Novel technologies, strategies and crops to sustain forage production in future climate

Abstracts of the 35th Meeting of the EUCARPIA Fodder Crops and Amenity Grasses Section in cooperation with the EUCARPIA Festulolium Working Group

Brno
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Edited by David Kopecký, Ivana Frei, Tomáš Vymyslický

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Opening address by Jan Nedělník, Agricultural Research, Ltd. Troubsko

Dear conference participants, dear supporters of fodder crops,

I warmly welcome you to Brno on behalf of all the host organizations. I welcome you to the city which, together with the whole world, celebrated last year the 200th anniversary of the birth of the founder of modern genetics, the abbot of Augustinians abbey Gregor Johann Mendel. I welcome you to Brno, where the Eucarpia Fodder Crops and Amenity Grasses section met exactly twenty years ago. It was an honor for us to welcome you together with my colleague Bohumír Cagaš at the Voroněž Hotel and spend a week with news about forage and turf breeding. You are coming back to Brno, a city with a university tradition and a strong research base. Our organization Agricultural Research, Ltd. Troubsko is geographically outside of Brno, however, we still belong to the regional research community. I cannot resist the opportunity to compare the program and focus of both Brno conferences. In short, the conference program shows a shift from phenotyping to genotyping, from classic methods to methods of modern genetics. In the course of twenty years, the breeding of forages and grasses has seen enormous research progress. Today, it is no longer a problem to read the genetic information of individual species of interest and to search for the characteristics in the genetic code that the breeder wants to emphasize. We live in a time when Europe is following the path of ever-increasing greening in agriculture. Here, forage crops can undoubtedly play an even bigger role than before, especially legumes, whose natural property of fixing atmospheric nitrogen is exactly what we need every day. Unlike other groups of crops, forage crops do not have such an obvious problem with elements of integrated protection because historically the amount of available active substances has never been high. For other crops, the permanent restrictions on the active substances of plant protection products emphasize even more the importance of genetics and the search for new materials with new properties of resistance or tolerance. Based on the discussions with you and other grass and legume breeders and researchers, I also dare to say that if the European Commission finally changes its previous negative attitude towards new genomic techniques, we are ready to start using them immediately.

In the era of digitalization and seamless online meetings, it seemed for a while that conferences with physical participation would be a thing of the past. However, personal contact cannot be easily replaced. Your participation here in Brno is a good proof. In addition to the exchange of information, conferences are a lively place to search for new partnerships and perhaps even new projects. I am grateful and fondly remember my active participation in the Eucarpia forage meetings since the last century. In addition to a whole range of friendships, they also brought new projects. One example for all is the recently completed project with the acronym Eucleg. I hope that this year's participation will also have these benefits.

Finally, let me thank all my colleagues from the organizing and scientific committee for the organization of this conference. I wish to thank all the sponsors for their financial contributions. I am glad that the Minister of Agriculture underlined the importance of the conference and forage breeding by granting patronage. Many thanks go to all of you for your active participation. Compared to previous conferences, we have changed the format a bit and there are no classic poster sections. There will be more space for your presentations. Enjoy your stay in the South Moravian metropolis while visiting the Augustinian Abbey, where Gregor Mendel worked, perceive the genius loci of this area and be proud that we can and have the opportunity to further develop the legacy of this geneticist. I wish you good health and success in your personal and professional life.

Jan Nedělník
Agricultural Research, Ltd. Troubsko

Preface

Dear colleagues and friends,

It is our great honor to welcome you to the 35th conference of Fodder Crops and Amenity Grasses (FCAG) section of EUCARPIA. We are meeting in person after four years as the conference in 2021 was organized online without the possibility of face-to-face meetings, backstage discussions and social program due to the COVID pandemic. Nevertheless, the organizers of the 34th conference, headed by Dr. Hartmann, did a great job and many thanks are due to them. This year we are meeting in person in Brno, just soon after the celebration of the 200th anniversary of the birth of Gregor Mendel, abbot of the Augustinian monastery in the old town of Brno and founder of a new biological direction – genetics. There is a certain amount of symbolism in this, as the conference is co-organised by Agricultural Research in Troubsko, just a few kilometres from the Augustinian monastery where Gregor Mendel spent most of his life and made his groundbreaking discoveries, and the Centre of Plant Structural and Functional Genomics of the Institute of Experimental Botany in Olomouc, where Mendel studied between 1840 and 1843 at the Faculty of Philosophy. Moreover, another co-organizer of the conference is the DLF Seeds, a breeding company from Hladké Životice (where our mid-conference excursion will be directed), located just twelve kilometers from Mendel's birthplace. Mendel himself was very interested in breeding, and his scientific career was shaped by the lectures of Johann Karl Nestler, who lectured on field management and plant and animal breeding. The care and precision of Mendel's experiments with plant crosses are still reflected in the work of every plant breeder today.

The theme of 35th FCAG conference is 'Novel technologies, strategies and crops to sustain forage production in future climate'. It is very topical. This year we have been witnessed of two heat waves caused by anticyclones Kerberos and Charon during the summer months, with temperatures in southern Europe reaching 45°C. It is obvious that we need to adapt to new climatic conditions, especially the unpredictability of climatic phenomena, including catastrophic summer droughts and heatwaves. The joint efforts of molecular biologists, geneticists, genomicists, physiologists, ecologists, biotechnologists and, last but not least, breeders must lead to the introduction of new approaches and technologies in plant breeding and production, including the development of new varieties. Forage grasses and legumes are mostly grown without artificial irrigation systems, so the search for new sources of tolerance to abiotic and biotic stresses in existing production is a key research direction. We hope that our conference will contribute to the search for new solutions to overcome the pitfalls associated with significant climate change, develop existing and start new collaborations between researchers and breeders, and contribute to deepening the knowledge of participants, to new ideas and themes for future research and breeding, and to strengthen our community of forage and amenity grass lovers.

We would like to thank all the authors for their contributions that made this conference possible. A large plethora of papers covering the topics of genetic diversity and the development of new phenotyping and genotyping technologies for plants were brought together. Students, enthusiastic young postdocs and breeders as well as experienced matadors from Europe, America, Australia and New Zealand are represented, a total of 25 countries. Such a wonderful example of biodiversity! Finally, we would like to express our gratitude to all members of the organizing and scientific committees for their perfect work and to all sponsors for their support. We wish you an interesting scientific conference, many new contacts and collaborations, interesting discussions and enjoyment of the accompanying program. We hope that you will find our conference an inspiration and a unique experience.

David Kopecký
Ivana Frei
Tomáš Vymyslický

Future agroclimatic conditions and implications for European grasslands

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Grasslands continue to play a significant role in livestock fodder production and thus, contribute to food security worldwide while providing numerous additional ecosystem services. In the same time grasslands are ever more valued as the type of agricultural landuse capable of increasing the soil carbon stock and thus potentially contributing to climate change mitigation. However, the agroclimatic conditions and adverse weather events relevant to grasslands will change across the European grassland areas and the impact of these changes has been the subject of intense research. In this contribution, the authors will present an analysis of agroclimatic conditions and likely vectors of their change over 476 European sites defined by climate stations. The analysis will address both changes in general agroclimate conditions indicators and also in the frequency of selected adverse weather events relevant to grasslands and how expected changes vary regionally across Europe. In general, we have found that by 2050, the exposure of the south and west European grasslands to heat and drought may double in comparison with today and that the area with frequent occurrences of heat and drought will expand northwards. Moreover, we will present marked changes in the overall thermal and water regimes across European regions projected for the coming decades.

Seed production of grasses and legumes in the Czech Republic and its impact on the agro and environmental sphere

Bohumír Cagaš

Association of grass and clover seed growers, Zubri, Czech Republic

The current production of grass and clover seed in the Czech Republic follows a domestic tradition established a century ago. Karel Holý and especially Ladislav Brada and Josef Demela expanded the cultivation of their own grass varieties adapted to the domestic environment into agricultural practice at the Grassland Research Station in Rožnov pod Radhoštěm (founded in 1920). After a temporary decline, this activity was revived in the 1960s. However, the area under grass cultivation over time is more like a sinusoid; currently, grasses are grown on about 10 000 ha, with annual production in the last decade ranging from 5 000 to 7 000 tonnes of certified seed. The range of about 20 grasses grown is dominated by species of ryegrass and fescue. Over the same period, the area under legume cultivation ranged from 7 to 14 000 ha and annual seed production from two to five thousand tonnes. The most important legumes are red clover, purple clover and alfalfa. Grass and legume seed is used for both domestic agricultural and non-agricultural purposes and, in particular, for export. A major problem today is attracting new growers – competition from cereals and rape is considerable. Our aim, however, remains to maintain and expand the area under grass and clover cultivation, given their indispensable role in agriculture and beyond.

