consist of several species, often represented by different cultivars. The species composition follows the substitution principle, where fast-growing species are gradually replaced by slower-growing species, stabilising forage mass over the years. Shifts in species and cultivar compositions are essential for long-term stability and productivity of swards and are thus of agronomical and ecological interest. While species composition can be monitored through visual assessment, detecting shifts in cultivar compositions is challenging and requires different approaches. Genetic methods can be used to analyse plant genetic diversity (PGD) and measure these shifts. Two common methods used to assess PGD are multispecies amplicon sequencing (MSAS), a method targeting conserved sequences among species, and genotyping-by-sequencing (GBS), a reduced genome representation method. Despite the potential, studies are scarce due to difficulties in collecting representative samples in grasslands and standardising experiments. Here we successfully applied MSAS and GBS to assess PGD of agronomically relevant grass species and detect shifts in cultivar compositions. Using MSAS on samples containing pools of multiple species, we could taxonomically separate five species (*Dactylis glomerata* L., *Festuca pratensis* Huds., *Lolium perenne* L., *Trifolium pratense* L., *Trifolium repens* L.) and accessions within species. For assessing further applications of MSAS, we simulated changes in cultivar compositions potentially occurring under field conditions based on an extended *L. perenne* sample set of six cultivars. Based on this greenhouse experiment, pure and mixed samples containing one and two cultivars, respectively, were prepared and analysed using MSAS and GBS. For the mixtures, cultivars were pooled at two different ratios based on length of leaf fragments. For both genetic methods, mixtures containing cultivars at 50% segregated from the corresponding cultivars according to this ratio. Additionally, GBS enabled separation of mixtures containing two cultivars at a 75:25-ratio from the corresponding cultivars and the 50:50-ratio mixtures. As a proof-of-concept, we applied both methods to samples from a field experiment where two cultivars were sown at a 50:50-ratio in drills. The genetic structure and the segregation pattern of these samples were very close to that of the 50:50-ratio samples from the greenhouse. Our results indicate complementing applications in PGD monitoring of the two approaches. While we anticipate that MSAS with its cost-effectiveness could be applied to large-scale PGD monitoring of multispecies grasslands, GBS with its lower detection limit could be applied to studies where shifts in cultivar composition are of interest.

Uncovering the role of phenotypic traits in shaping the genetic structure of alfalfa

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Understanding how phenotypic diversity relates to genetic structure is essential for optimizing crop breeding strategies. Traits such as productivity, biotic resistance, and quality contribute directly to genetic differentiation within species. Although previous studies have shown that geographic origin significantly shapes genetic diversity in alfalfa, the influence of agronomic traits on genetic structure remains unclear. The effect of human-driven selection on phenotypic variation may also drive genetic differentiation, warranting further investigation. Here, we examine whether phenotypic diversity significantly contributes to the genetic differentiation observed in alfalfa.

A panel of 395 alfalfa accessions from diverse geographic regions was analyzed. Phenotypic data, including 217 traits, were collected from two sites, focusing on yield, quality, growth seasonality, and anthracnose resistance. Genetic data, comprising 210 000 markers, were obtained through genotyping-by-sequencing (GBS), with allele frequencies for each accession. Seven genetic groups, previously identified by Pégard et al. (2023), were used as a reference for our analysis.

Phenotypic diversity was explored through PCA, with genetic groups overlaid onto the resulting space to examine a potential relationship. Correlations between phenotypic and genetic diversity were subsequently tested using a Mantel test.

A Boruta feature selection method with Random Forest was employed to identify the specific phenotypic traits that most contribute to genetic differentiation between the groups, helping to pinpoint the phenotypic variables that best predicted genetic group membership. Pairwise genomic differentiation (F_{ST}) was calculated between genetic groups, and the markers with the highest F_{ST} markers were analyzed further. Annotated genes from these markers were used to link high F_{ST} regions with potential functional traits under selection.

Results showed a weak correlation between genetic and phenotypic diversity (Mantel = 0.301, P = 0.001), with phenotypic structure not fully aligning with genetic groups identified by molecular markers. Despite this, as shown by Boruta method, some traits such anthracnose resistance, growth seasonality, and lodging resistance significantly contributed to genetic differentiation. Among genomic regions with high F_{ST} values, some were linked to genes involved in pathogen resistance, cell growth, carbohydrate metabolism, and pollen recognition (Figure 1). These findings provide valuable insights into the role of phenotypic traits in genetic differentiation and highlight new genomic regions that can be targeted in breeding programs.

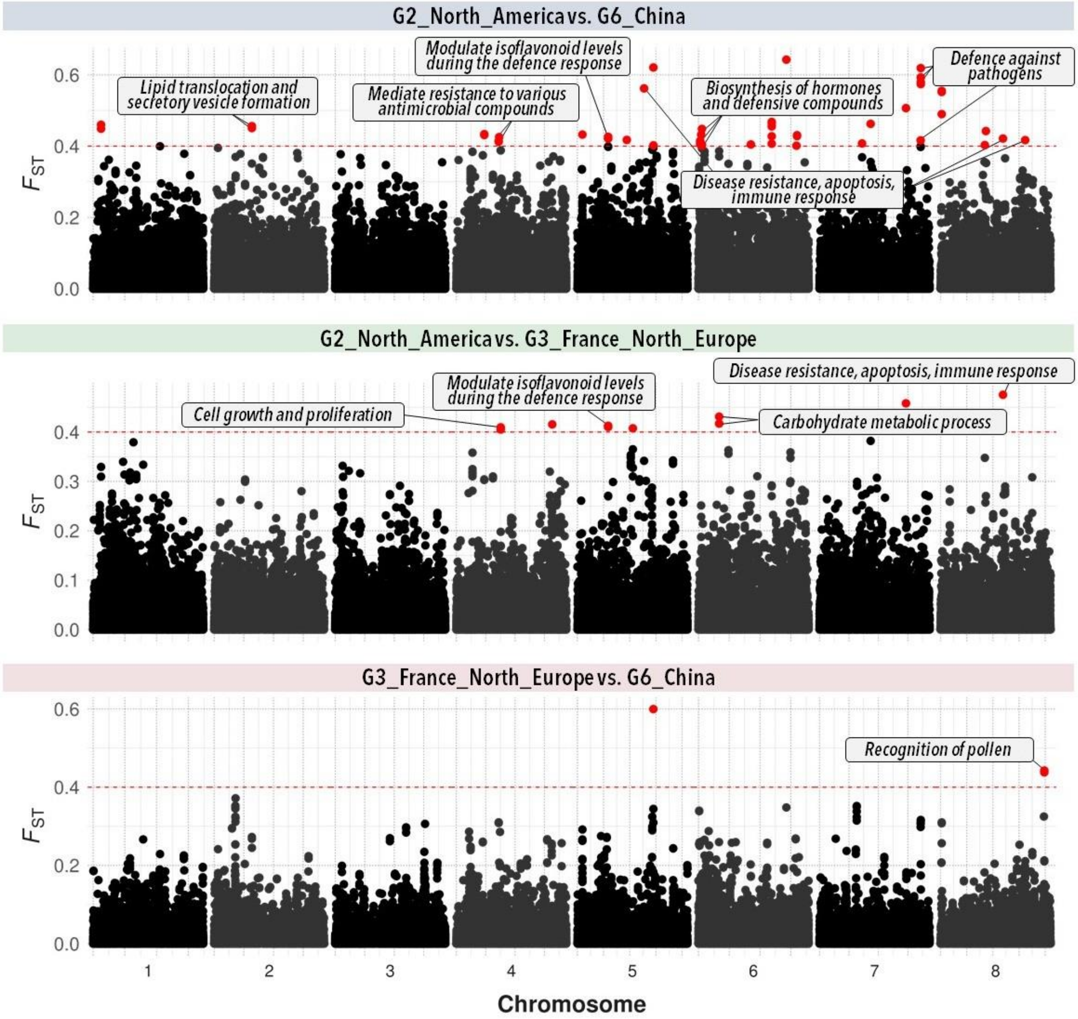

Figure 1. Genomic differentiation between genetic groups based on F_{ST} outliers.

Acknowledgements

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Reference

Pégard et al., 2023. DOI: 10.3389/fpls.2023.1196134

Genetic diversity of *Medicago* species within the German *ex situ* gene bank

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Alfalfa, an important leguminous fodder crop, belongs to the genus *Medicago*. *Medicago sativa* L. and *Medicago* × *varia* Martyn (alfalfa) with 744 accessions and further 38 *Medicago* species of crop wild relatives with 491 accessions are represented in the German federal *Ex situ* Gene Bank hosted at the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK).
The complete collection was genotyped by sequencing with bulk sampling per accession. The sequences were *de-novo* assembled, generating a high-quality dataset of 23,315 SNPs with less than 5.41 % missing data. Genetic diversity was studied via principal component analysis (PCA) and phylogenetic clustering.

Accessions generally clustered according to their taxonomic species, while sections according to Small (2011) were wider distributed and partially mixed. *M. sativa* and *M.* × *varia* clustered tightly together, reflecting the shared genetic composition and extensive geneflow. In contrast, wild crop relatives displayed a wider diversity. The section *Medicago* formed one major cluster, with three of the twelve species, *M. hybrida*, *M. suffruticosa* and *M. marina* (each represented by one accession), as outliers in the phylogenetic tree (Figure 1). Sections *Hymenocarpos*, *Carstiensis*, *Orbicularis* and *Dendrotelis*, which were represented by only one to two species each and a maximum of four accessions, were found to be monophyletic, respectively. The section *Dendrotelis* clustered near to the section *Medicago*. Species of the section *Spirocarpos* were found to be divided into several clusters.

The comprehensive analysis of the complete *Medicago* collection of the IPK gives first insights into intra- and interspecific genetic diversity within the genus and provides valuable assistance for gene bank management, targeted conservation efforts, and strategic breeding initiatives.

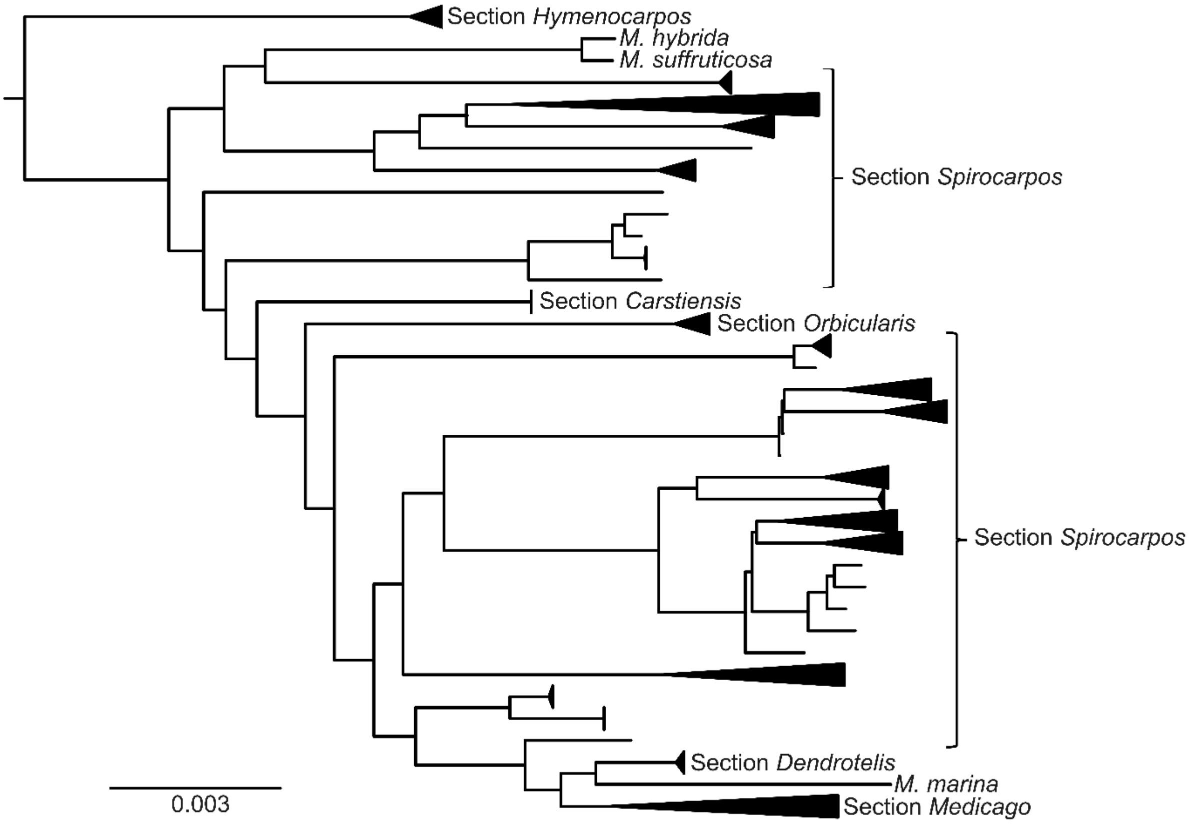

Fig. 1: Condensed Maximum Likelihood (ML) phylogenetic tree of *Medicago* germplasm. Species were grouped according to their respective sections.

Genome-wide analysis of genetic diversity in sainfoin (*Onobrychis viciifolia* Scop.) using genotype-by-sequencing

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Sainfoin (*Onobrychis viciifolia* Scop.) is one of the few forage legumes that produce condensed tannins- a trait that provides numerous benefits for livestock production and contributes to environmental sustainability. However, breeding progress has been significantly constrained by the limited availability of genomic data and advanced breeding tools. The objective of this study was to uncover allelic variation across diverse genotypes to enable downstream molecular-assisted selection and breeding of sainfoin. In 2023, a nursery comprising a diversity panel of 178 accessions, sourced from the USDA GRIN database, and breeding programs in Lethbridge and Saskatoon, was established in Lethbridge, Alberta. The experimental design was a randomized complete block design with three replications. Each plot consisted of five transplants of the same accession, spaced 1 ft apart, with 3 ft between plots. Agronomic data were recorded for traits of agronomic importance such as winter survival, plant height, flowering time, fall regrowth height, and biomass yield on the plot basis in year 2024. The phenotypic data was analyzed considering linear mixed model and BLUP values were used for downstream association with genotype. DNA was extracted from three individuals per accession using the E-Z 96 Plant DNA DS Kit (Omega Bio-tek Inc., Norcross, Georgia, USA), and sequencing was performed using genotyping-by-sequencing. Single nucleotide polymorphism (SNP) calling was conducted using the Stacks reference-based pipeline, mapped with in-house reference genome. Loci were filtered using the populations module with a per-accession coverage threshold of ≥50% followed by removing loci with >20% missing data. Approx. 7,800 high-quality SNPs were retained. These SNPs were used to classify accessions based on geographic origin or breeding population using Admixture. Preliminary results from genome-wide association analyses and genomic selection will be presented for selected agronomic traits.

Session 2: Advanced phenotyping and genotyping technologies

Predictive modeling for timothy grass yield under future climate scenarios in Norway using machine learning models

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Forage crop cultivation is dominant in Norwegian agriculture, comprising 68% of agricultural land and significantly supporting the dairy and meat industries. Introducing new grass varieties typically involves extensive breeding processes spanning around 20 years, and rapid climate changes pose significant challenges to crop yields. Timothy (*Phleum pratense* L.), a cool-season, hexaploid perennial grass, is particularly important due to its superior adaptation to cold climates. However, the anticipated climate changes necessitate evaluating how current Norwegian-adapted Timothy cultivars will perform in future scenarios. This study aims to enhance yield potential prediction for Timothy varieties under changing climate conditions. Comprehensive data, including temperature, precipitation, parentage, and dry matter yield, were collected from Timothy grass trials at five Norwegian locations over nine years (2003-2012). Initial descriptive analysis provided insights into the data for subsequent yield predictions under future climates. Two machine-learning/deep-learning models, ARIMA (Autoregressive Integrated Moving Average) and LSTM (Long Short Term Memory), were developed and tested. Model performance was evaluated using the Root Mean Square Error (RMSE) metric. The LSTM model demonstrated superior predictive capability with an RMSE of 90.37, thus being identified as the most reliable model for forecasting yields by incorporating key variables such as temperature, precipitation, parentage, location, and dry matter yield. Future climate scenarios were simulated by adjusting the original dataset according to the World Bank Climate Change predictions for 2030, 2040, 2050, 2075, and 2100. Applying the optimal LSTM model, yield predictions indicated that parental combinations "P1 – P2," "P3 – P4," and "P3 – P65" would yield increases of 10.88%, 7.90%, and 4.07%, respectively. Individually, parents "P1," "P89," and "P85" from the first parental pool showed predicted yield increases of 10.88%, 3.18%, and 1.69%, respectively, under future climate conditions. From the second parental pool, parents "P4," "P2," and "P85" were identified as optimal, with predicted yield increases of 7.90%, 6.26%, and 2.66%, respectively. These insights offer valuable guidance for developing Timothy cultivars optimized for future climatic conditions in Norway.