Session I:

Genetic resources and natural diversity

Use of genetic resources and natural diversity in forage breeding: a case study of annual legumes in Australia

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Much of southern Australia has a Mediterranean-type climate, best suited to annual pasture and forage legumes. Crops are generally rotated with pastures in 275–650 mm annual rainfall areas, while higher rainfall areas are dominated by permanent pastures. Annual legumes are important as a nutritious livestock feed and for the nitrogen they supply to other pasture components and subsequent crops. Australian native legumes are unsuited to ruminant livestock, resulting in imported legume species becoming domesticated.

Annual legumes native to the Mediterranean basin and surrounding areas were first introduced to Australia from the late 1700s by early European settlers as contaminants in fodder and wool fleeces or as weed seeds. Some became naturalised. The most important examples are subterranean clover (*T. subterraneum* L.) and annual medics (*Medicago* spp.), first commercialised in 1900 and 1938, respectively. Cultivar development commenced in the 1950s with the evaluation of germplasm collected from the Mediterranean basin and surrounding areas. Since then over 90 expeditions have been made by Australian scientists to collect pasture and forage legumes. This material is stored in the Australian Pastures Genebank (APG), which holds over 53,000 accessions of annual pasture and forage legumes (Smith *et al.* 2021). Subterranean clover and annual medics were the initial target species, whereas alternative species have been targeted since 1990 to introduce new pasture options for soil types and farming systems unsuited to traditional species. This has resulted in 37 annual legume species (or taxa) with registered Australian cultivars, with 27 of these first domesticated in Australia (Table 1).

Breeding programs, involving crossing and selection, operate for *T. subterraneum*, *T. michelianum*, *T. vesiculosum*, *T. resupinatum*, *T. purpureum*, *M. truncatula*, *M. littoralis*, *M. polymorpha* and *O. sativus*. New diversity for important traits has been introduced from wild accessions, including earlier or later flowering, resistance to important pests and diseases, novel morphological features, increased hardseededness (seed coat dormancy) for greater persistence, and increased biomass potential. Core collections, representing the species diversity, have been developed for *T. subterraneum*, *T. spumosum*, and *M. truncatula*, using accessions collected across their native habitat. These allow an assessment of

Table 1. Annual legume species with cultivars registered in Australia, showing those first developed in Australia and year of first cultivar commercialisation (from Nichols *et al.* 2012).

Species	Common name	Year commercialised	First developed in Australia
Astragalus hamosus L.	Milk vetch	1977	√
Biserrula pelecinus L.	Biserrula	1997	√
Lathyrus cicera L.	Dwarf chickling	1995	
L. sativus L.	Grass pea	2003	
Medicago littoralis Rhode ex Loisel	Strand medic	1959	√
M. murex Willd.	Murex medic	1988	√
M. orbicularis (L.) Bartal.	Button medic	2010	√
M. polymorpha L. var. *brevispina*	Spineless burr medic	1976	√
M. rugosa Desr.	Gama medic	1966	√
M. scutellata (L.) Mill.	Snail medic	1977	√
M. sphaerocarpos Bertol.	Sphere medic	1993	√
M. tornata (L.) Mill.	Disc medic	1969	√
M. tornata × *M. littoralis*	Hybrid disc medic	2001	√
M. truncatula Gaertn. var. *truncatula*	Barrel medic	1938	√
Melilotus albus Medik.	White melilot	2006	
Mel. siculus (Turra) Vitman ex B.D. Jacks)	Messina	2018	√
Ornithopus compressus L.	Yellow serradella	1958	√
O. pinnatus (Miller) Druce	Slender serradella	1988	√
O. sativus Brot.	French serradella	1988	
Trifolium alexandrinum L.	Berseem clover	1996	
T. cheleri L.	Cupped clover	1963	√
T. dasyurum C. Presl	Eastern star clover	2007	√
T. glanduliferum Boiss.	Gland clover	2001	√
T. hirtum All.	Rose clover	1965	√
T. incarnatum L.	Crimson clover	1998	
T. michelianum Savi	Balansa clover	1984	√
T. purpureum Loisel	Purple clover	1971	√
T. resupinatum L. var. *majus* Gib. and Belli	Persian clover	1995	
T. resupinatum L. var. *resupinatum* Gib. and Belli	Persian clover	1988	√
T. spumosum L.	Bladder clover	2008	√
T. subterraneum L. ssp. *brachycalycinum* Katzn. *et* Morley	Subterranean clover	1950	√
T. subterraneum L. ssp. *subterraneum* Katzn. *et* Morley	Subterranean clover	1900	√
T. subterraneum L. ssp. *yanninicum* Katzn. *et* Morley	Subterranean clover	1939	√
T. vesiculosum Savi	Arrowleaf clover	1997	
Vicia benghalensis L.	Purple vetch	1972	
V. sativa L.	Common vetch	1920	
V. villosa ssp. *dasycarpa* (Ten.) Cav.	Woolly pod vetch	1968	√

diversity for new traits and more efficient utilisation of the APG collection to select parents for crossing.

The climate in southern Australia is predicted to become drier and more erratic with climate change, posing a challenge to plant breeders to develop better adapted pasture and forage legumes. It may also lead to development of new legume species with important traits. The forage plant genetic resource centres will be crucial to this process.

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Home sweet home – the genetic and phenotypic adaptation of red clover to European growing environments

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The outcrossing forage legume red clover (*Trifolium pratense* L.) is an important component in natural and sown grasslands and has adapted to a wide range of climatic conditions across Europe. The high amount of genetic diversity present in red clover provides an invaluable, but often poorly characterized resource to improve key traits such as yield, quality, and resistance to biotic and abiotic stresses. In the framework of the Horizon 2020 EUCLEG project, the genetic and phenotypic diversity within a diverse set of 395 diploid red clover accessions was assessed via genome-wide allele frequency fingerprinting and multi-location field trials across Europe.

The genetic similarity of accessions based on 20,137 SNP markers mostly reflected their geographic origin (Figure 1A, B; Nay et al. 2023). Accessions from Northern Europe and Switzerland clustered separately from Central, Eastern and Southern European accessions. Interestingly, some accessions showed similarity with accessions from foreign breeding programs and provided evidence that breeders made use of the 'breeder's exemption', i.e., the right to use registered cultivars from the market to integrate desired traits into their own breeding pools. Nevertheless, the distinct grouping of the accessions according to their region of origin suggests that this is not frequently done and breeders mostly rely on their own, local genetic resources.

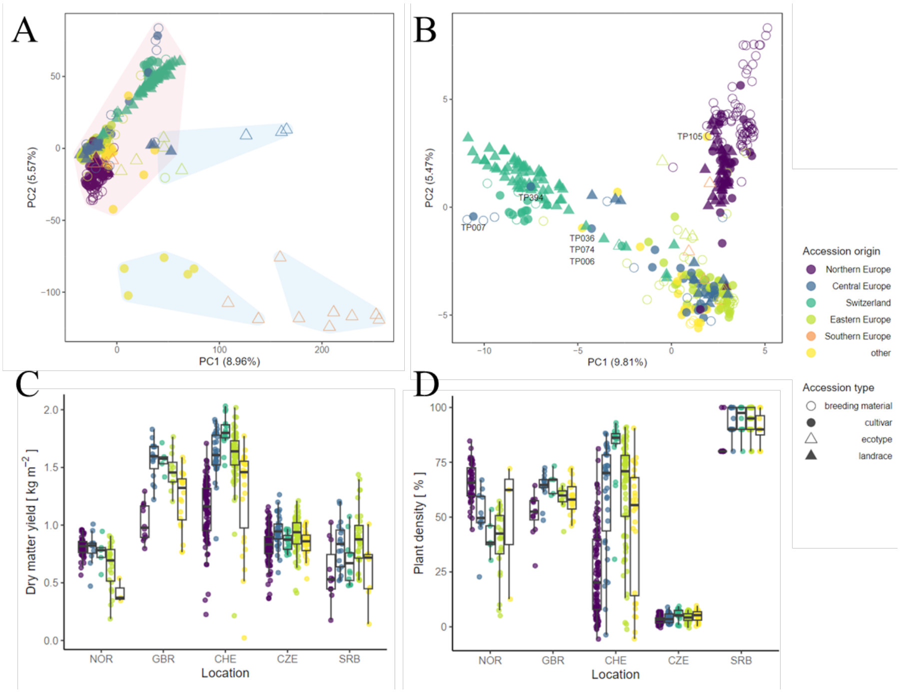

Figure 1: Upper panel: Genetic similarity of red clover accessions within the EUCLEG panel revealed by principal component analysis (PCA) of allele frequency data based on 20,137 SNP markers and pooled leaf samples of 200 individuals per accession (A) and a zoom into the group of 371 accessions shaded in red (B). Colors indicate the region of origin of the accessions and symbols indicate the accession type. Lower panel: performance of red clover accessions of the type 'cultivar' or 'breeding material' grouped by their region of origin for the traits total dry matter yield of the first year (C) and plant density after the first year (D; modified from Nay et al. 2023).