Improving forage nutritive value using harvester-mounted NIRS

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Near-infrared reflectance spectroscopy (NIRS) is an effective method for quantifying key plant constituents in dried and milled forage samples. However, the labour-intensive sample preparation required for laboratory-based NIRS limits its routine use for selection in forage breeding. By implementing higher throughput analysis through in-field NIRS measurements, the selection process for improved quality attributes in perennial ryegrass could be enhanced. To form the calibration dataset, perennial ryegrass samples were collected across seasons in 2022 and 2023. During plot harvest, samples were scanned using a Zeiss Corona Extreme at 2 nm intervals (950 -1690 nm). Various mathematical spectral treatments were evaluated for each calibration, ranked according to the root mean squared error of prediction (RMSE) and the ratio of percent deviation (RPD). In this study the most effective calibrations for dry-matter digestibility had an R_2 of 0.76 and an RPD of 2.18. By employing harvester-mounted NIRS, forage digestibility data can be routinely gathered throughout the growing season without the need for extra labour, thus aiding in the development of new forage grasses with improved digestibility.

Genomic insights into segregation distortion and heterozygosity in diploid alfalfa

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Alfalfa (*Medicago sativa* L.) is an outcrossing forage crop that carries a high genetic load with many deleterious alleles. This genetic load often presents itself physically in the form of inbreeding depression or genetically as a form of segregation distortion. Typically distorted chromosomal regions show inheritance patterns that deviate from expected Mendelian ratios favoring one parent's genotype, however, in the case of cultivated alfalfa and its diploid subspecies (*M. sativa* subsp. *caerulea* and subsp. *falcata, and their* hybrid, subsp. *hemicycla*) this distortion tends to favor heterozygosity across many regions of the genome.

To investigate the genetic basis of this pattern, we examined a broad collection of diploid alfalfa germplasm and generated F2 populations using parents of diverse geographic origin. Across all populations, we detected a large number of markers showing segregation distortion favoring heterozygosity across the genome, especially prevalent on chromosomes 5 and 7.

By assembling high-quality reference genomes for both diploid subspecies, we have identified a high degree of structural variations present in and between both subspecies as a way to better understand how and why these regions exhibit distortion skewed towards heterozygosity. We also performed RNA Iso-Seq across a circadian cycle to annotate these genomes and identify potential genes that are contributing to this observed pattern.

Previous studies have also shown correlation in physical maps of these distorted regions with consensus yield QTLs in tetraploid alfalfa, so the identification of these loci has potential to help breeders in the alfalfa industry to make informed decisions concerning through processes such as marker assisted selection.

Enhanced genome assemblies of French-bred *Dactylis glomerata* and *Medicago sativa*: achieving high-quality tetraploid genomes

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Having a reference genome for forage species is crucial for several reasons. It enables a deep understanding of the genetic structure and diversity of these species, essential for breeding and improvement programs. It is used to identify genes responsible for important agronomic traits, such as disease resistance, abiotic stress tolerance, and nutritional quality. A reference genome also facilitates the use of marker-assisted selection and genomic prediction, accelerating genetic improvement aimed at releasing robust and environmentally adapted varieties. It supports studies on natural populations to discover adaptive genes, aiding conservation and innovation in sustainable agriculture.

Haplotype-resolved assembly of autopolyploid genomes is challenging due to high sequence similarity and large repetitive regions. Recent evidence shows that combining HiFi (high-fidelity) long reads and Hi-C data achieves better contiguity and phasing accuracy. We present an assembly strategy based on post-processing Hifiasm results, significantly improving the final assembly of the 3.2 Gbp genome of *Medicago sativa* (lucerne) and the 7.49 Gbp genome of *Dactylis glomerata* (cocksfoot), both autotetraploids, using HiFi data and Hi-C reads. The assembly process began with an initial assembly using Hifiasm, which resulted in good quality assembly (For lucerne 3,415 Mbp across 5,696 contigs N50 = 94 Mbp, L50 = 147 and for cocksfoot 7,664 Mbp across 3,918 contigs N50 = 99 Mbp, L50 = 215) but with imbalanced haplotype lengths and inconsistent BUSCO scores. Hi-C scaffolding revealed inversions and misassemblies, requiring manual curation. Contigs were reassigned using Toulbar2, a combinatorial optimization tool, based on shared protein alignments. For lucerne, further refinement involved adjusting constraints by modifying the cost of shared protein and penalizing unlikely haplotype switches while preserving the original Hifiasm structure. Additional steps included the identification and removal of mitochondrial and chloroplast sequences from the assembly. Telomeric repeats were detected, although often present at the chromosome extremities. This method improved haplotype lengths, BUSCO scores, and Hi-C scaffolding accuracy, reducing manual corrections and resulting in more balanced final haplotype sizes. Final haplotype sizes were 759–779 Mbp across 451-753 contigs for lucerne and 1.84-1.92 Gbp across 347-587 contigs for cocksfoot, with BUSCO completeness over 98% for lucerne and 96% for cocksfoot, and duplication rates of 8.2–13.2% and 7-9.5%, respectively.

This study demonstrates the necessity of developing strategies to improve assemblies of challenging polyploid genomes. Having these two high-quality genomes is valuable for further fundamental and applied research in plant genetics, contributing to food security and the sustainability of agricultural systems.

Accelerating breeding in perennial ryegrass through artificial vernalization and speed breeding

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Perennial ryegrass (*Lolium perenne*) is an important forage species in temperate regions due to its high nutritional value and broad adaptability. However, genetic improvement is constrained by its extended generation time and the requirement for vernalization to induce flowering, which limits the number of achievable breeding cycles to one per year. Speed breeding (SB) protocols, based on the manipulation of environmental conditions to accelerate generation turnover, have been successfully implemented in several annual crop species. However, the application of SB to vernalization-dependent species such as perennial ryegrass remains a challenge.

This study investigates the integration of artificial vernalization into SB to enable multiple reproductive cycles annually. Several controlled-environment regimes have been tested to evaluate the effect of light composition, intensity and cold treatments on floral induction. Preliminary findings demonstrate that artificial vernalization can effectively induce early flowering across a broad range of genotypes. By integration of artificial vernalization into SB, this work aims to reduce the breeding cycle and improve the annual genetic gain. These advancements are expected to contribute to the development of more efficient breeding strategies for perennial ryegrass.

A virtual breeding system for sustainable forage mixtures: linking quantitative genetics and functional-structural plant modeling

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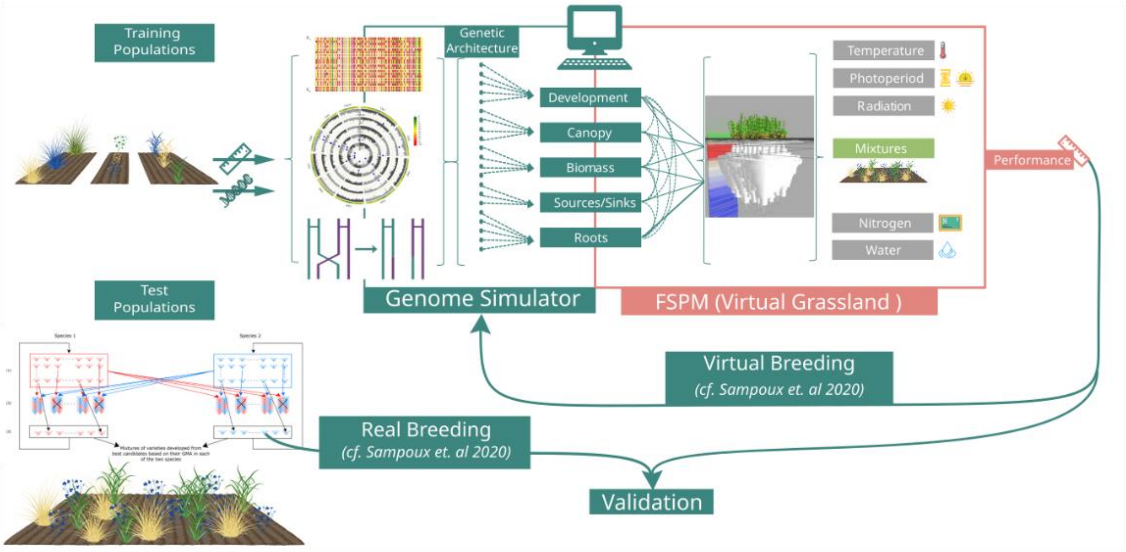
Forage mixtures including grasses and legumes can provide a forage of excellent quality with low inputs including nitrogen. However, their components are rarely bred in mixture, presenting a substantial opportunity for improvement. This study aims to develop a new virtual breeding system that couples quantitative genetics and ecophysiology approaches to optimize forage mixture breeding. Linking quantitative genetic models with ecophysiology models, such as Functional-Structural Plant Models (FSPM), will enable (i) the identification of key functional traits exhibiting heritable variability for productivity and stability in multi-species mixtures and (ii) the deciphering of the behavior of complex forage mixtures under selection. Specifically, we aim to determine whether input traits of ecophysiological models measured in isolated plants can predict mixture behavior, making the hypotheses that a substantial part of the differences in plants behavior between pure stand and mixture arise from different perceived micro-environmental conditions captured by the FSPM. We will then develop genomic prediction (GP) models for these crucial traits to reduce the experimental effort required for selection in species mixtures and explore new breeding strategies *in silico*. Using a training population of relevant pure stand forage species, we will incorporate the genetic architecture of essential traits into our predictive framework. Additionally, we will establish a connection between trait-centered approaches (measured from isolated plants) and quantitative genetics approaches -- such as those developed by Sampoux et al. (2020) -- that rely on the decomposition between direct and indirect effects of mixture components, whose ecophysiological origins remain poorly understood. Previous work in maize pure stands successfully validated the integration of quantitative genetics and ecophysiological traits using GP and crop growth models (CGM). This work highlighted the potential of this approach to unravel the evolution of genetic variance and heritability for flowering time under increasing temperatures in a simpler pure stand system and its potential transfer to mixed stands. Our long-term goal is to establish a selection program for diversified forage targets, collaborating with industry partners and academic institutions to validate our approaches and evaluate diverse field performances. This research will contribute to the development of forage mixtures, ensuring balanced and stable production systems.



Genome-wide association study (GWAS) reveals loci associated with forage biomass and development in hexaploid timothy grass

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Keywords: *Phleum pratense*, Timothy grass, Genetic diversity, Population structure, Admixture analysis, Dry matter, GWAS

Timothy (*Phleum pratense* L., 2n=6x=42) is a crucial forage crop widely cultivated in temperate regions across the globe. In this study, we evaluated a diverse panel of timothy accessions originating from Northern Europe under field conditions. We genotyped approximately 600 samples, representing 243 distinct wild and domesticated accessions. The resulting markers were employed to analyze population structure, detect admixture patterns, and identify genomic loci associated with key eco-agronomic traits. Admixture analysis revealed four distinct genetic clusters, with a high degree of admixture observed between wild and cultivated accessions. Genome-wide association studies (GWAS) identified significant marker–trait associations for dry matter yield on chromosomes 5PpD, 2PpB, and 7PpD. Additionally, significant associations were detected for developmental stages, specifically days to booting, on chromosomes 3PpB, 3PpD, and 6PpC. We also identified loci associated with canopy composition, which includes three tiller types: vegetative (VEG%), elongating (ELONG%), and generative (GEN%). Notably, markers associated with elongated tillers (ELONG and GEN) were predominant. These findings provide valuable genomic insights that can support pre-breeding and breeding efforts aimed at improving timothy for cultivation in Northern Europe, particularly in Sweden.

Exploring the expression and genetic control of polyphenol oxidases across a diverse range of red clover germplasm to guide the development of varieties suited to specific markets

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Red clover (*Trifolium pratense* L.) is a perennial forage legume that forms an important component of sustainable UK livestock systems as it fixes atmospheric nitrogen at an average rate of 250 kg^{-1} N ha^{-1} yr^{-1}. This species can express high levels of polyphenol oxidase (PPO) which catalyses the oxidation of polyphenols to produce highly reactive quinones that readily bind with proteins to produce coloured quinone-protein complexes. These stable complexes, formed following tissue damage, delay protein breakdown in the rumen and result in reduced loss of nitrogen as ammonia and N_2O to the environment. PPO is also reported to reduce both lipolysis and biohydrogenation of lipids leading to increased polyunsaturated fatty acid content in meat and dairy produce. While red clover is primarily grown as a forage for ruminants, extracted protein can also provide a feed for monogastrics. However, PPO has a negative impact on the digestibility of extracted protein and therefor limits the use of red clover as a source of protein for pigs and poultry.

We have already identified a red clover variant with low levels of PPO and used this to characterise enzyme expression both at a biochemical and genetic level in the cultivar Milvus. Through these studies we established that red clover has a large PPO gene family all closely located on the genome. As part of a bigger project, select red clover controls and 50 accessions lines collected from a range of elevations across Europe with varying phenotypes are currently being evaluated on an altitudinal gradient experiment for Distinctive Uniformity and Stability (DUS) breeding characteristics and other phenotypic characteristics of interest. An equivalent set of plants is being maintained in a frost-free glasshouse. As part of this research programme, we are evaluating PPO expression in this set of red clover using high-throughput genomic and biochemical techniques. Candidate red clover accessions displaying a range of PPO levels of expression will be compared with their counterpart along the gradient trials. Phenol profiles have also been evaluated as they provide substrate for PPO and have a role in the browning effect observed following tissue damage. The aim is to identify candidate red clover accessions for further development of cultivars tailored for two specific markets; red clover with high levels of PPO as forage for the dairy and meat sectors and varieties with low levels of PPO to produce feed for the monogastric sector (pigs and poultry).

Development of NIRS equations to predict biochemical composition of annual clovers

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Annual clovers (*Trifolium* sp.) include a wide number of species used either as forage crops (sown in spring or in autumn) or as cover crops (sown in summer or autumn). Recent regulations, requiring soil coverage between the harvest time of a cash crop and the sowing time of the next crop (autumn or spring), are encouraging the use of cover crops, which provide ecosystem services such as nitrogen sequestration or fixation and soil conservation. For example, in France, seed market for crimson clover (*T. incarnatum*) is second only to lucerne (*Medicago sativa*) among forage legumes. When annual clovers are used, the biomass obtained before the next cash crop sowing can be harvested as forage or left in the field as green manure. In both cases, the biochemical composition of the biomass affects its value. The biochemical composition of annual clovers is poorly described, and any breeding programme that aims at increasing the nutritional value of annual clovers requires NIRS equations. Such NIRS equations have already been developed for lucerne and red clover (*T. pratense*). In this study, we tested whether the available equations could be used to predict biochemical composition of annual clovers or whether a dedicated equation could give better predictions.

About 220 samples of annual clovers *(T. incarnatum*, *T. michelanium*, *T. resupinatum*, *T. squarrosum*, *T. subterraneum*, *T. vesiculosum*) were collected at two sites, with a total of three trials sown in either spring or autumn, with different harvest dates in spring, summer or autumn. Samples were dried and ground to pass a 1 mm sieve and NIRS spectra were collected on a Brucker instrument with three repetitions. NDF, ADF and protein contents, as well as digestibility were measured on the samples with two repetitions. Lucerne and red clover equations were used to predict the biochemical composition that was compared with the measured one. A subset of 193 annual clover samples was used to develop specific NIRS equations that were used to predict a test set consisting of the 34 remaining samples. The predictions were compared with the measurements.

The lucerne equations gave correct predictions of ADF and protein contents of annual clovers but the predictions of NDF content and digestibility were poor. The predictions were somewhat better with the red clover equations than with the lucerne equations but a strong bias and a structure between sites and cuts was observed (Figure 1, top). With new equations based on annual clovers, R^2 reached 0.89 and the bias was close to 0 (Figure 1, bottom). More samples, covering a wide range of sites, seasons and stages, will progressively enrich the equations. This offers the prospect of developing NIRS equations to predict the biochemical composition of annual clovers for use in breeding programmes and variety evaluation.

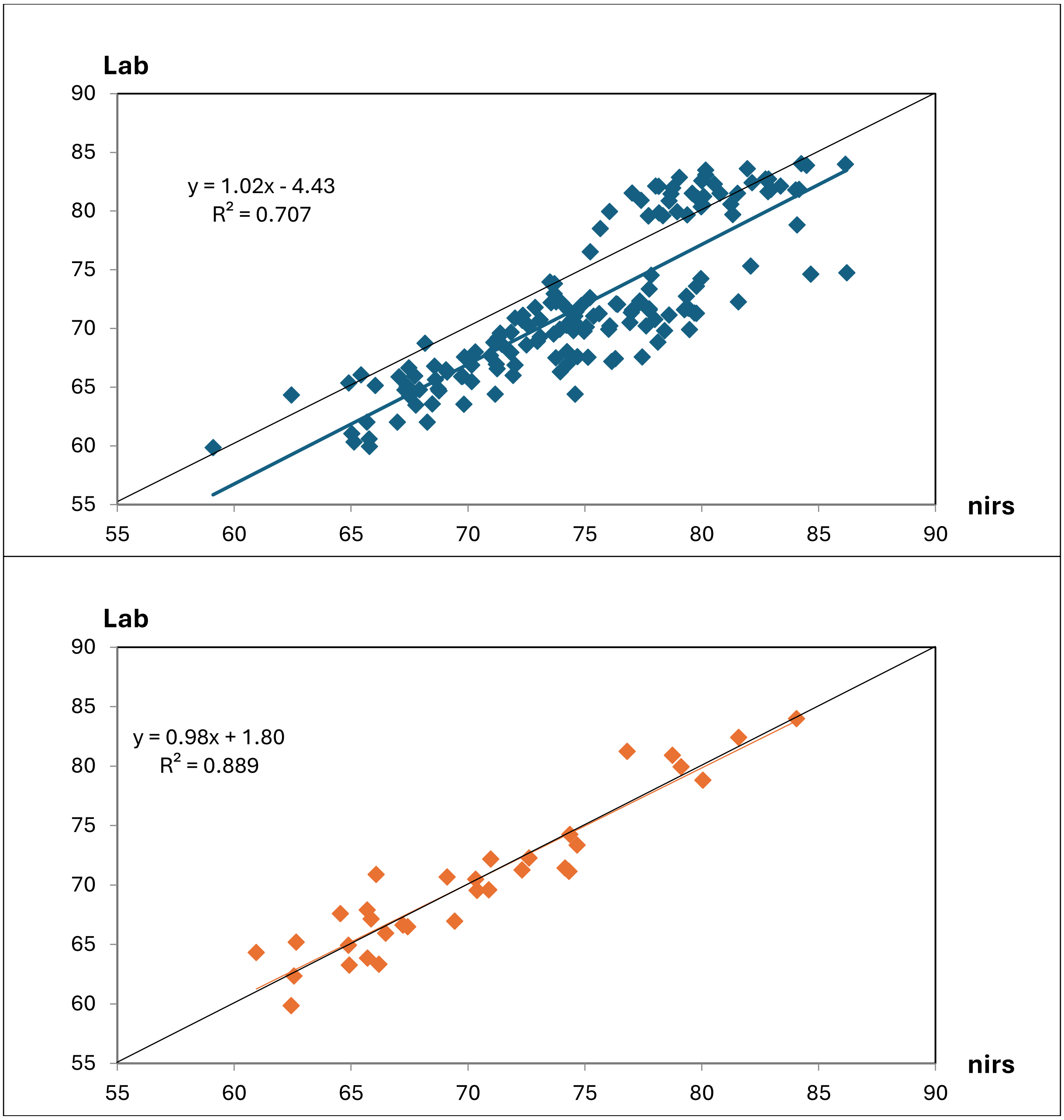

Figure 1. Comparison of digestibility (% dry matter) measured in the lab and predicted by a red clover equation (top) or an annual clover equation (bottom).

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Towards marker-assisted selection for anthracnose resistance, clover rot resistance and freezing tolerance in red clover (*Trifolium pratense* L.)

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Marker assisted selection (MAS) allows to efficiently select genotypes with resistance and/or tolerance to various abiotic and biotic stresses. Several QTL mapping and genome-wide association studies (GWAS) in red clover have revealed associations of genetic regions with stress resistance or other agronomic traits. However, only few publications present molecular markers that can be readily implemented in red clover breeding programmes.

Here, we have used previously published, allele-frequency based GWAS and bulk segregant analyses to select candidate SNPs for MAS for resistance against two fungal diseases, southern anthracnose (*Colletotrichum trifolii*) and clover rot (*Sclerotinia trifoliorum*), and for freezing tolerance. In total, 11, 7, and 6 candidate SNPs were selected for anthracnose, clover rot and freezing tolerance, respectively. For 20 of these SNPs, KASP (Kompetitive Allele-Specific PCR) markers could successfully be designed and genotyped. Greenhouse trials were conducted with 5 accessions each for anthracnose resistance in Switzerland, for clover rot resistance in Belgium, and for freezing tolerance in Norway. Per accession, 180-792 plants were tested, and the 25-30 most resistant and 30 most susceptible plants were genotyped. Allele frequency distributions within the resistant (R) and susceptible (S) pools were assessed. For 18 out of 20 KASP markers, the R pools had higher allele frequencies of the beneficial allele than the S pool. For anthracnose resistance, visual assessment of the phenotypes in relation to the genotypes revealed that two markers located on chromosomes 2 and 3 showed potential for MAS as they were co-segregating with putative recessive anthracnose resistance loci in all tested accessions. Two additional markers on chromosomes 4 and 6 were segregating with a susceptibility loci for clover rot resistance and freezing tolerance, respectively, in at least one accession. The remaining markers did not segregate with stress tolerance at sufficiently high frequencies to be useable for MAS.

Despite observing an accumulation of beneficial alleles within R pools, the linkage of the trait and the KASP marker seem to be frequently lost due to due to low linkage disequilibrium (LD) caused by recombination between the marker and the trait. In addition, the observed resistance to clover rot and freezing tolerance may be caused by several quantitative loci with minor effects. With our approach, the power to detect loci with small effects may not have been sufficient. Overall, our study highlights the difficulty of transferring insights from research to advance red clover breeding and calls for increased molecular marker densities and validation of candidate genes and regions.

Generation of *Festuca glaucescens* plants with mutations in the gene coding chloroplastic lipocalin by the use of CRISPR/Cas9 technology

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Plant lipocalins can be distinguish on two families: chloroplastic lipocalins (CHLs) and temperature-induced lipocalins (TILs). They play a protective role against oxidative stress by reducing the level of lipid peroxidation. Preliminary analyses revealed that expression of the gene coding chloroplastic lipocalin is regulated under drought and salinity but not under low temperature in the members of *Lolium-Festuca* complex.

F. glaucescens (*F. arundinacea* Schreb. subsp. Fenas (Lag.) Arcang.) (2n = 4x = 28) is a allotetraploid containing G1 and G2 sub-genomes (G1G1G2G2). It reveals unique drought tolerance strategy related to metabolism deceleration and a reduction of growth, associated with ‘a quiescence’ under water deficit, followed by a further regrowth after stress cessation. In *F. glaucescens* genome three homologs of chloroplastic lipocalin gene (*FgCHL*) were identified using whole genome sequencing. Each homolog consists of five exons and four introns. The differences in intron length between homologs were observed.

To investigate functions of chloroplastic lipocalin under oxidative stress in *F. glaucescens*, transgenic plants with mutations in chloroplastic lipocalin gene (*chl*) using *Agrobacterium tumefaciens* transformation and CRISPR/Cas9 technology, were obtained and applied. The modular vector system for RNA-guided Cas endonucleases in monocots was used to obtain CRISPR/Cas9 construct. The prepared construct consisted of four guide RNAs driven by individual promoters (OsU3, TaU6) targeting the second, third and fourth exon of chloroplastic lipocalin gene, *cas9* gene under ZmUbi promoter and *hpt* conferring hygromycin resistance as a selection marker. *Agrobacterium*-mediated transformation of callus derived from mature embryos was performed with vacuum infiltration. To identify mutations in *chl* gene, PCR and Sanger sequencing were performed. That approach allowed to obtain mutants with deletions in *chl* gene over 400 bp in length. Moreover, we observed intron retention phenomenon. Transgenic plants revealed different phenotypic traits and delayed growth in comparison to the wild type plants.

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Phenomic prediction for perennial ryegrass: initial findings and future directions

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Phenomic prediction is an innovative technique that uses phenotypic data, such as reflectance spectra, to predict complex traits. Unlike traditional genomic prediction methods that rely on molecular markers, phenomic prediction leverages the biochemical composition of tissues, which is genetically controlled, to build relationship matrices that capture genetic signals. This approach has been primarily applied to annual crops and explored in perennial species such as poplar and grapevine.

Phenomic prediction offers a low-cost alternative to genotyping, making it an attractive option for predicting complex traits. It has been successfully tested for various traits, including yield, phenology, and berry composition in grapevine, demonstrating stable predictive abilities across different tissues and years. While phenomic prediction has not been applied to perennial ryegrass, most advancements have been seen in genomic prediction and high-throughput phenotyping techniques. Genomic prediction models have shown significant potential in improving traits like dry matter yield (DMY) in perennial ryegrass, with various machine learning and statistical models being employed to enhance predictive accuracy. Given this performance and the fact that techniques like non-destructive spectroscopy have been employed to measure nutritive value parameters in perennial ryegrass, we can expect phenomic prediction to work.

Although phenomic prediction is a promising and cost-effective method for predicting complex traits, its application in perennial ryegrass needs to be proven. This study represented a test of phenomic prediction on perennial ryegrass, a first for this species. To achieve this, we tested the prediction of various phenotypic traits related to phenology, such as heading date, start and end dates of pollen emission, and certain growth characteristics. These predictions were made using a portable NIRS (Near Infra-Red Spectroscopy) device. This characterization was carried out on individuals belonging to perennial ryegrass populations that were representative of the species' phenological diversity and installed in two different locations. Initial results reveal that the genetic variability can be captured with the NIRS at the individual and population level on fresh leaves. The phenological state of the tiller carrying the leaf used to take the measurement is also important for this characterization.

Further research is needed to explore the application of phenomic prediction in perennial ryegrass, in comparison to genomic prediction frameworks. In conclusion, while phenomic prediction holds promise, its application in perennial ryegrass is still in its infancy, with important breeding outcomes for this important forage species but also for lesser species where genomic prediction is not yet feasible.

Session 3: Qualitative and quantitative traits

A possible future for alfalfa production

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Alfalfa production in the USA has declined significantly over the past 20 years, including a precipitous decline in California, the leading alfalfa hay production state just several years ago. The reasons for the decline are varied, from displacement by higher value crops (including nuts and grapes) to shifts in dairy ration components (e.g., to corn silage and almond hulls). But some of the decline has to do with the crop itself, with high labor requirements for multiple harvests per year and the necessity of maintaining stands for multiple years for profitability. As climates shift and irrigation water becomes less reliable, what is the future for the crop? I will discuss several ways we can use germplasm, genetics, and genomics to strengthen breeding programs, drawing examples from my own research program as well as those of colleagues. I will argue that despite these advances, we need to do more to create a future for alfalfa production. And then I will propose the need for a new alfalfa plant ideotype and discuss what a research program might require to develop this new alfalfa paradigm.

Expected genetic gain of the most important traits in the breeding process of the meadow fescue

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Keywords
meadow fescue, Nested Design I, expected genetic gain.

Meadow fescue (*Festuca pratensis* Huds.) is an important perennial forage grass for animal feed production on grasslands and pastures, with high yield potential and good-quality biomass. During the breeding process, it is often necessary to predict, based on the results of the initial selection cycles, how much a certain trait can be improved. To that end, it is necessary to determine the expected genetic gain (ΔGe), a parameter that tells us how much a trait can be improved by breeding it alone in the following period. Individual plants for hybridization, to obtain progeny by the method of Comstock and Robinson, were selected completely randomly. To examine the obtained progeny, the study was performed in an experimental field of the Institute for Forage Crops, in two replications. The experiment was set up according to the Nested Design I, with sets within the replications. In cv. Kruševački 21 (K-21), 60 full-sib progeny were studied in two sets, while in the cv. Pradel a total of 39 full-sib progeny were studied. Within each full-sib progeny, 60 plants were analyzed.

In both cultivars, the highest expected genetic gain was determined for the number of generative and vegetative tillers per plant and dry matter yield per plant. The least success can be achieved by improving the heading date and crude protein content in the 1st cut in cv. K-21, and in cv. Pradel for traits leaf length and crude protein content in the 1st cut.

By breeding individual traits, based on the obtained results, a large genetic gain is expected in many studied traits. Considering the existence of correlations between the most important agronomic traits, it is observed that often, by breeding one trait, a negative response is encountered in another trait. For this reason, the expected indirect genetic gain from selection was calculated, i.e., the effect of breeding one trait on other ordered traits. In the cv. K-21, achieving the expected genetic gain in plant height, would lead to a decrease in dry matter yield and leaf length. By creating later cultivars, the result would be a cultivar with a smaller number of generative tillers and a lower protein content in the first cut. Successful breeding for increased crude protein content in the first cut of 9.9 gkg-1 would result in a significant reduction in dry matter yield, followed by a reduction in the number of vegetative and generative tillers, but also earlier maturity. In cv. Pradel, a positive effect of the selection of individual traits on other studied traits was recorded for most of the traits. The only realization of the expected genetic gain in the increase in leaf length would result in a decrease in the number of vegetative tillers per plant.

Expected genetic gain (ΔGe) of the most important traits of meadow fescue

Traits	Heading date*	Plant height (cm)	Leaf length (cm)	No veg. tillers	No gen. tillers	DMY (gplant^{-1})	CP 1st cut (gkg^{-1})
	K-21						
Heading date*	**2.58** (9.06%)	0.02	2.23	0.59	-2.28	0.89	-1.57
Plant height (cm)	0.07	**9.11** (10.02%)	-5.68	3.91	10.17	-3.80	3.49
Leaf length (cm)	3.81	-3.14	**4.32** (13.88%)	0.58	-0.12	2.73	1.09
No veg. tillers	4.35	9.24	2.46	**21.36** (34.66%)	4.24	2.74	-4.28
No gen. tillers	-30.98	44.79	-0.98	7.90	**62.94** (28.17%)	27.97	-21.59
DMY (gplant^{-1})	20.37	-27.99	36.31	8.51	46.81	**55.49** (18.45%)	-44.82
CP 1st cut (gkg^{-1})	-0.45	0.33	0.19	-0.17	-0.46	-0.57	**9.9** (8.78%)
	Pradel						
Heading date*	**5.64** (13.45%)	5.42	1.74	5.30	4.03	0.704	4.37
Plant height (cm)	11.50	**11.56** (11.43%)	4.94	6.35	2.81	10.55	6.82
Leaf length (cm)	1.52	2.03	**1.38** (5.61%)	-2.20	2.19	0.69	0.79
No veg. tillers	39.14	22.07	-18.55	**42.87** (45.59%)	37.11	21.35	-8.24
No gen. tillers	81.93	26.93	50.84	102.21	**115.88** (45.93%)	98.09	22.27
DMY (gplant^{-1})	12.66	89.22	14.18	51.97	86.69	**97.96** (40.34%)	71.13
CP 1st cut (gkg^{-1})	0.97	0.71	0.20	0.25	0.24	0.88	**10.1** (7.46%)

* Number of days from April 1st

Acknowledgments

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RNAseq based evaluation of how perennial ryegrass vernalization and flowering responds to a warming climate

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Keywords
Climate, Change, Grass, Perennial, Cultivar, Growth, Flowering, RNAseq, Vernalization

Most land use in Ireland is dedicated to the production of perennial ryegrass (*Lolium perenne*) Crop usability is dictated by heading date, after flowering, ryegrass becomes unpalatable to cattle and undesirable for silage. Using historic data, we show that heading date has been getting gradually earlier as the climate warms. Before heading, plants must go through a period of vernalization to enter reproductive growth. It has been widely accepted that this process takes eight weeks below-10ºC, under short day conditions. The vernalization requirement of early, intermediate, and late flowering cultivars were examined across both natural and controlled conditions. Results show that there is a variance in vernalization requirement, with some cultivars commencing reproductive growth after as short as a four-week vernalization period, while others remained vegetative up to the end of the more widely accepted 8 weeks, splitting cultivars into groups of weak and strong vernalization types. Preliminary RNA-sequencing results have shown key vernalization and flowering genes are behaving differently on a varietal and thermic basis. As conditions suitable for vernalization are predicted to become shorter due to climate change a greater understanding on the mechanisms underpinning these processes will help grass breeders to create cultivars better equipped for future conditions.