Field plot trials were conducted in Switzerland (CHE), Czech Republic (CZE) and with a reduced set of 100–110 accessions in Norway (NOR), Wales (GBR) and Serbia (SRB). The trials were managed according to common practice for the respective locations with 3–5 cuts per main harvesting year. The mean dry matter yield of the first main harvesting season ranged from 0.74 kg/m^2 in SRB and NOR to 1.34 kg/m^2 in CHE. Many accessions showed declining plant densities under the selective trial conditions with hot summers in SRB, cold winters in NOR and high disease pressures (southern anthracnose in CHE, fusarium root rot in CZE). Phenotypic performance of accessions revealed a strong accession x location interaction. Generally, breeding material and cultivars survived and performed well "at home", the climate and management conditions they were developed in (Figure 1C, D). This local adaptation is especially prominent in northern accessions, which show highest yields and survival rates at location NOR, but underperform in all other locations. The traits vigor, dry matter yield and plant density were even negatively correlated between the location NOR and the locations GBR, CHE, SRB and CZE.

Overall, our results confirmed that red clover cultivars were bred from regional genetic resources and show a specific adaptation to regional conditions. The phenotypic data of multi-location trials can serve as a valuable basis for identifying interesting materials that express the desired characteristics and contribute to the adaptation of red clover to future climatic conditions.

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Assessment of vulnerability to climate change in natural populations of perennial ryegrass across Europe from allele frequencies at adaptive loci

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Global climate change is expected to have substantial impacts on natural and agricultural systems and we are already seeing impacts on standing populations of many plant species, including grassland species such as perennial ryegrass. In many regions of Europe, stressful summer conditions during which forage production is almost null have increased in duration and the recovery after stress is more and more unsettled. This study aimed to assess the extent to which extant European natural populations of perennial ryegrass could be expected to adapt to climate change and assess the need for human interventions to reduce maladaptation. We built from data and results from a previous project that discovered climate adaptive loci in perennial ryegrass using 385 genebank accessions from the natural diversity of perennial ryegrass (GrassLandcape project). This previous project provided population allele frequencies at more than 300,000 SNPs, phenotype characterization of populations at three trial sites with contrasted climates and fine resolution climate norms at sites of origin of populations. A canonical correlation analysis using these genomic, phenotypic and climate data was implemented to recover outlier loci putatively involved in climate adaptation. Here we completed the canonical correlation analysis with new investigations aiming to accommodate nonlinear relationships between allele frequencies and climate as well as spatial distances between populations and to predict maladaptation of local populations in future regional climates of Europe. We applied two additional methods, GDM (Generalized Dissimilarity Modeling) and GF (Gradient Forest), that can account for strong nonlinearity in gene-environment relationships, handle datasets containing many rare and infrequent alleles, provide insight into regions of the genome under local selection, and generate maps of suitability or maladaptation of local populations in future foreseen climate. These methods allow us to estimate the distance between the optimal genetic composition and the actual genetic composition. With this information, we can identify endangered populations and attempt to determine whether existing populations exhibit preadaptation to expected future climates. These analyses will help to inform assisted migration to reduce vulnerability to climate change and help breeders identify regions their cultivars are likely to perform best.

Securing adaptation of timothy cultivars under climate change and during seed multiplication

Odd Arne Rognli[1], Trygve Sveen Aamlid[2], Muath Alsheik[3],
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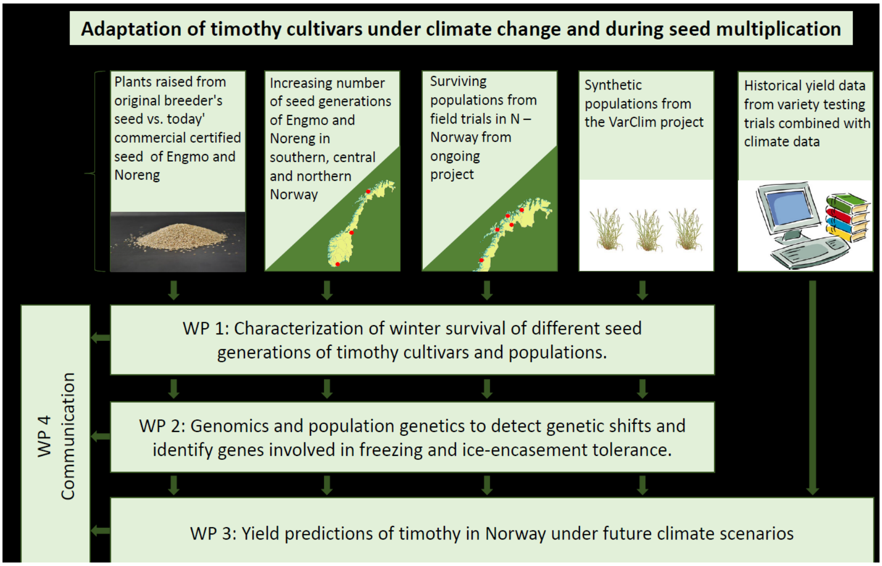
Grassland based forage production is the cornerstone of agriculture in Northern Norway. Timothy (*Phleum pratense* L.) is the most important forage species in Norway, especially in the north. The predicted climate change will lead to higher temperatures and more variable precipitation patterns. These changes are expected to be most rapid in the north. Development of timothy cultivars that are adapted to the future climate is crucial for a sustainable forage production.

Production of commercial seeds of timothy cultivars bred for Northern Norway is located in the South-Eastern part of Norway. This can cause genetic shifts in the cultivars and thus lead to changes in key traits that are important in the north, e.g., winter survival. Studies on genetic shifts and their phenotypic effects during seed multiplication are limited.

The aims of the project presented here are to: i) Estimate freezing and ice-encasement tolerance in different seed generations of northern timothy cultivars and breeding populations; ii) Perform transcriptome profiling by RNA-sequencing to capture genes and their expression levels related to frost and ice-encasement tolerance; iii) Develop a draft genome sequence of timothy; iv) Study genetic shifts in seed lots of different generations using GBS-SNP markers; v) Combine historical yield data from official variety testing with climate data to



predict yield of timothy in Norway under future climate scenarios. An overview of the project is presented in the figure. The results of the transcriptome studies and genome sequencing will be described in other presentations.

Special focus is devoted to the two cultivars 'Engmo' (an old landrace from ~1930) and 'Noreng' (synthetic cultivar from 1990). Both are very winter hardy. 'Noreng' was bred to replace 'Engmo', but the farmers in Northern Norway are complaining that the winter hardiness and persistency of 'Noreng' has decreased over years. We want to find out whether seed multiplication at southern locations is affecting the genetic composition and winter stress tolerance of the cultivars. Diverse plant materials of these two cultivars and others are being compared, i.e., pre-basic seed lots vs. later generations of seed multiplication, new seed multiplications (3 generations) at a northern, intermediate, and southern location, and surviving plants and Syn2-populations from earlier field experiments (see the figure). A statistical prediction model for timothy yield based on temperature and precipitation has been develop using data from multi-location-year official variety testing (8 locations, period 1988–2017, 166 combinations of location and establishment year, 199 cultivars). This model was used to predict DMY of timothy for the decades 2050–2059 and 2090–2099 at 8 locations in Norway.