Multiple selection of red clover based on symbiotic nitrogen fixation

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Current trends in agriculture are shifting towards sustainable and ecological farming systems that aim to reduce dependence on synthetic inputs, particularly industrial nitrogen fertilizers. One of the key strategies to achieve this goal is the utilization of natural biological nitrogen fixation (BNF), a process carried out by red clover (*Trifolium pratense* L.) and other leguminous plants in symbiosis with root-nodulating bacteria of the order *Rhizobiales*. Red clover is a cross-pollinated species characterized by considerable intraspecific variability, which includes variation in the ability of individual genotypes to fix atmospheric nitrogen. Our study focuses on the recurrent selection of red clover genotypes with the aim of enhancing their BNF capacity. The selection of beneficial plants was based on measurements of nitrogenase enzyme activity using the acetylene reduction assay (ARA). Four selection cycles were conducted. In each cycle, plants with the highest nitrogenase activity were selected, intercrossed, and their progeny were subsequently re-evaluated using the ARA method. The final evaluation of progeny, along with comparison to parental population and the initial population, was conducted in 2025. The results indicate that during each selection year there was a noticeable increasing trend of nitrogen fixation and the trait variability was lower compared to parental populations. These findings are consistent with the assumption that nitrogen fixation is a quantitative trait governed by polygenic inheritance. The resulting plant material represents a valuable resource for the development of new cultivars with improved and more stable nitrogen fixation capacity.

Genetic basis and improvement strategies for nitrogen use efficiency in perennial ryegrass (*Lolium perenne* L.)

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Perennial ryegrass (*Lolium perenne* L.) is a key forage grass valued for its nutritional quality. Improving its nitrogen use efficiency (NUE) is crucial for productive and sustainable agriculture, particularly in Norway's challenging climate. This study investigates the genetic basis of NUE in three ryegrass cultivars: a Dutch cultivar with high NUE (Barhoney) and two Norwegian cultivars (Fagerlin and Figgjo), under varying nitrogen levels. Hydroponic experiments allowed precise manipulation of nitrogen treatments. Samples of shoots and roots were collected at multiple timepoints to monitor gene expression changes. Key nitrogen transport and assimilation genes (*NRT1.1, NRT2B, NAR2.1, GS1, GS2, NiR, and AlaAT*) were analyzed using quantitative PCR (qPCR). Leaf nitrogen content was measured with a handheld N-tester. Phylogenetic analysis, incorporating sequences from *L. perenne* and other species, revealed conserved transporter genes among temperate grasses but highlighted lineage-specific differences indicative of functional diversity. Gene expression analysis indicated that nitrogen-responsive genes differed in timing and magnitude of response, varying by gene and cultivar. *AlaAT* and *NiR* exhibited rapid responses to nitrogen shifts, whereas *GS2* and *GS1* generally increased at later stages, notably differing between the Dutch and Norwegian cultivars, suggesting distinct genetic strategies for nitrogen metabolism. Genotype influenced leaf nitrogen content more significantly than nitrogen treatment or sampling time, despite some measurement constraints. CRISPR/Cas9 Level II knockout constructs targeting NUE-related genes (*AlaAT, GS1, GS2, NRT1.1*, and *NAR2.1*) were successfully assembled and sequence-verified, providing tools for future functional validation studies. Overall, this research offers new insights into the temporal and genetic dimensions of nitrogen responses in perennial ryegrass, laying foundational groundwork for targeted genome-editing strategies to enhance NUE.

Variability and heritability of dry matter yield components of perennial ryegrass (*Lolium perenne* L.) genotypes

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Keywords
perennial ryegrass, breeding, variability, heritability

Perennial ryegrass (*Lolium perenne* L.) is one of the most important perennial forage grasses in the northern climate. It is characterized with high quality, digestibility and dry matter yield. The aim of breeding of this species is obtaining cultivars with high stabile yield, quality and field resistance, with different time of maturity. Wild populations are good initial breeding material, which have high variability and adaptability what is important prerequisite for selection of superior genotypes. The main disadvantage of that material is lack of uniformity, which is hard to achieve before cultivar registration. In the directed breeding process, despite decreasing of some traits total variability, some desirable level of heterogeneity remain what gives the genotype better adaptability. Nevertheless, improvement of some important agronomic traits interact with value of other ones.

Investigations of variability parameters (variance components and coefficients of variation) and heritability of perennial ryegrass genotypes for agronomic most important traits are shown here, as well as their correlations. Prosperous perennial ryegrass genotypes originated from wild populations were investigated in space-plant nursery (60x60cm). Time of tillering, plant height, sward diameter, number of tillers per plant, dry matter yield (DMY) and crude protein content were investigated. For plant height and DMY the highest differences between genotypic and phenotypic variance components were determined and the lowest heritability (16.4% and 25.4%), respectively. Highest heritability is determined for time of tillering (83.26%) and sward diameter (79.87%). According the data, breeding of genotypes for improvement of these traits could provide effective results in short period. Also high heritability was detected for number of tillers per plant and crude protein content.

It was determined that generally highest variability parameters between genotypes were for number of tillers per plant and DMY. Phenotypic coefficients of variation for these two traits were about 15%, while genotype of plants is responsible for 80% of total phenotypic variations. Also, 91% of variability for time of tillering originated from genotypes, although total variability is relative low (3.77%).

Highest coefficients of correlation were estimated between sward diameter and plant height (0.86), and between plant height and crude protein content (-0.87), respectively. According to determined coefficients of correlations, time of tillering is negatively connected with plant height and number of tillers per plant, while DMY is increasing with late tillering of plants. Higher plants and plants with wider swards showed higher DMY, confirming that breeding of these two yield components in this perennial ryegrass genotypes collection may indirectly result in increasing of DMY. It can be concluded that investigated perennial ryegrass genotypes represent promising germplasm for the further breeding.

Table 1. Indicators of variability and heritability of the agronomically most important traits of perennial ryegrass genotypes

Traits	Genetic CV (%)	Phenotypic CV (%)	Genetic variance	Phenotypic variance	Heritability
Time of tillering	3.44	3.77	4.92	5.92	83.26
Plant height	1.85	4.57	1.83	11.16	16.43
Sward diameter	4.98	5.57	5.95	7.45	79.87
Number of tillers per plant	11.87	14.91	1708.9	2694.3	63.43
DMY	7.51	14.89	64.74	254.96	25.39
Crude protein content	6.56	9.16	1.31	2.56	51.26

Acknowledgment: This research was financed by Ministry of science, technological development and innovation of Republic of Serbia, contract No 451-03-136/2025-03/200217

Genetic and phenotypic diversity of lucerne (*Medicago sativa*) for optimising its role as a living mulch in agroecological systems

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Keywords
Agroecological systems, living mulch, lucerne, genetic diversity, competition

Lucerne (Medicago sativa), a drought-tolerant forage legume, is increasingly used in agroecological systems as a service plant, particularly in intercropping with annual crops such as cereals. As a perennial crop, lucerne forms a living mulch that potentially offers multiple benefits, including weed control, nitrogen enrichment, and support for reduced tillage practices, thus saving energy consumption and preserving biodiversity. However, recent studies have highlighted a significant challenge: current lucerne varieties, selected for forage production, are overly competitive and negatively impact the productivity of interplanted crops such as wheat.

This study aimed at analysing the genetic and phenotypic diversity within the *M. sativa* complex to identify traits that enhance lucerne's effectiveness as a living mulch, focusing on competition for light and nitrogen among lucerne, wheat, and weeds, and later their genetic determinism.

Thirty diverse lucerne accessions, representing different subspecies, autumn dormancy levels, and plant architectures (ranging from prostrate to erect forms), were evaluated. In the first phase of the study, the effects of lucerne dormancy and growth habit on wheat dominance during early stages and weed abundance were assessed. Later in the season, at the wheat heading stage, the impact of lucerne height and lodging on wheat biomass and nitrogen status was evaluated. In the second phase, forty plants of each accession were genotyped using Genotyping-by-Sequencing (GBS) and phenotyped in a nursery for plant height, growth habit, and lodging susceptibility. Genetic variance was estimated using the REML model, and broad-sense heritability was calculated. Genome-Wide Association Studies (GWAS) with a Multi-Locus Mixed Model (MLMM) were used to identify candidate QTLs.

The results suggest that lucerne varieties with slow growth, moderate height, and low lodging are most effective as living mulches. However, no variety in the panel exhibited all these desirable traits, requiring dedicated breeding programmes to create this ideotype. A total of 100K SNPs covering all eight lucerne chromosomes were identified. Genetic structure analysis revealed distinct separation between wild and cultivated forms, as well as between the *falcata* and *sativa* subspecies. A high heritability of the traits was observed, varying from 0.47 for lodging to 0.69 for growth habit. Variation around the correlations between traits suggested that it is possible to combine favourable traits in a single variety. QTLs were obtained; they could be used to speed up the genetic progress in breeding programmes.

These findings provide a foundation for the genetic improvement of lucerne as a living mulch, contributing to effective and sustainable agricultural practices. Further analyses are ongoing to determine how to introduce molecular markers in the selection of lucerne varieties adapted to living mulch use.

Yield of white clover (*Trifolium repens* L.) differs remarkably between monoculture and grassclover

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White clover (*Trifolium repens* L.) is predominantly used in mixtures with forage grasses and grassland herbs. A good white clover cultivar is adapted to competition with companion grasses and ensures a high-yielding grassclover mixture with a stable clover content. Although these traits are only visible in mixed swards, some breeders and variety trials continue to evaluate white clover candidate varieties exclusively in monoculture trials, which may not be representative for practice conditions.

We evaluated the correlation between performance in monoculture and grassclover. A 3-year yield trial was established in May 2020 with 24 white clover accessions, including cultivars, candivars developed at ILVO and 2 wild populations from Belgium. The trial was established as a split plot design with two replicates. The main plot factor was the mixture (monoculture *vs.* grassclover), the subplot factor was the accession. Plot size was 7.8 m². Each plot was sown with 7000 germinable clover seeds, and grassclover plots were amended with 20g seeds of the late-flowering perennial ryegrass (*Lolium perenne* L.) 'Agosto'. Monoculture plots were fertilized with 12/35/260 NPK units in year 1 and 12/40/320 units in years 2 and 3. Grassclover plots received the same fertilization amended with 55 units N in year 1 and 100 units N in years 2 and 3. Plots were harvested using a Haldrup forage harvester: two cuts in 2020, four in 2021 and four in 2022. The dry matter yield (DMY) was determined for each plot and cut, and we calculated correlations between the total annual DMY in monoculture vs. grassclover for the three harvest years (Figure 1).

Annual yields in monoculture were only 54%, 57% and 67% of the grassclover yields in harvest years 1, 2 and 3, respectively. Yields in monoculture and grassclover were not correlated in year 1 ($r = 0.07$, $p = 0.752$), weakly correlated in year 2 ($r = 0.45$, $p = 0.027$), and moderately correlated in year 3 ($r = 0.68$, $p < 0.001$) (Figure 1). Although some accessions yielded well in monoculture and grassclover over the three years (e.g. accessions 8, 14 and 22), the best accessions in monoculture were generally not the best in grassclover. In addition, performance varied considerably over the years, e.g. accessions 19 and 23 performed poorly in grassclover in year 2 but well in year 3. Only accession 14 ranked in the best-performing quartile for total yield in both monoculture and grassclover.

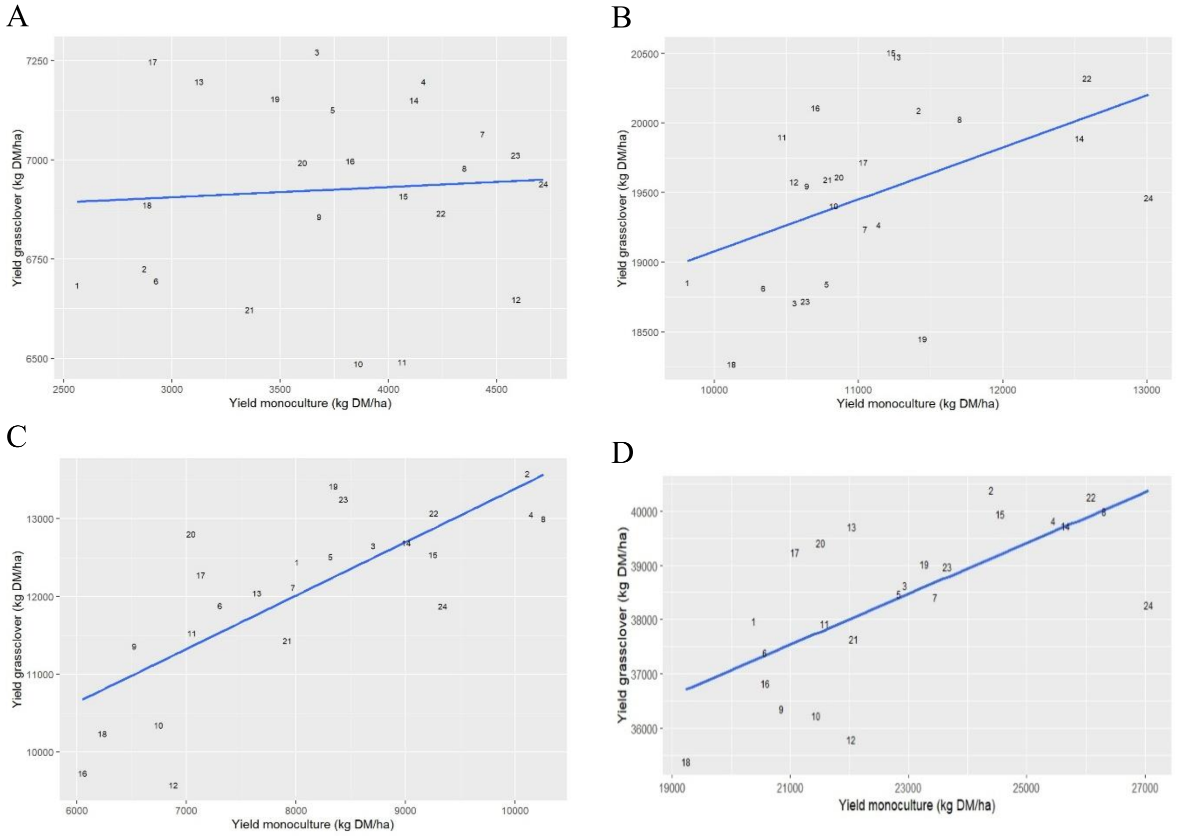

Figure 1: DMY of each accession in monoculture and grassclover in year 1 (A), year 2 (B), year 3 (C) and in the three years combined (D) in kg/ha.

Although our single-trial results should be interpreted carefully, they show that monoculture trials deliver little information on performance of white clover in mixtures. Our study underlines the importance of performing yield trials in grassclover mixtures to identify white clover candivars that ensure a high-yielding grassclover mixture with a stable clover content throughout the years. The same message holds true for perennial ryegrass: the best yielding varieties in monoculture are not the best in mixtures with white clover (Cougnon et al., 2024). In line with this, breeders should select genotypes in mixtures to develop cultivars adapted to mixtures. ILVO has already implemented this approach in its white clover and perennial ryegrass breeding programmes.

References

Cougnon M., Ghesquiere A., Aper J. (2024). Breeding perennial ryegrass varieties with an improved combining ability with white clover. In: Why grasslands? Grassland science in Europe 29, 514-517.

Predicting seed set of perennial ryegrass (*Lolium perenne* L.)

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Seed set describes early growth of embryo and endosperm after successful fertilization. It exemplifies the utilization of the potential seed yield by determining the ratio of developed seeds of total floret of a spikelet. In perennial ryegrass, there is a high potential seed yield but low realized seeds which may be due to incompatibility of deposited pollen attributable to self-incompatibility, seed abortion or seed shattering. Predominantly, traits such as, forage dry matter yield, disease resistance, nutritional value and persistency have had more attention in plant breeding of forage crops in comparison to seed yield hence this study seeks to bridge knowledge gap and to reveal how potential seed yield components (number of spikelet and floret) influences seed set.

In order to study the relationship between the yield components spikelet and floret number and their relationship with seed set we initiated a field experiment. In 2022 we got access to 384 genotypes of *Lolium perenne* L. from DLF. They were all diploid forage types heading group six. The experiment was conducted with four pseudo replications in a randomized complete block design. Inflorescence samples were harvested at the dough stage (BBCH 80) for evaluation. Three spikelets at the central position of the spike were assessed for developed and undeveloped seeds. Data such as number of spikelet per spike, number of floret per spikelet, developed seed per floret, undeveloped seed per floret, seed set and weight of spike were collected and analyzed.

The study predicted that seed set was influenced negatively by the number of spikelets and number of florets (Figure 1) but was positively associated to weight of spike. Thus, high number of spikelets and florets significantly reduced seed set. However, spikes with high seed set had high weight. The 384 genotypes were transplanted to AU-Flakkebjerg in autumn 2022. Data from 2023 experiment revealed similar trends and interactions between traits (data not shown). Results from this study will be discussed with plant breeders and combined with their prediction on seed yield. The perspective is to guide breeders on the next steps to select for seed yield in their breeding program.

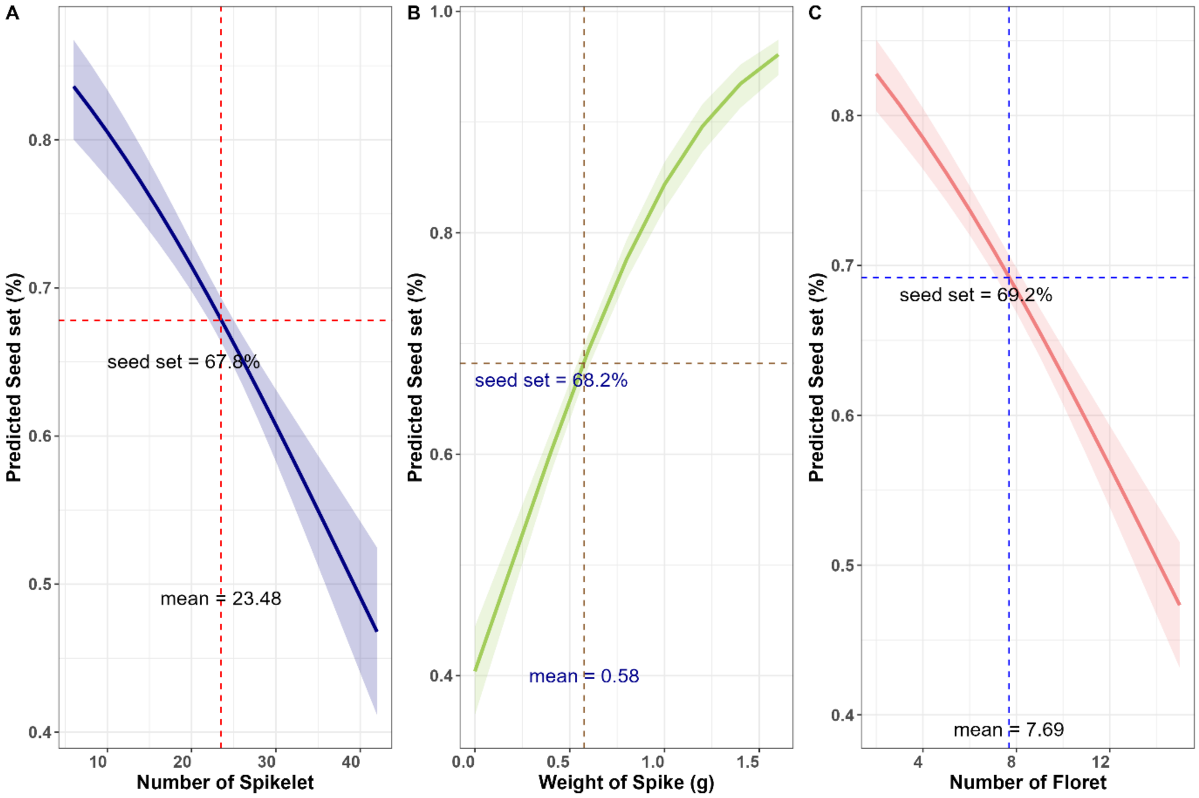

Fig 1. Prediction of seed set percent on A. number of spikelet. B. weight of spike. C. number of floret from 384 *Lolium perenne* genotypes, 2022.

Acknowledgement

We would like to thank DLF for access to the plant material and to the breeders Morten Greve Pedersen and Niels Roulund for their participation in the project.

A first nested association mapping population for Italian ryegrass reveals candidate genes for seed shattering and seed yield-related traits

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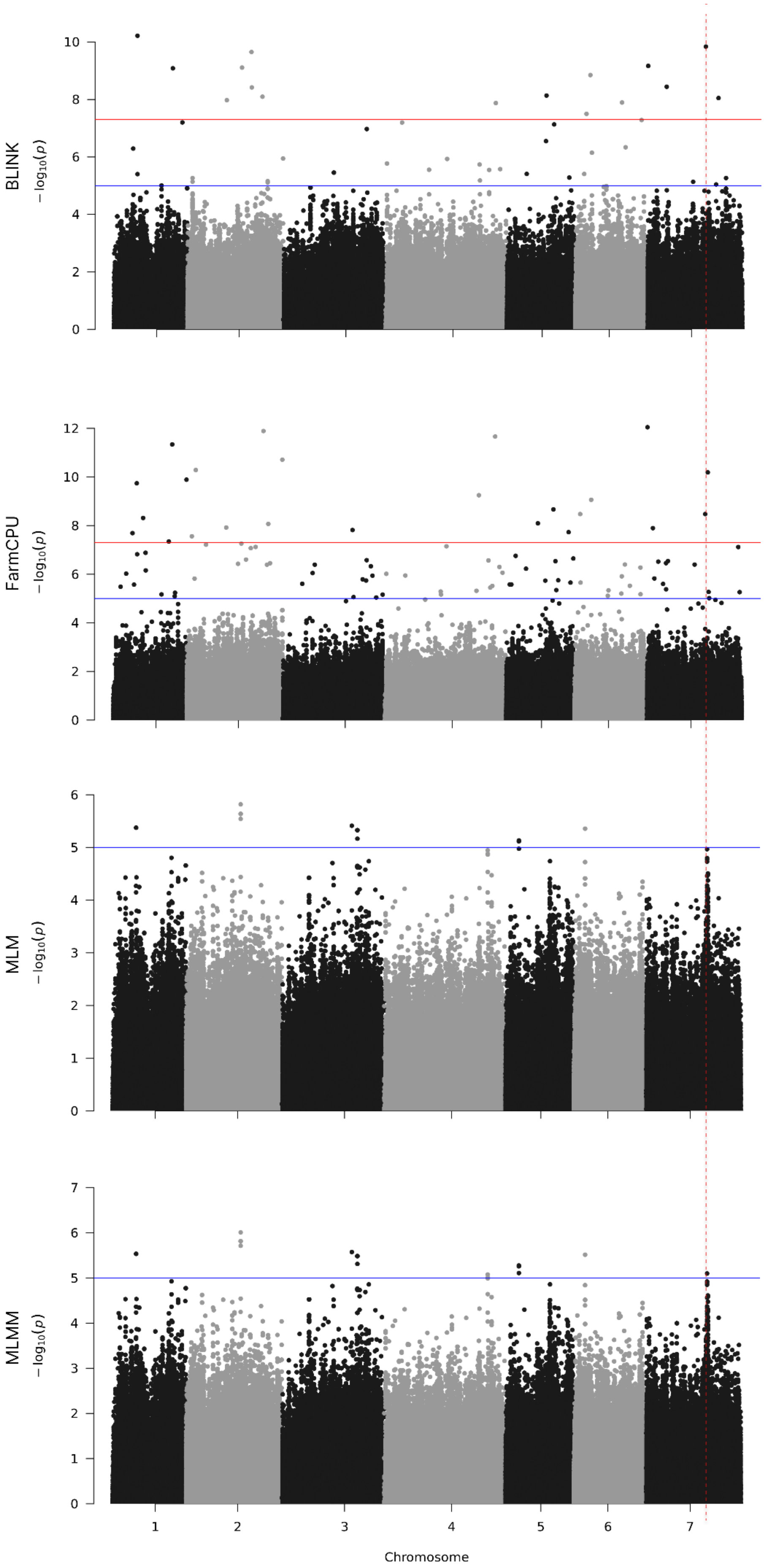
Seed shattering, or the loss of seeds shortly before or during harvest, is a major concern in forage crops because it significantly decreases seed yield. Seed shattering and other traits related to seed yield are described as complex, highly quantitative traits. For studying complex traits, nested association mapping (NAM) offers high detection power and mapping resolution by combining the strengths of both linkage and association mapping. Therefore, we established the first NAM population for the outcrossing forage crop Italian ryegrass (*Lolium multiflorum* Lam.). In a first step, 23 diverse founder genotypes (cultivars, ecotypes, and breeding material) were crossed with one common founder. Open pollination among the resulting F_1 plants gave rise to 708 F_2 plants, denoted as the NAM population. Double digest restriction-site associated sequencing of the 708 F_2 plants, combined with whole genome sequencing of the 24 founder genotypes, revealed a total of 3,199,253 polymorphic SNPs in the NAM population. Phenotypic data for seed shattering and seed yield-related traits were collected in three different environments, each in duplicate. Combining genotypic and phenotypic data in genome-wide association mapping, seven QTL for seed yield, seed shattering, spike length, flag leaf length and flowering time were consistently detected using BLINK, FarmCPU, MLM and MLMM. For seed shattering, one significant association was found on chromosome 7, which explained 10.03% of the phenotypic variance (Fig.1). The most significantly associated SNP was located inside the gene *Evm.model.chr7.26897*, which is predicted to be a ripening-related protein. Ripening-related genes might play a role in seed shattering because early harvesting of the same plants compared to later harvesting are known to reduce the percentage of seed shattering in Italian ryegrass. Among other candidate genes found for seed yield-related traits, this ripening-related gene can be used for functional validation. The SNPs associated with the traits can be directly used for marker-assisted breeding or genomic selection. The discovery of these candidate genes underscores the utility of NAM populations in uncovering the genetic basis of complex traits.


BLINK
$-\log_{10}(p)$
0
2
4
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FarmCPU
$-\log_{10}(p)$
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2
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12
MLM
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6
MLMM
$-\log_{10}(p)$
0
1
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6
7
1
2
3
4
5
6
7
Chromosome

Figure 1: Manhattan plot showing SNP markers significantly associated with seed shattering in a nested association mapping (NAM) population of Italian ryegrass (*Lolium multiflorum* Lam.), comprising 708 F_2 plants. The x-axis represents the chromosomes, while the y-axis displays the $-\log_{10}(p)$ values obtained from BLINK, FarmCPU, MLM, and MLMM. The red horizontal line indicates the genome-wide significance threshold based on Bonferroni correction ($\alpha = 5\%$), and the blue line denotes a suggestive significance threshold ($p = 1.0 \times 10^{-5}$). The red dotted line marks the SNPs most strongly associated with seed shattering, consistently identified across all four methods (BLINK, FarmCPU, MLM, and MLMM).

Genomic improvement of persistence in white clover

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Keywords
White clover, Persistence, Pan-genome, Rhizomatous growth, Hybrid cultivar

White clover (*Trifolium repens*) is an allotetraploid forage legume that plays a pivotal role in sustainable farming due to its nitrogen fixing capacity and soil-improving properties. However, most current cultivars lack robust persistence—particularly under cold or grazing conditions—in low-input environments. Closely working with Germinal breeders, our project employs a genomic pre-breeding strategy using 188 diverse white clover accessions, as well as a hybrid population between Caucasian and white clover aiming to elucidate the genetic basis of persistence-related traits and accelerate cultivar improvement.

My PhD project comprises three objectives. First, I will construct a white clover pangenome along with their diploid ancestors to capture their underlying genomic diversity. We aim to detect sub-genome homoeologous exchanges, gaining a better understanding of the dynamics that arose during polyploidy speciation, and to identify any introgression from wild populations. Second, I will utilise a white clover diversity panel (n=188) to explore its population history, QTLs and underlying causative genes associated with persistence-related traits. We believe that the integration of pan-genomics can bolster association mapping and discovery of useful variants. Our analysis will also benefit from the multi-environment phenotyping provided by our partners in The Legume Generation Clover Innovation Community. Third, we will use a white clover × Caucasian clover (*T. ambiguum*) hybrid population to identify genomic regions associated with its characteristic rhizomatous growth habit using bulk-segregant analysis. These loci will inform marker-assisted selection to produce hybrids adapted to temperate, low-input farming systems.

Overall, our integrated approach aims to reveal key genomic features underpinning the persistence of white clover, laying a foundation for next-generation cultivars that enhance resilience and productivity in sustainable agriculture.

Session 4: Biotic and abiotic stresses

Winter survival in red clover - cold acclimation, growth and interactions among stresses

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Winter survival depends not only on freezing tolerance, but also on the ability to tolerate numerous other stresses such as anoxia due to ice encasement and water-saturated soils, freeze-thaw cycles, winter pathogens, drought due to frozen soils, high light combined with low temperatures as well as long periods with little or no light. A range of tolerance mechanisms are involved, and, given the complexity of both stresses and tolerance mechanisms, there are strong genotype by environment interaction effects on winter survival. The ability to withstand winter stresses increases during the cold acclimation process occurring in the autumn and are lost in spring, processes that align with changes in growth and biomass allocation. This annual cycle of growth and stress tolerance is regulated by environmental and internal signals. Climate change has an effect on winter stresses, particularly in northern Europe, where winters are affected more than summers, with increased temperatures, precipitation and variability. Less protective snow cover, more severe freezing and more freeze-thaw events and ice cover may be expected in some regions.

I will summarize some of the research that has been done on winter survival of red clover in the last decade or so, focussing mostly on Nordic conditions. This comprises studies of genetic variation, tolerance mechanisms, regulation of cold acclimation and de-acclimation, relationships with growth and interactions among winter stresses.

Metabolic changes associated with the selection for freezing tolerance in alfalfa affects spring regrowth and the recruitment of soil microbiome

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Alfalfa is the most important perennial forage crop in Canada. However, the survival of the species is jeopardized by harsh autumn and winter conditions that either interfere with the cold acclimation process or directly cause winter injuries and mortality. Thus our team has dedicated important breeding efforts to increase the freezing tolerance of alfalfa by applying a scheme of recurrent selection under controlled conditions to improve the tolerance to freezing (TF) in two alfalfa cultivars: Apica and Caribou. We obtained, after three to six cycles of selection, the populations Apica TF3, Caribou TF3, and Apica TF6. When compared to their initial cultivars, the TF populations were shown to have a more vigorous regrowth after a severe freezing stress under controlled conditions, which were linked to several metabolic changes in perennial organs related to cell protection against frost.