This presentation will focus on the results of the phenotypic and genetic shifts during seed multiplication and the prediction of timothy DMY in future climates.

Triploid hybrids *Festuca apennina* × *Lolium* occur rarely in nature but show high colonizing potential without dominating the sward excessively

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Tetraploid *Festuca apennina* De Not., a broad-leaved species related to diploid *F. pratensis* Huds. and hexaploid *F. arundinacea* Schreb., has been shown to hybridize easily in nature with *F. pratensis* to form a triploid, sterile hybrid (Fape x Fp) of very high vigour and a tendency to dominate multispecies natural pastures by vegetative expansion through rhizomes. Here, we report on occurrence of another type of hybrids resulting from natural hybridization between tetraploid *F. apennina* and diploid *Lolium*, presumably *L. perenne*, as shown by genomic in situ hybridization (GISH). *F. apennina* × *Lolium* (Fape x L) is triploid and sterile, and relatively easy to recognize in reproductive stage by single spikelets attached on short branches, as opposed to sitting spikelets of *Lolium* (spike type) or multiple spikelets on ramified branches of *Festuca* inflorescences (panicle type).

We surveyed 185 locations in the Swiss Alps, at most of which Fape x Fp was frequent, and were able to confirm presence of Fape x L in 21 locations. Species (hybrid) identity was confirmed with the help of species-specific DArTseq markers and GISH. DArTseq was also used to identify clonal replicates.

At most sites, less than five distinct genotypes of Fape x L were identified (Table 1). Individual genotypes often had many clonal replicates, expanding for dozens or even hundreds of meters. However, their estimated contribution to biomass of the sward rarely exceeded 5 %. This is in contrast to triploid Fape x Fp hybrids which, when present, usually reaches a biomass contribution to the sward of 20 % or more.

The largest population of Fape x L was found at locality Zinggen. At this location, two genotypes with numerous clonal replicates were found (Fig. 1). Genotype Z1 was concentrated in the eastern part of the site, a field limited by forest and a paved road. Genotype Z2 was more widespread and was found on both sides of the paved road. All genotypes without clonal replicates (Z3 to Z8) were found in close vicinity to the paved road. We hypothesize that Fape x Lp hybridization occurs during transient presence of *L. perenne* after disturbance of the sward e.g. by road construction. Genotypes with numerous, widespread clonal replicates must be well adapted to local conditions and highly persistent to allow for rhizomatous expansion over many years.

Table 1: Sites at which hybrids *Festuca apennina* × *Lolium were* found

Site	Municipality	Ct.	no. of Fape x L plants found	No. of distinct geno-types	Clone with most numerous replicates	Expansion (m) of largest clone
Zinggen	Wildhaus-Alt St. Johann	SG	57	10	31	440.3
Risipass	Nesslau	SG	39	2	38	150.0
Gruoba	Grüsch	GR	18	1	18	73.8
Matossa	Flums	SG	12	3	9	42.4
Chüesitz	Hundwil	AR	11	5	6	41.6
La Filistorfena	Plasselb	FR	11	1	11	16.9
Naserina	Flums	SG	7	4	3	1.6
Stürfis	Maienfeld	GR	7	1	7	9.1
Chessler	Wildhaus-Alt St. Johann	SG	6	4	2	5.7
Hengstschlund	Guggisberg	BE	6	2	4	13.5
11 more sites with < 5 individual plants			23	15	4	6.0
Total			197	48		

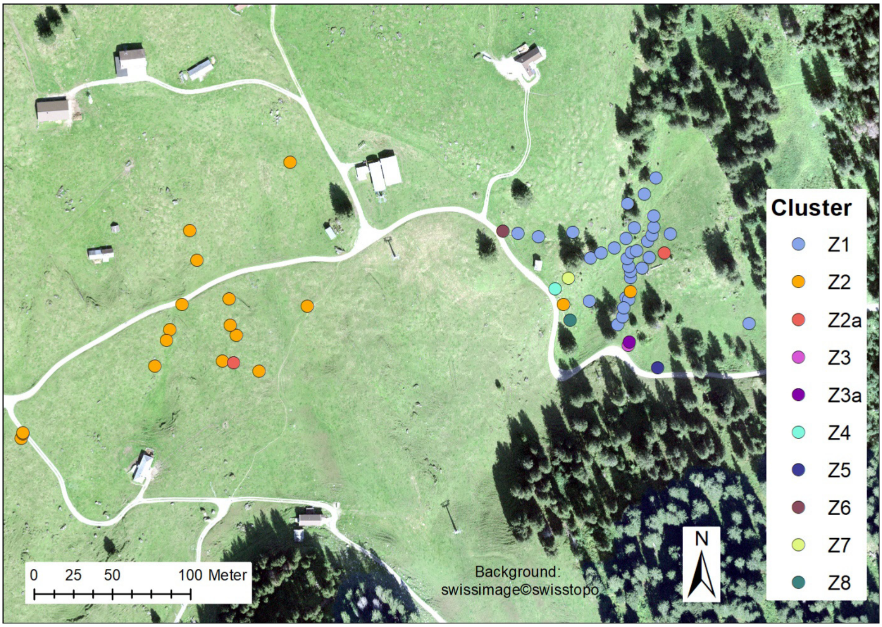

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Figure 1: GPS localization of 57 individual *Festuca apennina* × *Lolium* plants found at locality Zinggen and belonging to 10 distinct genotypes. Genotypes Z2a and Z3a seem much more closely related (potentially full sibs) to genotypes 2 and 3, respectively, than to other genotypes.

We conclude that *F. apennina* × *Lolium* hybrids are of interest due to their good adaptation to high altitude, their persistence and potential of rhizomatous expansion. In order to utilize their agronomic potential, procedures should be sought to multiply elite genotypes vegetatively, or to restore fertility by polyploidization.

Genetic variation and genome-environment association of alfalfa (*Medicago sativa* L.) populations originating from long-term grazing sites

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Information on alfalfa adaptation to long-term grazing is useful for alfalfa genetic improvement in grazing tolerance. In this study, 14 alfalfa populations were collected from long-term grazing sites (> 25 yrs) across four soil zones in western Canada. Six agro-morphological and three nutritive value traits were evaluated from 2018 to 2020. The genotyping-by-sequencing (GBS) data of the alfalfa populations and regional environmental data were used for genotype-environment association (GEA). Plants from the Black soil zone produced the highest forage mass, plant height, and stem number, while the lowest values for these traits were recorded plants from the Brown soil zone. The alfalfa populations from the Black soil zone had higher percentage of *M. sativa* genome compared to the plants from the Brown soil zone based on STRUCTURE analysis using 19,853 SNPs. The linear regression of STRUCTURE membership probability on phenotypic data indicated genetic variations for forage dry matter yield, spring vigor and plant height were low, but the variations of regrowth, fall plant height, days to flower and crude protein were still high for the alfalfa populations originating from the long-term grazing sites. The GEA identified 31 SNPs associated with 13 candidate genes that were mainly associated with six environmental factors. Candidate genes underlying environmental factors were associated with a variety of proteins, which were involved in plant responses to abiotic stresses including drought, cold and salinity stresses.

Genetic gain from simulated forage breeding programs with genomic selection

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Most forage breeding programs are based on phenotypic selection on traits such as yield or persistence and do not extensively use genomic data. Field trials are done in competitive sward conditions lasting several years, and well over a decade is required to release a new cultivar. Leveraging genomic data to inform selection decisions (i.e., genomic selection) has the potential to reduce breeding cycle time and cultivar development time, increase the accuracy of selection and ultimately increase the rate of genetic gain. However, implementing genomic selection in forage species poses special challenges as compared to other crop species. At different stages of the breeding cycle, forages are evaluated at a plot level in swards or at an individual level in the nursery, and the optimal entry point for genomic selection is not known. Being obligate outbreeders, forages have weak linkage disequilibrium, an important parameter dictating the accuracy of genomic selection. Moreover, outbreeding perennial forage crops, such as red clover and timothy, are affected by inbreeding depression, which requires that a genomic selection approach account for dominance effects. In our work, we investigate the relative effectiveness of different entry points for genomic selection. Using stochastic simulations to model breeding programs and assess their performance, we contrast different genomic selection scenarios in terms of their genetic gain and predictive accuracy. We also investigate whether dominance effects compromise the effectiveness of genomic selection, or if they can be included in genomic prediction models to improve their predictive accuracy.