The present study was designed to compare the responses of TF populations under natural conditions of photoperiod and temperature, and to delve into the relationship between plant biochemistry and associated microbiomes (nodule and root) by growing alfalfa in agricultural soil. Plants of each TF population were potted in soil from an alfalfa-rotation field and transferred in an unheated greenhouse for cold acclimation, overwintering, and spring regrowth under field conditions. We sought to 1) validate that the TF populations had an improved spring vigor compared to their initial cultivars; 2) quantify metabolic changes in crowns and roots; and 3) validate if these metabolic shifts have an effect on nodule and root microbiomes. Plants were sampled three times during the experiment that lasted eight months, in autumn - non-acclimated (NA), in winter - cold-acclimated (CA) and in spring - deacclimated (DA).

Our results showed that plants which had been selected for freezing tolerance had increased spring shoot and root biomass. Biochemical analysis revealed that changes related with improved freezing tolerance such as higher concentrations of starch and total amino acids were linked with intensity of selection, particularly in the spring, and were associated with changes in the root and nodule microbial communities. Recurrent selection and season interact to cause major changes in allocation of plant metabolites that explain a large degree of variation in microbial community structure.

Resistance breeding of red clover for selected fungal and viral diseases

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The aim of our project is to enhance clover resilience and increase its production by breeding resistance to major fungal (Fusarium) and viral (WClMV, RCMV) pathogens. Here, we perform strategy which employs employs modern genetic tools and selection based on favorable genetic variants, with a focus on marker-assisted selection in cross-pollinated crops. Genotypes are evaluated after inoculation with specific pathogen strains under both natural field conditions and controlled growth chamber experiments. Genetic and transcriptomic analyses, including whole-genome genotyping and RNA-seq, will be used to investigate resistance traits. A genome-wide association study (GWAS) of phenotypic data and detection of selection signatures will identify candidate genes associated with resistance. Resistant plant material will be further validated under laboratory and field conditions, and individuals will be selected for the pre-breeding phase based on integrated genotypic and phenotypic data to support the development of new, modern clover varieties.

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Membrane lipids and primary metabolites accumulated in crown tissue affects winter-hardiness and frost tolerance in *Festuca arundinacea*

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To endure winters, plant species in temperate regions, such as forage grasses, have to recognize signals of low temperatures, prompting the activation of cold acclimation (CA) mechanisms that enhance frost tolerance. However, global climate change is associated with an increase of temperatures. Upon exposure to higher temperatures, plants recover vegetation which triggers de-acclimation (DA) and consequently loss of frost tolerance. Furthermore, it has been also recognized that plants may regain frost tolerance during a process of re-acclimation (RA), after the next period of lower temperature. Except the leaves, another grass organ important during the exposure of plants to environmental stresses is thought to be the crown tissue. Moreover, overwintering and frost tolerance of grasses are both dependent on the crown survival. The crown tissue could be also a reservoir of assimilates and an important 'source of energy' to survive adverse environmental conditions.

Tall fescue (*Festuca arundinacea)* is one of the important grass species, which has been proven as excellent forage grass model to decipher mechanisms of resistance to a wide range of environmental stress conditions, including winter stresses. For this project, we have selected: 3 lines of *Fa* – each one with a different level of winter-hardiness. Plants were subjected to the simulated experimental conditions with oscillating temperatures of pre-acclimation (7 days in 12 °C), cold acclimation (21 days in 4/2 °C day/night), de-acclimation (7days in 12 °C), and re-acclimation (21 days in 4/2 °C) and were then analyzed according to the level of frost tolerance. Crown tissue was collected for further molecular analyses.

We hypothesize that alterations observed in the crown cellular lipidome and primary metabolome is associated with physiological indicators of frost tolerance. The obtained results indicated that physiological and molecular parameters revealed significant differences between particular genotypes in the analyzed experimental time-points. We could observe strong correlation between the level of frost tolerance and accumulation of lyso-lipids and triacylglycerol. Moreover, the frost tolerant genotype was also characterized by higher accumulation of proline, maltose, glucose, fructose, sucrose and raffinose which are the known osmoprotectants and can significantly enhance the frost tolerance. Although, the complete mechanism of winter-hardiness in the analyzed *F. arundinacea* genotypes has not been fully recognized yet, some its crucial components have been indicated in this work.

The research was funded by National Science Center (2021/41/B/NZ9/00782).

Red clover's battle against root rot and clover rot: an *UDP-glycosyltransferase* gene turns clover into a root rot warrior

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Keywords
RNA-seq, Root rot, *Trifolium pratense*, *UDP-glycosyltransferase*

Red clover (*Trifolium pratense*), a key forage legume in Northern Europe is constrained by weak persistence due to soil-borne pathogens. Root rot (*Fusarium avenaceum*) and clover rot (*Sclerotinia trifoliorum*) fungal pathogens remain the main challenge for red clover cultivation. Understanding the molecular mechanisms of disease resistance is crucial for breeding more resilient cultivars. We performed RNA-Seq analysis on red clover genotypes exhibiting contrasting resistance levels to root rot and clover rot pathogens. Analysis revealed differential expression of numerous defense-related genes. Based on their function annotations and expression values, we selected several candidate genes for validation. Among them, a potential detoxification gene *UDP-glycosyltransferase* (UGT) emerged as a strong candidate associated with enhanced disease tolerance to root rot. Detoxification of fungal-derived toxins is a critical component of plant defense. Plant UGTs are family 1 Glycosyltransferases catalyze the process of glycosylation by transferring sugar moieties from activated donor molecules to acceptor molecules including phytohormones and diverse specialized metabolites. They have important functions in detoxifying mycotoxins such as deoxynivalenol produced by *Fusarium* spp. through glycosylation. Functional validation through overexpression of red clover UGT gene in susceptible lines significantly improved resistance to *Fusarium* root rot in a controlled hydroponic infection assay. Quantitative PCR analysis showed considerably reduced amounts of fungal DNA in susceptible lines. Our findings provide new insights into the defense strategies employed by red clover against root rot pathogen. Next step is to integrate RNA-Seq data with SNPseq data for marker design.

Breeding drought tolerant forage crops in Mediterranean regions. Annuals or perennials? Pure stands or mixtures? Which legume plant ideotype?

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Questions as those addressed in this work can be crucial for the challenging endeavour of forage crop breeding for drought-prone environments of the Mediterranean basin, where rainfed agropastoral systems show a deficit in forage supply from late spring to late summer. With the ultimate goal of providing a clearer framework to forage breeders in the region, our study, which included 21 forage crops belonging to seven species (Fig. 1), aimed at: (i) comparing annual vs. perennial legume-based mixed crops and their respective monocultures for annual average dry-matter yield (DMY) and farmers' acceptability score (on a 1-5 scale) attributed by visiting farmers prior to harvesting; (ii) verifying the effect of mixture complexity; and (iii) comparing different plant ideotypes within alfalfa and pea.

The average annual DMY of all crops in the two test locations are shown in Fig. 1, while specific comparisons were statistically assessed by linear contrasts partitioning the variation for the 'crop' factor. The main outcomes from the data analysis were the following.

(i) Adapted alfalfa, such as the cultivar Mamuntanas (L1 in Fig. 1), can be a remarkable perennial forage resource for Mediterranean environments. Adapted alfalfa appears by far more performant and preferable than Mediterranean-type forage grasses (such as the cultivars of cocksfoot and tall fescue used here), although some useful complementarity may occur between alfalfa and grasses in mixture (Fig. 1), and selection of drought-tolerant alfalfa must continue to be a priority for Mediterranean conditions (Annicchiarico et al., 2011, Field Crops Res. 120: 283–291).

(ii) Valuable binary mixtures with high forage yield and appreciation by farmers compared to monocrops can be found in annual crops. Pea can be a promising alternative to the more widely used vetch (Fig. 1). An efficient selection strategy for pea competitiveness in intercropping (Annicchiarico et al., 2021, Front. Plant Sci. 12: 731949), combined with a specifically targeted breeding for pea adaptation to harsh Mediterranean conditions (Annicchiarico et al., 2020, Int. J. Mol. Sci. 21: 2414), should favour the availability of ever more suitable pea cultivars for productive and resilient annual forage mixtures.

(iii) Forage mixtures do not necessarily have to be complex (multi-species) (Fig. 1).

(iv) The cultivar adaptation to the environmental conditions tended to be more important than the plant ideotype *per se* for crop performance, both in alfalfa (where only Mamuntanas was specifically adapted) and pea. The fact that the pea cultivar Kaspa (P1) represented a rather tall variant within the semi-dwarf germplasm of the species may have contributed, though, to the similar behaviour between the two pea cultivars.

Acknowledgements

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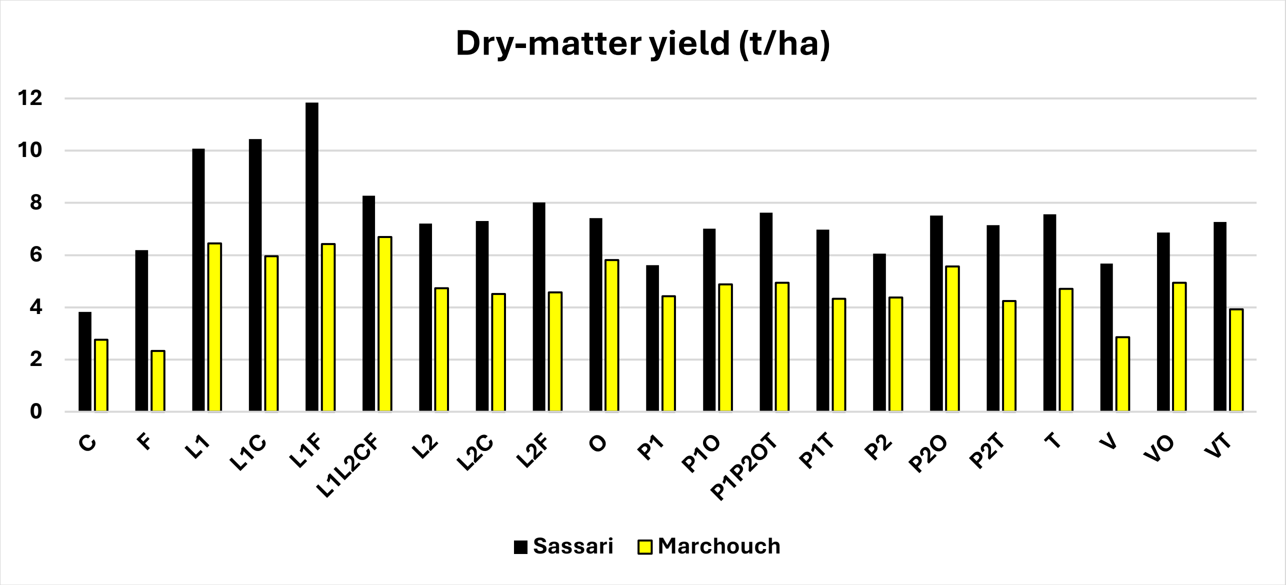

Figure 1. Mean values of annual average dry-matter yield for 21 forage crops in two Mediterranean locations (Sassari, Italy, and Marchouch, Morocco). Perennials: C, Kasbah cocksfoot; F, Flecha tall fescue; L1, Mamuntanas erect, drought-tolerant alfalfa; L2, Bulk semi-erect, grazing-tolerant alfalfa. Annuals: O, Genziana oat; T, Vivaciò triticale; P1, Kaspa semi-dwarf pea; P2, Pifor tall pea; V, Barril common vetch.

Identification of the genetic architecture of stem rust resistance in perennial ryegrass

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Keywords
Lolium perenne, Stem rust resistance, *Puccinia graminis* f. sp. *lolii*, Genome-wide association study (GWAS), Biparental mapping populations

Perennial ryegrass (*Lolium perenne*) is a major species in temperate grassland systems, important for its use in forage and turf production. However, foliar diseases, such as stem rust (*Puccinia graminis* f. sp. *lolii*), severely reduce seed yields in Denmark. The expected intensification of disease pressure due to climate change, coupled with restrictions on pesticide use in Europe, highlights the urgent need for sustainable control strategies. Developing durable resistant varieties offers a sustainable strategy for stem rust control. This study aims to dissect the genetic architecture underlying resistance and identify resistant genotypes to stem rust. A collection of 390 *Lolium* genotypes from diverse backgrounds was phenotyped for resistance to a Danish stem rust isolate, based on assessments of pustule type and disease severity. A range of responses, from complete resistance to high susceptibility, was observed. A genome-wide association study (GWAS) revealed multiple loci associated with the traits, and candidate genes were identified. To complement these findings and detect rare alleles potentially involved in resistance, two biparental mapping populations were developed from parental genotypes exhibiting contrasting resistance phenotypes against three European isolates, including the Danish isolate previously used, and one American isolate. Mapping analyses are in progress to refine the identification of resistance loci. Altogether, this work provides new insights into the genetic architecture of stem rust resistance and lays a foundation for the future characterization of the molecular pathways involved in grass species. In parallel, the identified resistance loci will support pre-breeding programs aiming to develop durable resistant varieties of perennial ryegrass.

Climate-resilient forage crops: identifying molecular hubs for multi-stress responses

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Climate change is a major threat to Irish agriculture, especially affecting perennial ryegrass and white clover which are essential forage crops for its pasture-based dairy industry. However, previous breeding strategies led to high-yielding perennial ryegrass and white clover varieties at the expense of abiotic stress resilience. Therefore, it is critical to climate proofing these crops to tackle the devastating effects of climate change.

In order to mine for more climate resilient crops, we combined the evaluation of plant growth responses under specific environmental conditions associated with a future climate in Ireland. Twelve ecotypes of perennial ryegrass (6 from the North of Europe and 6 from the South of Europe) will be grown, separately or in combination with white clover varieties, under different environmental conditions that replicate future Irish summers and winters. We will then assess specific physiological responses including, shoot biomass, photosynthetic parameters, starch content, total protein content, and stomatal analysis. In addition, we will determine the expression levels of previously identified components of the drought and high temperature responses in the different ecotypes grown under combined stress. Our approach will also include transcriptomics and proteomics to identify specific and novel regulators (genes, long non-coding RNAs, proteins) of growth and development under distinct abiotic stress conditions. We will then assess their specific biological functions using gene editing and by generating transgenic overexpression lines. The identified regulators will then be tested in field conditions.

We expect our findings will uncover new genetic variability which could inform breeding strategies to generate novel varieties able to thrive under future climate conditions to ensure the sustainability of Irish agriculture.

Genetic control of anthracnose resistance in lucerne, consequence in breeding

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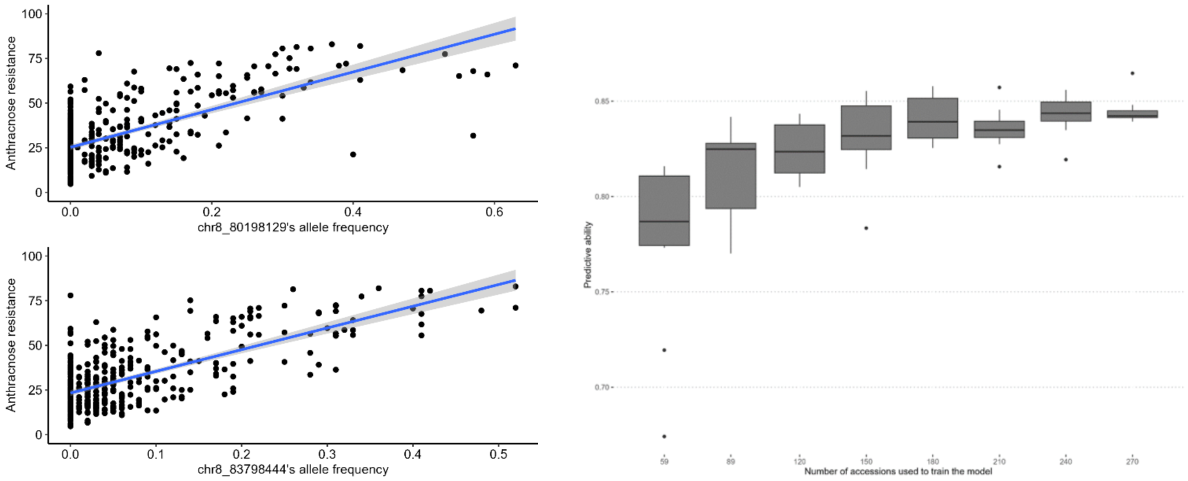
Some ecosystem services provided by lucerne (*Medicago sativa*) are related to forage production and persistence but may be affected by disease susceptibility. Resistance to anthracnose is a breeding objective, and selection is based on surviving plants after tests in controlled conditions. Molecular markers could help breeders to select plants carrying the resistant alleles, possibly in four copies on this autotetraploid species. In a study of 400 lucerne accessions that comprised varieties, landraces and breeding materials, genotyped with GBS markers, anthracnose resistance was scored as the frequency of resistant plants. QTL detection by genome-wide association study (GWAS) and genomic prediction were performed. A wide range of variation for anthracnose resistance was observed, with newer varieties and breeding materials exhibiting a greater resistance than old varieties and landraces. Accessions from America showed the highest resistance, although some European accessions also displayed notable resistance. Six QTL, controlling 58% of the variation, were identified by GWAS. Among them, two major QTL were found on chromosome 8, within a region already identified in an alfalfa mapping population. Four other QTL, each controlling less than 5% of the variation, were also found, including one near a major QTL on chromosome 4 in the model species *M. truncatula*. The predictive ability of this set of accessions reached the very high level of 85%. These results are promising for the use of markers to improve lucerne for anthracnose resistance in breeding programs. Kasp markers, developed in the region of the main two QTL, could be used to compose polycross with plants carrying 3 to 4 doses of the alleles conferring the resistance. A genomic prediction could also be conducted on a breeding pool and the plants predicted to be the most resistant ones could be intercrossed. With one or two selection cycles, resistant varieties would be obtained from the desired breeding pool.



(Left) Percentage of resistance for anthracnose depending on the allele frequencies for the main two QTL. (Right) Predictive ability as a function of the number of accessions in the training population.

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Transcriptional signatures underlying freezing tolerance in perennial ryegrass genotypes

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Climate change may extend the growing season in temperate and Nordic-Baltic regions, offering potential for increased productivity and the introduction of new forage species. However, increased climatic variability—including warm spells in late autumn and reduced snow cover—poses serious risks to perennial forage crops by disrupting cold acclimation and increasing susceptibility to freezing damage. Cold acclimation is a complex, temperature-driven process involving transcriptional reprogramming, hormonal changes, and physiological adaptations such as membrane stabilization and carbohydrate accumulation. While much of the current understanding of cold response comes from model species like Arabidopsis, cold-regulated gene expression in perennial grasses such as Lolium perenne remains underexplored, particularly at the transcriptomic level. We studied the transcriptional responses of genotypes differing in freezing tolerance when exposed to low and sub-zero temperatures. A panel of 160 genotypes was screened for freezing tolerance using electrolyte leakage (EL) assays after 24-hour exposure to -12 °C and -14 °C. Substantial variation in freezing tolerance was observed among the tested genotypes, particularly at -12 °C, with significantly higher mean EL at -14 °C ($p < 0.0001$, Student's t-test). Based on these results, two tolerant and two sensitive genotypes were selected for transcriptomic analysis. Crown tissues were sampled at six time points: prior to cold acclimation (control), at the onset of acclimation, after one and three weeks of acclimation, and following exposure to -5 °C and -10 °C. Differential gene expression analysis revealed 11,125 DEGs in sensitive genotypes and 12,937 in tolerant genotypes, with 3,323 and 5,135 genes being unique to the sensitive and tolerant groups, respectively, while 7,802 were shared. Variations in electrolyte leakage between tolerant and sensitive genotypes appear to be associated with differential expression of genes such as cold shock protein CS120-like, dehydrin DHN3-like, heat shock protein A-2a-like, LEA proteins, glycine-rich cell wall structural protein 1, and those involved in lipid oxidation. Overall, sensitive genotypes exhibited fewer DEGs during both cold acclimation and the early stages of freezing stress. These findings suggest that the slower and less robust transcriptional response in sensitive genotypes compromises their cold acclimation capacity, ultimately reducing their ability to withstand freezing temperatures. Notably, suppression of genes related to fatty acid elongation and glutathione metabolism suggests that cold response mechanisms in perennial ryegrass may include pathways that are yet to be fully characterized. Further functional studies on the DEGs identified here are needed to clarify the molecular pathways conferring freezing tolerance in perennial ryegrass.

Binucleate *Rhizoctonia* AG-A pathogen of red clover in Serbia

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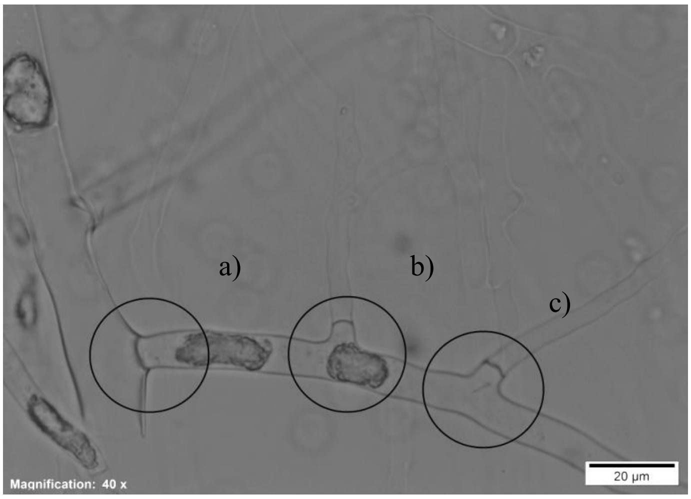
Red clover (*Trifolium pratense* L.) in Serbia has a long history of cultivating due to its favorable growing characteristics and its high nutritional value, but it is susceptible to the variety of fungal pathogens that can limit its yield and shorten the plants lifespan. *Rhizoctonia* spp. represent a very important genus of phytopathogenic fungi that inhabit the soil and can infect a vast verity of cultivated plants (Vojvodić, 2021). Sampling of red clover plant F2 with symptoms of stunting and necrosis of leaves and stems was done on 05.11.2020. on red clover monoculture plot in Globoder, Serbia (43°34'56.9"N 21°12'08.2"E). Plant F2 root surface was dark colored with sporadical cracks in the root epidermis. The root cross sections showed dark brown necrotic tissue of central cylinder, which was used for pathogen isolation. Obtained isolate had an average growth of 75,67 mm after seven days at 25° C and was designated F2B. After acquiring a hyphal tip fungal colony macromorphological and micromorphological features were examined on PDA medium. The culture had a woolly structure, mycelium color was paled beige with shades of light brown, sclerotia structures were not present. Microscope examination showed septate hyphae (average width 9,3 µm) with characteristic 90° branching, constriction at the branching point and a septa in the immediate vicinity (Picture 1.). Obtained morphological features were in accordance with the features of the *Rhizoctonia* genus. Pathogenicity of the isolate was confirmed on red clover plants and detached plant parts by three pathogenicity tests. The first test consisted of detached red clover leaves inoculation and measuring of lesion diameter (average 6.23 mm), the second was inoculation of red clover stem fragments (40 mm) with measuring of necrotic stem part length (average 27,65 mm) after seven days of incubation at 25° C. The third pathogenisity test consisted of inoculation of 150 days old red clover plants in semi controlled environment (Yli-Mattila et al., 2008). Inoculation spot was on the main clover roots, about 20 mm from the plant crown and scoring was done 60 days after. Leaves and stems symptoms were visible on seven plants, but the root symptoms were present in all 12 treatment plants, while control plants remained symptomless. Average width of the root necrotic tissue was 2,36 mm and length was 24,28 mm. Species level identification was done by isolating genomic DNA followed by PCR amplification of the internal transcribed spacer (ITS) using ITS1/ITS4 primer pair. BLAST analysis of the nucleotide sequence revealed that isolate F2B was identical with several reference sequences of binucleate *Rhizoctonia* AG-A deposited in NCBI GenBank. Based on morphological, pathogenic and molecular features of the isolate F2B, the pathogen of red clover that caused rot root was identified as binucleate *Rhizoctonia* AG-A.



Picture 1. Isolate F2B septate hyphae with characteristic 90° branching a), b) and c)

References

Vojvodić, M. (2021): Species diversity of the genus *Rhizoctonia* in Serbia. Doctoral thesis, Faculty of agriculture, Belgrade, Serbia.

Yli-Mattila, T., Kalko, G., Hannukkala, A., Paavanen-Huhtala, S. and Hakala, K. (2008): Prevalence, species composition, genetic variation and pathogenicity of clover rot (*Sclerotinia trifoliorum*) and *Fusarium* spp. in red clover in Finland. European Journal of Plant Pathology, 126(1): 13-27.

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Session 5: Polyploidy and interspecific hybridization

Applied systems biology and predictive breeding in pastures and forages

German Spangenberg

From sequence to cross-compatibility prediction: a functional analysis of self-incompatibility haplotypes in forage grasses

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Hybrid breeding forms the basis of seed production for many crop species. In forage grasses, however, self-pollination and inbred line production are naturally prevented by gametophytic self-incompatibility (SI), a genetic mechanism that promotes outcrossing. In grasses, SI is controlled by two multi-allelic loci, *S* and *Z*. At each locus, three genes are involved in self-recognition: two genes encoding a transmembrane protein (DUF247) expressed in the pollen, and one gene encoding a small, secreted peptide expressed in the stigma. Yet, knowledge of their sequence diversity, allelic variation and physiological functions remains limited. Understanding how these genes interact to regulate SI could unlock opportunities to control pollination and support the development of hybrid breeding strategies.

To build a model predicting cross-compatibility based on genotypic data, we established an F_1 population of perennial ryegrass (*Lolium perenne* L.), derived from a polycross of five parental plants. Consequently, the population comprises a restricted number of haplotypes at the two SI loci *S* and *Z*. To identify the different SI alleles present within the population, we used a probe capture assay, followed by next-generation sequencing and *de novo* assembly of alleles. Based on the translated amino acid sequence, we clustered the different SI alleles with 99% similarity to form putative SI haplotypes. To link the genotypic data to phenotypic data from semi-*in vivo* pollination assays, we performed more than 2,900 targeted crosses between 30 plants within the F_1 population in 2023 and 2024: an example semi-*in vivo* pollination is shown in Figure 1. Currently, we are repeating the crosses for a subset of ten genotypes, for which we were able to fully recover the putative SI haplotypes at both loci. This fully characterized subset was used to train a model for cross-compatibility predictions. We were able to assess the overlap between the predicted cross-compatibility and the observed cross-compatibility in the 2023 crosses. In 30 out of 58 crosses, the predicted and observed cross-compatibility was identical. However, twelve crosses deviated by at least 50% in observed cross-compatibilities. By analyzing the phenotypic data from 2024 and 2025, we seek to better understand the sources of variation between the predicted and observed cross-compatibility.

Ultimately, we aim to define which sequence characteristics of the SI alleles determine SI recognition, with a view to defining functional haplotypes. This knowledge would enable the development of a tool for breeders to assess the allelic diversity of SI in their breeding pool and to predict cross-compatibility between genotypes.

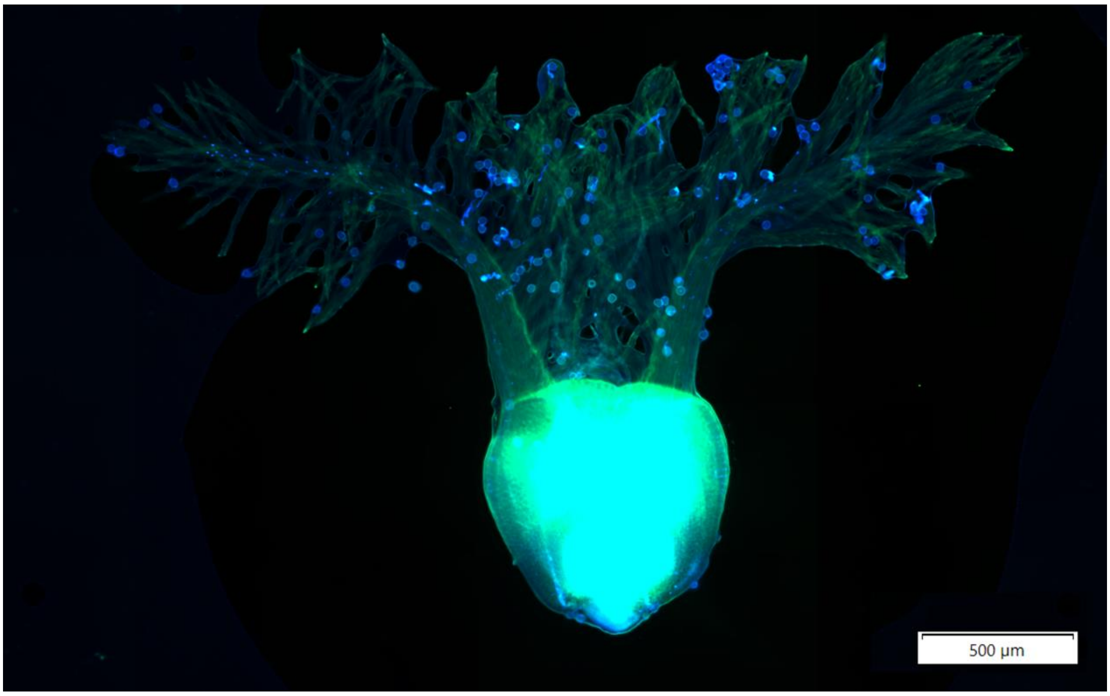

Figure 1. Image showing an incompatible cross, from a semi-*in vivo* pollinated stigma, stained with aniline blue.

Physiological traits of cellular membranes associated with frost tolerance in *Festuca arundinacea* and *Lolium multiflorum* × *F. arundinacea* hybrids distinct in winter hardiness

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Winter hardiness in forage grasses is a multifaceted trait involving physiological, biochemical, and morphological adaptations that allow plants to endure adverse winter conditions. Among them, frost tolerance plays a central role, being acquired through cold acclimation (CA), disrupted by de-acclimation (DA), and potentially restored during re-acclimation (RA). However, the mechanisms regulating these processes remain insufficiently understood. This study aimed to assess the physiological basis of winter survival in hexaploid genotypes of *Festuca arundinacea* and pentaploid hybrids of *Lolium multiflorum* × *F. arundinacea* with contrasting field winter hardiness. The experiment was conducted under controlled environmental conditions in a phytotron and comprised four distinct phases: pre-hardening, cold acclimation, de-acclimation, and re-acclimation. During pre-hardening, plants were maintained at 12°C for 7 days under a 10-hour photoperiod and 200 μmol $m^{-2}s^{-1}$ PPFD. Cold acclimation was then induced for 21 days at 4°C/2°C (day/night). Afterward, a 7-day de-acclimation phase was carried out at 12°C, followed by a 21-day re-acclimation (RA) under conditions identical to CA. Throughout the experiment, light conditions remained constant, and a control group was maintained at 22°C for comparison. Physiological analysis focused on cellular membranes stability, evaluated using the TEL_{50} parameter – the temperature at which 50% electrolyte leakage occurs in leaf tissue at particular time-points of the experiment. In turn, molecular analyses were associated with lipidome and primary metabolome profiling at the same experimental time-points. The results revealed significant differences in membrane injury dynamics among the studied plants. The accumulation of triacylglycerols and reduced amount of lysolipids content at hardening temperatures was shown to have a key role in frost tolerance. Significant differences between genotypes were also observed in the proportions of structural lipids in chloroplast membranes and other biological membranes, which could have a significant influence on membrane stability. Analysis of physiological and lipidomic data shows that the frost tolerance of forage crops depends on genotype-specific adaptation pathways. Species- and genotype-dependent changes in the lipid profiles highlight the key role of biochemical adaptation in tolerance to low temperature stress, providing a basis for the development of breeding strategies aimed at increasing winter hardiness.

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The effect of various species of arsenic on plant growth, major elements accumulation, physiological processes two *Miscanthus* × *giganteus* genotypes

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Keywords

arsenic species, *Miscanthus* × *giganteus*, phytoremediation, arsenic uptake, genotype selection, soil contamination, physiological response, forage and amenity grasses

Miscanthus × *giganteus*, a perennial grass with high biomass productivity, is increasingly recognized for its dual-purpose role as both a renewable energy crop and a phytoremediation agent. Additionally, due to its striking appearance and architectural growth form, it is also cultivated as an amenity grass in ornamental landscaping and urban green spaces.