Shattering resistance in genetic resources of the fodder grasses *Lolium perenne* L. and *Festuca pratensis* Huds.

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Introduction

Seed shattering is the detachment of mature seeds for plant dispersal. High shattering reduces the harvestable seed yield and therefore the commercial success of a variety, *e.g.* in the grass family. Especially in fodder grasses, there was only little selection for seed retention until now. We screened genetic resources of the important fodder grasses *Lolium perenne* and *Festuca pratensis* for seed retention potential and explored the variability between species and between and within populations. Additionally, the anatomy of the abscission zone as the site of disarticulation was examined in contrasting plants of *Lolium perenne*.

Material and Methods

In a two-year field trial, seed retention of 10 plants of 150 accessions of *Festuca* and 286 accessions of *Lolium* was assessed in the first year, and of 30 plants of 17 contrasting *Festuca* accessions and 32 contrasting *Lolium* accessions in the second year. Inflorescences were covered with perforated plastic bags after flowering. Weights of field shattered seeds and hand-stripped seeds were determined. For histological studies, grain bases of different developmental stages were fixed, embedded in resin and longitudinal sections examined with a light microscope.

Results

The variability in seed retention was high in both species. Accessions of *Lolium* had, based on population averages, higher variability and seed retention than *Festuca*, ranging from 12 to 94 % in *Lolium* and 13 to 71 % in *Festuca* in 2018 (average: 49 % and 35 %, respectively). Seed retention, based on replicated plants, was lower in the second year. Variability within populations was very high in *Lolium* and *Festuca*. In *Festuca*, two tested cultivars were within the group with highest seed retention. In *Lolium*, all tested cultivars had lower seed retention than some of the genetic resources.

The abscission zone anatomy was slightly different between *Lolium* plants with high seed retention and plants with low seed retention: Two to three rows of loosely connected cells were already distinguishable during flowering in the

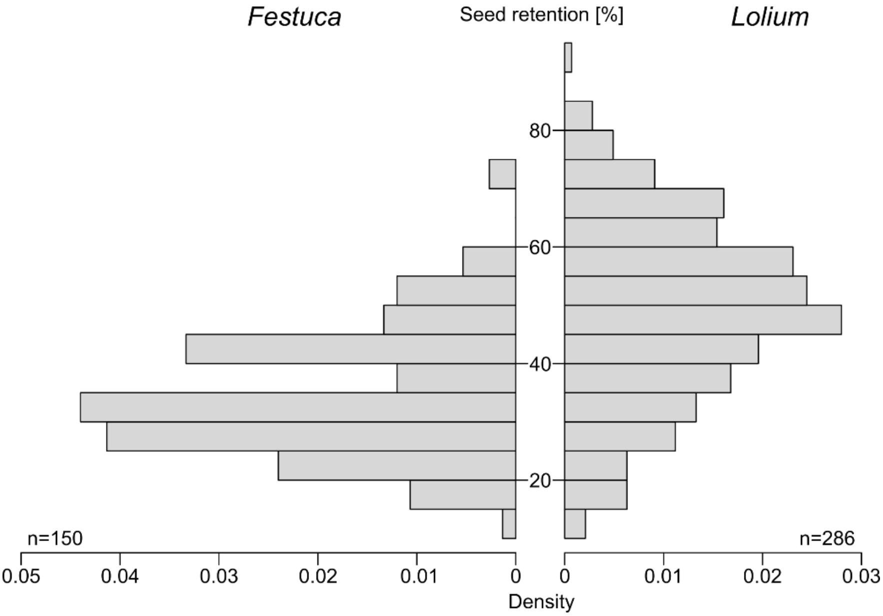

Figure 1: Distribution of seed retention within *Festuca pratensis* and *Lolium perenne*, based on the mean values of 10 plants per population.

plant with low seed retention, while the cells were more nested in the plant with high seed retention.

Conclusion

Plant genetic resources of *Lolium* – but not *Festuca* – have high breeding potential for seed retention, and shattering resistant genebank material has already been integrated into the breeding program of the project partner Saatzucht Steinach. Shattering resistant genotypes were selected, multiplied and integrated as new populations into the IPK Gene Bank collection and can be used by breeders and for further scientific projects.

Acknowledgements

This project was funded by the German Federal Ministry of Food and Agriculture under grant number 2818203115. We thank Christine Luckmann and Daniel Gransow for excellent technical assistance.

Allelopathic effect of herbage water extracts on seed germination and seedling development of some perennial grassland species

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In grasslands ecosystems, different allelopathic interactions are established between the plant communities, with influence on density and distribution of species and which are affected by different forms of environmental stress, including climate changes.

The phenomenon of allelopathy can occur also through of the decomposition and leaching of plant residues. The purpose of this work is to evaluate the allelopathic effect of two concentrations (5% and 10%) of herbage cold water extracts of two species of perennial grasses, of *Dactylis glomerata* and *Festuca arundinacea* and two species of perennial legumes, *Trifolium repens* and *Lotus corniculatus* on seed germination (%) and seedling development (shoot and root lenght) *Dactylis glomerata, Lolium perene, Trifolium repens, Lotus corniculatus, Festuca arundinacea și Phleum pratense*). The experiment was carried out in petri dishes and the measurements were performed after 14 days. In general, seed germination and root development were inhibited on the studied extracts, while shoot length was least affected. The negative effect was stronger at the higher concentration. The inhibitory effect of the tested extracts was the strongest on the species *Dactylis glomerata* and *Trifolium repens* regarding both seed germination and seedling development, and the least affected were *Lolium perenne* and *Lotus corniculatus*. Positive allelopathic effect was noted at the concentration of 5% on the seedling development at *Lolium perenne* species.

Agronomical traits, seed color and protein content of protein pea (*Pisum sativum* L.) cultivars grown in European conditions

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Protein pea (*Pisum sativum* L.) is a protein-rich legume (up to 33% of seed protein). Using pea in the human and animal diet provides one of the best solutions for the long-term lack of plant-based protein. Although soybean is one of the primary plant protein sources, the advantage of growing pea is their wider geographical area and colder climates adaptability.

The objective of this study was to determine the impact of different agroecological conditions in 64 protein pea cultivars on seed protein content and agronomical traits related to seed yield. A two-year trial on two European sites (Serbia and Belgium) was done using an augmented block design. The average temperatures and total precipitation amount during the vegetative period (March-August) in Serbia trials were 18.4 °C and 381.4 mm for 2019 and 18.1 °C and 488.9 mm for 2020, and in Belgium trials 14.9 °C and 268.9 mm for 2019 and 14.7 °C and 184.6 mm for 2020. Agronomical traits (flowering duration FD, plant seed yield PSY, thousand seed weight TSW, and seed per pod SPP) were determined using ten plant samples, while seed protein content (SPC) was determined using near-infrared spectroscopy (Table 1). Statistically significant differences between localities were determined by T-test, and Pearson's correlation coefficients were determined between traits. Multivariate analysis was performed based on the examination of mean values of traits for both localities, in order to investigate the population structure of 64 pea genotypes differing in color and seed type. Significant positive correlation was observed between TSW and PSY (0.60), and between SPP and SPC (0.25). A significant negative correlation was determined between SPC and TSW (-0.66) and between SPC and PSY (-0.41). The results of multivariate analysis based on seed color show the separation of pigmented seeds from mixed non-pigmented seeds by the first axis (35.9%) and yellow non-pigmented seeds by the second axis (17.9%), with no clear grouping in relation to seed type (smooth, wrinkled, dimpled).

The similar values of the main agronomic traits that affect the yield confirm the great ability of protein pea to adapt to different agroecological conditions.

Table 1: Pea agronomic traits in two trials

	Serbian trial			Belgian trial		
	Mean	Min	Max	Mean	Min	Max
FD **	22.3	14.6	27.5	14.9	11.4	18.8
PSY **	8.4	4.2	11.6	6.3	4.1	9.7
TSW	182.6	57.5	256.4	185.3	109.2	288.4
SPC	27.3	25.0	29.8	27.4	25.1	30.2
SPP	4.4	3.7	5.4	3.8	2.5	5.2

$p \leq 0,01$ (**)
FD-flowering duration (days); PSY- plant seed yield (g); TSW- thousand seed weight (g); SPC-seed protein content (%), SPP- seed per pod (#)

Isoflavones profiles of some diploid and tetraploid red clover cultivars (*Trifolium pratense* L.) at flowering stage

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Isoflavones are one of the best studied groups of phytoestrogens. Phytoestrogens are plant compounds that can exhibit a biological effect similar to the reproductive hormone estrogen. From nutritive and pharmaceutical aspect, red clover is one of the most important sources of these compounds, and they are often used as a raw material for production of dietary supplements used for alleviation of menopausal symptoms.

The objective of this study was to determine the concentrations of isoflavones in 11 diploid (2n) and 10 tetraploid (4n) cultivars of red clover (*Trifolium pratense* L.) from different origine, in a whole plant and separately: in stems, leaves and flowers at the stage of full flowering. Herbage samples from whole and different parts of plant were dried at 60 ˚C for about 48 hours, homogenized and grounded to a particle size of Æ = 0.8 mm as well as mixed with 2 ml of water on 37°C. After that, HCl and ethanol are added and mixture is heated to boiling. Obtained extract are purified by solid phase extraction on HLB cartridges and analyzed on HPLC after filtration. Zorbax SB C 18 reversed phase HPLC column is used for separation of main isoflavones present in red clover: formononetin, biochanin A, genistein and daidzein. Isoflavones are identified by comparing the retention times in HPLC chromatograms and UV spectral patterns with those of standard compounds. Isoflavone concentrations are quantified by external standard method using five-point regression curves of formononetin, biochanin A, genistein, and daidzein standard compounds.

The content of total isoflavones ranged from 3.83 (**Marieta**-2n cultivar) to 17.11 mg g^{-1} DM (**Margot**-4n cultivar). Red clover plant parts were significantly different according to isoflavone content. The leaf was the richest source of isoflavones (6.54 mg g^{-1} DM), followed by flowers (1,96 mg g^{-1} DM) and stem (1.16 mg g^{-1} DM). Biochanin A and formononetin were the predominant isoflavones in leaves, while genistein was mostly present in flowers.

Tetraploid red clover cultivars (4n) had significantly higher isoflavones content compared to diploids (2n). On average, the content of isoflavones in tetraploids (11.0 mg g-1 DM) is statistically significantly higher than in diploids (8.44 mg g^{-1} DM).

Grass and forage legume genetic resources in the Czech Republic and their practical utilization

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Genetic resources of cultivated plants are the irreplaceable wealth of our planet, created by nature and later by the purposeful creative work of man. Cultivated and restricted varieties, regional and primitive forms of cultivated plants, breeding lines, parental components of hybrids and crop wild relatives (CWR) are designated as genetic resources (Maxted & al., 2012; Ulukan & al., 2011).

Gathering, evaluation and research of plant genetic resources has a long tradition in the Czech Republic (Holubec & al., 2017). The Czech Republic is very diverse in ecological conditions and habitat types, thus the amount of CWR on its territory is very high. Unfortunately, germplasm erosion was very intensive in the case of landraces and old cultivars (Holubec & al., 2010).

Genetic resources of grasses have been studied in the Grassland Research Station in Zubří since 1920 (Ševčíková & al., 2002). Genetic resources of forage legumes have been studied in the Research Institute for Fodder Crops in Troubsko since 1953 (Vymyslický & al., 2003). Since the fifties, crop varieties of both the Czech and World assortments have been gathered, evaluated, conserved in the Czech Gene Bank and used both in research and breeding (Knotová & al., 2020). Very important role both in grasses and legumes play the CWR, local ecotypes and landraces. The first collecting expeditions for CWR and landraces were organised in the seventies and eighties. Systematic and modern collecting of CWR have been started in 1993. Altogether 32 collecting expeditions have been organised and 5 029 seed samples of domestic origin have been collected. More than half of the number belongs to grasses and forage legumes. These materials successfully fulfilled the gap in the Czech Gene Bank.

National collection of grasses consists of 2 596 gene bank accessions. National collection of forage legumes consists of 2 592 gene bank accessions. The collection of 172 vegetatively propagated genetic resources of ornamental grasses is kept directly in the gene pool garden at the Grassland Research Station in Zubří. The most abundant grasses collections are *Lolium* (703 accessions), *Festuca* (541 accessions), *Poa* (286 accessions), *Dactylis* (191 accessions) and *Phleum* (159 accessions). The most abundant forage legumes collections are *Medicago sativa* (585 accessions), *Trifolium pratense* (416 accessions), *Trifolium repens* (295 accessions) and *Trifolium* other species (274 accessions).

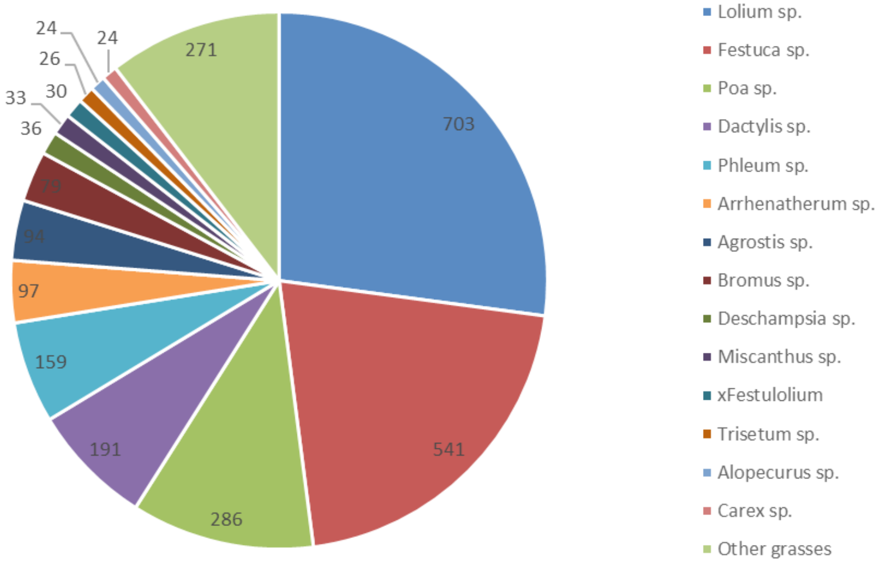

Figure 1: The Czech gene bank collection of grasses

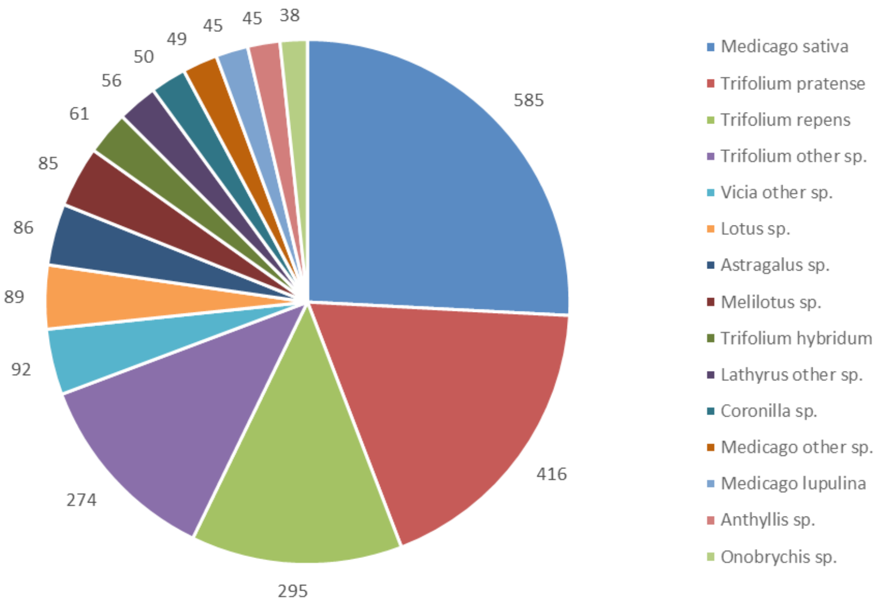

Figure 2: The Czech gene bank collection of forage legumes

Gathered seed samples, especially those of CWR and landraces, are important input materials for breeding, for increasing the diversity of agricultural landscape, for regional seed mixtures, as feed sources for pollinators or in case of legumes as fixators of atmospheric nitrogen. These materials are also valuable inputs for resistance breeding programmes. Many currently registered varieties of grasses and forage legumes have their origin in CWR and landraces (Knotová & al., 2020).