This study assessed the response of two distinct genotypes, 'Illinois' and 'D-116,' to arsenic (As) contamination in soil (10, 50, and 100 $mg \cdot kg^{-1}$), applied in three chemical forms: As(III), As(V), and dimethylarsinic acid (DMA). We evaluated biometric traits, arsenic and mineral uptake (Ca, K, Mg, Na), and physiological parameters, including photosynthetic activity, chlorophyll content and fluorescence, and membrane stability.

Plants demonstrated the ability to uptake arsenic depending on its chemical form. The organic form (DMA) was most effectively accumulated in above-ground parts, while inorganic species, particularly As(III), showed limited translocation from roots to shoots. Arsenic concentrations in shoots reached up to 40 $mg \cdot kg^{-1}$ dry weight. Arsenic exposure also influenced the uptake of other mineral nutrients, disrupting their balance and suggesting the activation of detoxification mechanisms within plant tissues.

The 'Illinois' genotype exhibited stronger stress responses, especially under As(III) and As(V) exposure, which significantly impaired membrane integrity and nutrient uptake. Although *M.* × *giganteus* cannot be classified as a hyperaccumulator due to moderate As accumulation, it demonstrated substantial tolerance to arsenic-induced stress while maintaining growth and biomass production.

The species' perennial growth habit, high abiotic stress tolerance, and stable biomass yield offer strong potential for long-term phytoremediation on contaminated or marginal soils. The observed genotypic variation in arsenic tolerance and physiological performance underscores the importance of targeted genotype selection in breeding programs for resilient forage, energy, and amenity grasses.

Our findings highlight *M.* × *giganteus* as a promising candidate for integrated environmental and biomass applications, with added value as a low-maintenance ornamental grass suitable for polluted urban and peri-urban environments. The results also lay the groundwork for further research into the physiological and molecular mechanisms underlying its tolerance to trace metal stress.

Use of seedling root fluorescence for high throughput detection of *Lolium multiflorum* introgressions in *L. perenne* breeding populations

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Perennial ryegrass (*Lolium perenne*, Lp) and Italian ryegrass (*L. multiflorum*, Lm) form a species complex with no reproductive barriers between the two species. This can be beneficial in breeding the species hybrid (*L.* × *boucheanum*, Lb), aiming to combine the persistence and forage quality of Lp with the yield potential of Lm. However, hybridization can also be problematic in Lp breeding, as even small introgressions of Lm can result in so-called "off-type" plants, which differ from the Lp phenotype through traits such as presence of awns, erect growth, or broader leaves. Lp varieties bred by Agroscope in Switzerland largely originate from ecotypes found in Swiss natural meadows, where interspecific hybridization is limited due to differences in flowering time (Lp earlier than Lm). Nevertheless, natural hybridization has occurred, and some degree of Lm introgression is unavoidable in ecotype-based Lp varieties. To prevent the occurrence of off-types, which may lead to disqualification of seed lots and financial losses, a method to detect Lm introgressions associated with off-type traits is needed.

Seedling root fluorescence (SRF) under UV light, caused by exudation of the alkaloid annuloline, is an established method for detecting contamination of Lp (non-fluorescent) with Lm (fluorescent) in seed purity analysis. This study aims to evaluate the potential of SRF for (conservation) breeding to detect Lm introgression in Lp breeding material to prevent off-types. Additionally, published molecular markers known to distinguish between Lp and Lm are tested and compared to SRF.

First, the SRF protocol was optimized for breeding applications by minimizing false-negative rates and shortening the growing period until measurement to ease later transplanting. Germination on moist, folded filter paper at 30/25 °C day/night temperatures and a two-day waiting period between root emergence and measurement provided optimal results. Second, the optimized protocol was applied to all Lp, Lm, and Lb varieties currently recommended in Switzerland. In Lp varieties, SRF frequency ranged from 0% to 56%, averaging 17% per variety. Varieties known for off-types, such as cv. Salmo, showed higher frequencies. In Lb varieties, minimum, maximum, and average SRF frequencies were 78%, 98%, and 92%, respectively; in Lm varieties, 99%, 100%, and 99.8%. The high SRF frequency in Lm is a prerequisite for detecting potential outcrossing in Lp breeding material. Finally, the relationship between SRF, molecular markers, and off-type morphology is being explored. Single plants from different Lp populations known for off-types are analyzed for SRF, genotyped, and grown in a nursery where inflorescence and other morphological traits are assessed. Correlating SRF and molecular data with morphology will enable a final assessment of SRF's suitability as a tool in Lp species purity breeding.

Deciphering the complex genomic architectures, origins and evolution of timothy grasses [*Phleum* species]

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Phleum species are globally important perennial grasses that play a crucial role as key flora and biodiversity in temperate grasslands. The genus comprises various diploid and complex polyploid timothy grasses. Among them, the hexaploid forage timothy (*P. pratense*, 2n=6x=42) is an agriculturally and ecologically important perennial forage grass and presumed to have been created through natural hybridization of diploid *Phleum* species: *P. nodosum* [2n=2x=14] and *P. alpinum* [2n=2x=14]. Despite its immense importance as a forage crop, exhibiting higher productivity and quality under diverse environmental conditions, nitrogen sequestration capacity, and substantial value for the wildlife biodiversity, research and breeding in timothy lagged substantially behind. To fill these gaps and revolutionize timothy research and breeding programs, the Pär Ingvarsson Lab (https://www.pkilab.org) at the Swedish University of Agricultural Sciences (SLU) Uppsala has developed haplotype-resolved chromosome-scale reference genomes and transcriptomes for four important *Phleum* species, *P. nodosum* [2n=2x=14], *P. alpinum* [2n=2x=14], *P. alpinum* [2n=4x=28] and *P. pratense* [2n=6x=42] using PacBio HiFi long reads sequencing technology. The developed genomes were used to study key research questions in timothy grasses: dissecting and understanding the complex genomic architectures, the origins and evolution, and polyploidization events in *Phleum* species. Using state-of-the-art OMA (Orthologous MAtrix) reference database, algorithms and pipelines, we constructed hierarchical orthologous groups (HOGs) from the four assembled timothy grass genomes and five phylogenetically related grasses species from the *Pooideae* subfamily (*Brachypodium distachyon*, *Triticum urartu*, *Hordeum vulgare*, *Lolium perenne*). The inferred HOGs revealed extensive levels of genomics changes, such as gene expansion, retention and duplication events, in the *Phleum* genus. The results suggest potential processes underlying genome evolution, speciation, and phenotypic diversification, which are crucial for understanding differential adaptive evolution in diverse timothy grasses. This knowledge will be vital for future climate-resilient breeding, with significant implications for understanding the evolution of temperate grasslands.

Advances in the interspecific *Urochloa* breeding program 25 years after the release of its first apomictic cultivar – transitioning to the omics era

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Keywords
Brachiaria, genomic selection, forage breeding, high-throughput phenotyping

The task to develop apomictic interspecific *Urochloa* hybrids began at the Alliance of Bioversity International and CIAT started in the late 1980's. The main goal of the breeding program was, and continues to be, to contribute to the world's food security through the development of forage cultivars adapted to the growing conditions of the Tropics and Subtropics with a focus on smallholder livestock producers of the Global South. Since the release of its first apomictic hybrid in 2001, the program has released cultivars to meet the growing demand of livestock farmers focusing on biotic stress tolerance, productivity and climate change adaptation and mitigation. High rates of genetic gain have been estimated for the tolerance and antibiosis against spittlebugs (7.4 % and 2.3 %, respectively), the main insect pest in the neotropics. However, traits like dry matter yield, crude protein content and seed yield, have shown slower gains throughout the years (0.5 %, 1 % and 2.8 %). For this reason, an important effort is being made to introduce state-of-the-art technologies for high-throughput phenotyping (HTP) and genotyping. A strong pipeline for image-based data capture and analysis has allowed the development of biomass and ground cover prediction models, pest tolerance and seed production with $R2 > 0.7$ improving phenotyping accuracy and reducing time. Near-infrared Spectroscopy models have been designed and are used routinely for forage quality assessment. Currently, the application of GWAS is being studied for different traits, such as spittlebug tolerance, for which significant SNPs have been identified. Genomic Selection models are being tested, and a mid-density panel is being designed. The integration of genomic tools with HTP will aid in more accurate selections, reduce breeding cycle times and increase genetic gains.

Induction and evaluation of tetraploids in annual ryegrass

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Keywords
Annual ryegrass, colchicine, polyploidy, flow cytometer, plant breeding

Induced polyploidy by application of mutagens such as colchicine is a well-known technique with a long history. It has been used successfully on many plant species. Generally they have different morphological, physiological, and genetic characteristics than their diploid progenitors. As a result of these differences, they have higher biomass yield than their diploid progenitors as they can be more tolerant to biotic and abiotic stress. Therefore, induced polyploidy is an important breeding method, especially for forage species. Approximately, 50% of the annual ryegrass cultivars registered in the world are autotetraploids although it is a natural diploid with 2n=14 chromosome. Based on the results of previous studies, it has been determined that characteristics and performance of autopolyploids depend greatly on their diploid progenitors. Therefore, it has critical importance to evaluate diploid germplasms in the conditions of target regions and select the best performing ones for poliploidy induction. The objective of this study was to develop an efficient method for our laboratory to induce chromosome doubling in annual ryegrass and develop a tetraploid population. Approximately 1200 seeds of a diploid population developed by 3 cycles of selection from an annual ryegrass collection including 140 gene bank accessions based on their performance in Tekirdağ conditions. Five different doses of colchicine (0.05, 0.1, 0.2, 0.3, 0.4 %) and 3 different period of application (4, 8 and 24 h) were used in the study. Flow cytometry was used in ploidy analysis after 8 weeks of colchicine application. The highest recovery of autotetraploids (15%) was obtained by treatment of one week old germinating seedlings with 0.2% colchicine for 8 hours. A high incidence of mixoploidy was obtained following colchicine treatment. Rarely hexaploids were also encountered. It was found out that ploidy varied among the tillers of the same plants when ploidy analysis repeated after 4 months of colchicine applications. Autotetraploid tillers propagated vegetatively and used in polycrosses to develop an autotetraploid population. After a two-year selection process, the performance of the polyploid population was compared with that of the diploid population and other standards.

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High productivity of *Festuca apennina* x *pratensis* hybrids is not linked to inferior forage quality

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High productivity in terms of dry matter yield is often linked with poorer forage quality (Casler 2001). We reported previously about extremely high heterosis for dry matter yield exhibited by the triploid hybrid (3x Fape × Fp) *Festuca apennina* De Not. (4x Fape) × *F. pratensis* Huds. (2x Fp). It seems attractive to exploit agronomic potential of such hybrids, which are well adapted to growth conditions at high altitude; however so far no information is available about their forage quality.

We therefore analyzed selected samples collected in the performance trial details of which are reported in Boller and Kopecký (2020). The first cut at the site Maran and the third cut at the site Früehbüehl were analyzed for the content of digestible organic matter (DOM) and crude protein (CP) using Near Infrared Reflectance Spectroscopy (NIRS).

In the first, reproductive cut, CP and DOM of the triploid hybrid were slightly lower than both parents (-9 to -11 g/kg for CP, -11 to -25 g/kg for DOM). However in the third, vegetative cut, the forage quality of 3x Fape was superior to both parents (+23 to +30 g/kg for CP, +5 to + 42 g/kg for DOM), see Table 1. On average over both cuts, 3x Fape had forage quality similar to 4x Fape and slightly superior to 2x Fp.

Table 1. Crude protein (CP) and digestible organic matter (DOM) content of *Festuca apennina*, *F. pratensis* and two triploid hybrids

	First cut		Third cut		Average	
Ploidy and species	CP g/kg	DOM g/kg	CP g/kg	DOM g/kg	CP g/kg	DOM g/kg
2x *Festuca pratensis*	106.3	719.7	118.3	682.5	112.3	701.1
4x *Festuca apennina*	115.8	706.2	125.9	719.3	120.9	712.8
3x *F. apennina* × *F. pratensis*	104.8	695.0	148.6	724.4	126.7	709.7
3x *F. apennina* × *Lolium perenne*	115.0	764.0	124.0	719.5	119.5	741.8

One of the 3x genotypes included in the trial later turned out to be a hybrid *F. apennina* × *Lolium* (presumably *perenne*). This genotype was analyzed separately and had forage quality similar to 4x Fape, except in the first cut where it was markedly better digestible than all other species.

Although these results are preliminary, it seems clear that the very high productivity of the hybrid 3x Fape × Fp is not linked with a drawback in forage quality. The lower digestibility in the first cut is probably a consequence of the high fiber content of the stalks, which are numerous and grow very tall. We observed that these plants are only reluctantly grazed by cattle. This disadvantage is not present in the *F. apennina* × *L. perenne* hybrid, which grows much less tall and produces only a limited number of fertile tillers.

We conclude that the triploid hybrids of *F. apennina* with *F. pratensis* and *L. perenne* deserve efforts to overcome sterility in order to exploit their potential in view of utilizing them as high -quality forage plants for high altitudes.

References

Boller, B., Kopecky, D.: Triploid forage grass hybrids *Festuca apennina* x *pratensis* display extraordinary heterosis for yield characteristics. Euphytica 216, 143 (2020).

Casler, M.D. 2001. Breeding forage crops for increased nutritional value. Advan. Agron. 71:51-107 (2001).

Genome assembly and genomic selection to accelerate timothy grass breeding programs

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Timothy (*Phleum pratense* L.) breeding significantly impacts livestock nutrition, economic stability, and sustainable agriculture. However, breeding this perennial forage species poses considerable challenges, including complex genetic backgrounds, prolonged breeding cycles, and environmental interactions. This study explores genomic approaches, specifically genome assembly and Genomic Selection (GS) to optimize timothy grass breeding programs. Timothy grass, a hexaploid species with a genome size of approximately 4.3 Gb, was sequenced using PacBio HiFi, Oxford Nanopore Technologies (ONT), and Hi-C data, resulting in chromosome-level assemblies with significantly improved contiguity. These genome assemblies facilitated efficient single nucleotide polymorphism (SNP) discovery, yielding 30,698 high-quality SNPs identified through genotyping-by-sequencing (GBS) of 889 FS2 individuals. Ridge regression models integrated genotype data from SNP markers and phenotypic data from yield and forage quality traits (e.g., crude protein, digestibility, fiber content), collected across multiple environments between 2003-2012. The resulting predictive models were validated on an independent dataset consisting of 213 full-sib families planted in 2015. The validation demonstrated strong correlations between observed and predicted phenotypes for yield traits, confirming the accuracy and effectiveness of genomic selection. The genetic relatedness between training and validation populations was crucial for reliable predictions. Smaller, closely related training populations yielded accurate GEBVs, optimizing resource efficiency. Incorporating genome assemblies and GS into timothy breeding programs significantly accelerates genetic gains, enabling the development of robust, high-yielding varieties essential for sustainable livestock production.

Alien introgression revisited – what they are and how they are maintained in the host genome

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Introgression breeding via interspecific hybridization followed by several steps of backcrosses is one of the ways to introgress traits of interest into the elite cultivars. This approach has been widely used in cereals, including many lines of wheat with alien introgressions of different origin (rye, *Thinopyrum*, *Agropyron*, *Aegilops*, barley and various *Triticum* species) has been developed. In forages and turf grass species, this approach has been utilized mainly in Festulolium (*Festuca* x *Lolium*) breeding. Up to now, there are dozens of introgression cultivars released and many lines developed without registration. There are two types of them – *Festuca arundinacea* type, having introgressions from *Lolium* species (sometimes called *Festulolium krasanii* in case of *L. multiflorum* and *Festulolium holmbergii* in case of *L. perenne*) and *L. multiflorum*/*L. perenne* types having introgressions from various *Festuca* species (mainly *F. pratensis*, but also *F. arundinacea*, *F. glaucescens* and *F. mairei*). In my presentation, I will revisit the genome constitution of these cultivars with the focus on the composition of the introgressed segments and I will show how they are transmitted to the successive generations and how stable these cultivars and lines are. I will also show the possibilities how to modify the rate of their transmission via playing with the ratio of the parental genomes or via genome editing.