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Assembling forage grass genomes in the long-read sequencing era: a case study with *Lolium perenne* L. and *L. multiflorum* Lam.

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Recently, the availability of long-read sequencing technologies, such as PacBio HiFi and Oxford Nanopore Technologies (ONT), has enabled telomere-to-telomere gap-free genome assemblies for plant species. However, it is still very challenging to correctly assemble the large, highly heterozygous and repeat-rich genomes of *Lolium* spp.. The reason behind this is current long reads are not long (PacBio HiFi) or accurate enough (ONT) to separate allelic or repetitive sequences to resolve haplotypes or complex repetitive regions. Besides, current assemblers either produce partially phased contigs with phase switches or fully phased unitigs not binned to individual haplotypes. Therefore, extra phasing steps are required to reconstruct each haplotype.

Here, we present a high-quality chromosome-level haploid assembly for a doubled haploid *L. perenne* L. genotype (hereafter referred to as Kyuss) and a haplotype-resolved diploid assembly for a highly heterozygous *L. multiflorum* Lam. genotype (hereafter referred to as Rabiosa). We assembled Kyuss with 30× coverage ONT data using NextDenovo, resulting in an assembly of 2.26 Gb with a contig N50 of 120 Mb. After scaffolding with high-throughput chromosome conformation capture (Hi-C) data, 99% of the 2.26 Gb were anchored to 7 chromosomes. Quality assessment of the assembly revealed a very high completeness (99% BUSCO score), a very high base-level accuracy (QV 50) and a very accurate chromosome structure (correct Hi-C contact map), suggesting that we have produced a *L. perenne* genome assembly with the highest quality. However, as a doubled haploid genotype, Kyuss only contains one half the genetic information of the original diploid parental genome, and to construct haplotype-resolved assemblies for *Lolium* spp., alternative assembly strategies still need to be explored and established. Therefore, we extended the haploid genome assembly workflow to a reference-based phasing workflow to assemble Rabiosa with 60× ONT data and 50× Hi-C data. This resulted in a 2.4 Gb unphased haploid assembly with 2.2 Gb anchored to 7 chromosomes plus a haplotype-resolved diploid assembly with 1.8 Gb for each haplome. BUSCO analysis of the diploid assembly showed a high completeness (97%) with more than 82% duplicated BUSCOs, suggesting that the diploid assembly indeed contains both haplotypes.

The assembly workflows presented here are designed to work with both PacBio HiFi and ONT data and can be readily applied to other sequencing projects involving highly heterozygous diploid plant genomes. In addition, the genome assemblies will serve as references to conduct variant discovery, marker development, genome-wide association studies and genomic selection, unlocking the potential of molecular plant breeding approaches in forage grasses.

An initial metabolomic study on Hungarian clover

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Hungarian clover (*Trifolium pannonicum* Jack.) is the youngest member of the genus *Trifolium* and is classified as a minor species in terms of cultivation areas. It has a long lifespan, up to 15 years (Nechaeva et al., 2020), is resistant to freezing, diseases, and drought, has good antioxidant potential, and produces one stable harvest annually. Despite these advantages, however, it is not used enough in the production of fodder. Previous research indicates that this species has a favorable antioxidant potential that is unrelated to the total content of phenolic compounds (Petrović et al., 2016). The detailed 1H NMR spectroscopic analysis of its chemical profile was carried out on a Bruker 500 AVANCE III NMR, Fällanden, Switzerland, system equipped with a 5 mm BBI probe head and BVT unit at 298K system equipped with 5 mm inverse broad bend probe head (BBI) and variable temperature unit (BVT), at 298K. The results of Simca software's principal component analysis (PCA) are presented here. By applying the 1D NOSEY pulse sequence, the obtained ^{1}H NMR experimental data were used to identify biomarkers of different species as well as their metabolome in general. The material used in the study was collected from individual plants in an ongoing experiment (started 2019). The samples were collected from the only European variety – Pannon and genotype from the nature. As a control species, Swedish clover (K50 variety) was used. The spectral data that were obtained represent the metabolic profiles of single samples (upper parts of particular plants during the bud development stage) at a specific time. The obtained graphic illustrates the first two main components (PC1 and PC2) as well as the distribution of individual samples from the PCA model (individual points on the graph). Swedish and Hungarian clover species were separated based on the first principal component, while the variety and wild material were split based on the second principal component (Figure 1). PC2 separation illustrates differences in the chemical profiles of the tested samples that can be linked to a specific genotype. Apart from the visual grouping and separation of individual species or genotypes, the validity of the presented model is additionally confirmed by the R2 model's high coefficient of determination of 0.822. The results show that the methodology can be used successfully and statistically analyzed further using other more complex

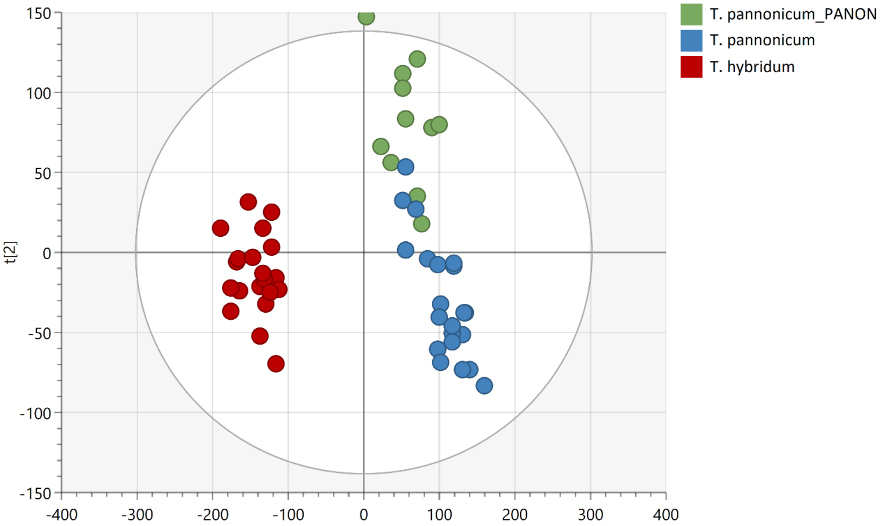

Figure1: PCA score plot of 1H NMR data from two *Trifolium* species: *T. pannonicum*, variety Panon, wild material and *T. hybridum*, variety K50.

models (orthogonal partial least squares regression (OPLS-DA) and analysis of 1D and 2D NMR spectral data) to identify individual chemical compounds.

Acknowledgments

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Evaluation of alfalfa populations selected for reduced fall dormancy in a frequent cutting trial

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Alfalfa cultivars adapted to northern Canadian prairies, while winter hardy, are very dormant leading to low seasonal yield. Breeding for reduced fall dormancy is an efficient approach to increase the annual yield of alfalfa by extending its growing season from late summer through late fall. While fall dormancy and winter hardiness are highly correlated in alfalfa, the genotypic correlation between the traits is low, suggesting that fall dormancy and winter survival can be improved simultaneously, resulting in higher yields and retained winterhardiness.

Using a selection method for reduced dormancy under controlled conditions developed by our team, three cycles of recurrent selection (D3) were performed within two winter-hardy alfalfa cultivars, Peace and Yellowhead. Our previous work has shown this method to be successful in increasing fall height, which resulted in one unit increase in the fall dormancy class of Peace and Yellowhead after one cycle of selection (Claessens et al. 2022). Here we aimed to determine the yield potential of the new reduced-dormancy populations under a frequent cutting management. To reach this goal, a field test was conducted in Swift Current (SK, Canada) using a grazing-type control cultivar (Rambler), a hay-type control population (ScL4803), the two initial cultivars (Yellowhead and Peace), and the two reduced-dormancy populations (Yellowhead D3 and Peace D3). Plants were cut three times during the growing season and yield and height were recorded at each cut during the two first production years.

At first and second cuts as well as for total biomass, Yellowhead D3 had significantly higher yield than Yellowhead and had similar yield to the controls, whereas, Peace D3 had similar yields to Peace, but significantly lower yields than the controls. At the third cut, however, all cultivars and populations had comparable yields.

In accordance with yield, Yellowhead D3 was significantly taller than Yellowhead at 1st and 2nd cuts whereas Peace D3 was equivalent to Peace, but shorter than ScL4803. Both reduced-dormancy populations were similar in

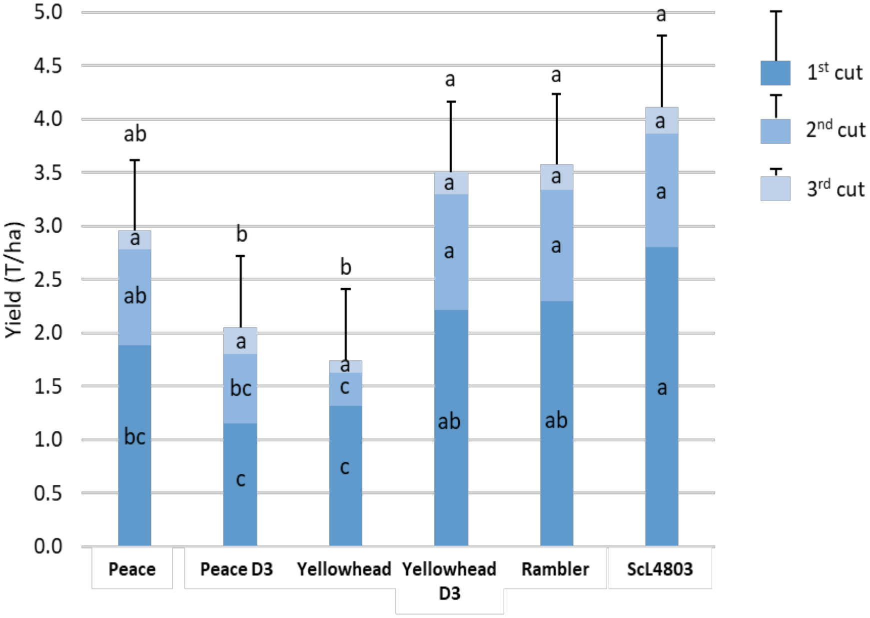

Figure 1: Forage yield at 1st, 2nd, and 3rd cut, and total yield of two cultivars (Peace and Yellowhead) and of two populations selected for reduced dormancy (Peace D3 and Yellowhead D3), and of two controls (hay-type ScL4803 and grazing-type Rambler). Yield is the average of the two first production years (n=12). Error bars correspond to the SEM associated to total yield (bars) or to each cut (legend).

height to control grazing-type Rambler at all cuts, except at the third cut where Yellowhead D3 was taller than the control.

Our results show that our selection method for reduced dormancy was effective in increasing the yield and height of cv. Yellowhead under semi-arid conditions. This response could be due to the fact that this cultivar is a *falcata* × *sativa* hybrid and that our selection method likely favored linkage groups associated with subsp. *sativa* traits at the expense of the subsp. *falcata* traits thus favoring taller and higher yielding plants. In Peace, low responsiveness to selection could be due to a lower number of stems per plant, but this data was not evaluated in the present experiment. Our selection method will be revised to take this variable into account.

Variability of sapogenins among *Medicago falcata* entries

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Lucerne saponins are receiving increasing attention in terms of their antinutritional properties and other utilization (Tava et al. 2022). In contrast to common *Medicago sativa* L., yellow-flowered *Medicago falcata* L. has been recognized as providing higher total saponin content; however, there is a lack of information about the variability of particular sapogenins within this subspecies of *M. sativa* complex.

The experiment with 22 *M. falcata* entries (genetic resources of gene bank in CRI in Ruzyně) was planted at the breeding station Želešice in 2020. The number of plants varied from 21 to 38 for a particular entry, with a spacing arrangement of 50 cm within the row and 100 cm between rows. Blue-flowered *M. sativa* was used as a reference population. Sampling was realized in the full-bloom stage in the first cut on 17th June 2021. A sampling of aboveground biomass was taken from five randomly selected yellow flowered plants within each entry; ten fully developed stems were selected from the sample, separated into leaves and stems, dried at 55 C, and milled through a 2 mm screen. Aglycone moieties (medicagenic acid, bayogenin, zanhic acid, soyasapogenols A and B) were obtained from the corresponding saponins by acid hydrolysis, identified by GC/MS and quantitated by GC/FID using an internal standard method (Tava et al. 2017).

Average content and sapogenin variability with *M. falcata* entries are shown in Figure 1. Key sapogenin was a medicagenic acid in leaves whilst soyasapogenol B and medicagenic acid were equal in stems. Variability was higher in leaves, where the coefficient of variation ranged from 37 to 66 % for soyasapogenol B and bayogenin, respectively. In stems, the lowest coefficient of variation was observed for soyasapogenol B (17 %) in contrast to 48 % at medicagenic acid. A comparison between *M. sativa* and *M. falcata* is presented in Table 1 where the highest difference was observed in medicagenic acid.

We conclude there is significant variability of sapogenin contents among *M. falcata* entries, especially in the leaves. Differences in sapogenins between *Medicago* subspecies could be observed mainly for medicagenic acid.

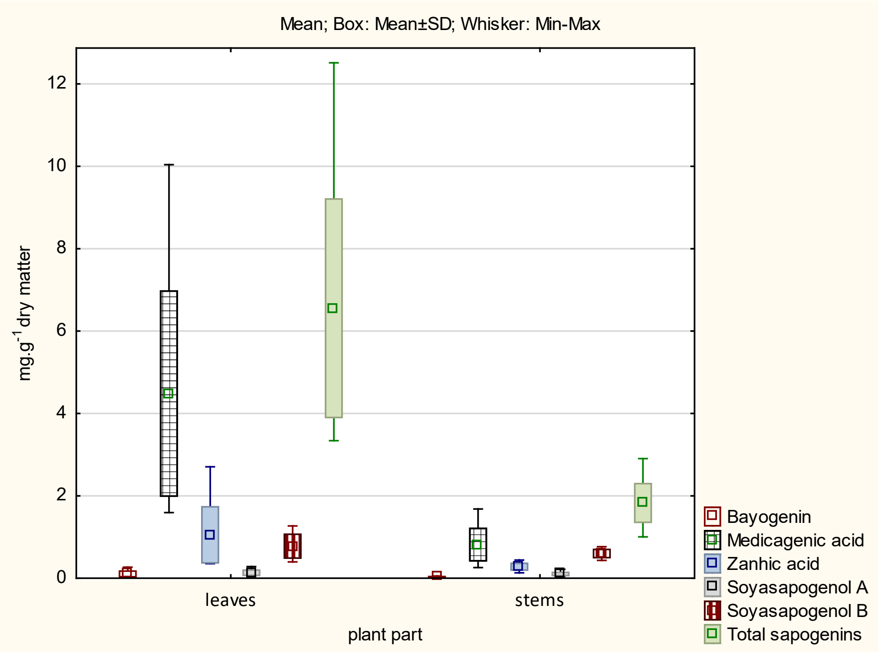

Figure 1: Box and whisker plot for variability of sapogenins in *Medicago falcata* L. during full bloom stage.

Photo 1: Yellow-flowered *Medicago falcata* in the sampling date (June 2021).

Table 1: Average sapogenins (mg.g^{-1}) content in leaf and stems of 22 *M. falcata* entries compared with *M. sativa* population.

Part	Medicago	Bayogenin	Medicagenic acid	Zanhic acid	Soaysapogenol A	Soaysapogenol B
Leaves	*sativa*	0.03	1.19	0.98	0.23	0.83
	falcata	0.10	4.48	1.05	0.13	0.78
Stems	*sativa*	0.02	0.49	0.26	0.13	0.51
	falcata	0.03	0.82	0.28	0.10	0.60

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Genome assembly and annotation of timothy (*Phleum pratense* L.)

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Timothy (*Phleum pratense* L.) (2n=6x=42), a cool-season perennial grass species, is the most important forage grass species in Norway because of its good winter hardiness, ability to thrive in short growing season and resistance to frost and ice-encasement. However, the predicted increase of temperatures during autumn and winter at high latitudes in the coming decades and the associated variable temperature and precipitation patterns might negatively affect cold acclimation and winter survival of timothy, therefore potentially affecting the forage production in Nordic regions. A combined effort of improved agronomy and breeding of high-yielding and persistent cultivars is needed to secure stable supplies of high-quality forages for meat and milk production. However, timothy breeding is a long and slow process hampered by limited genomic and molecular resources. Genomic resources such as a complete genome sequence and transcriptome will be important for research and breeding of this high value forage crop. *P. pratense* is a hybrid, most likely involving the species *P.* nodosum (2n=2x=14) and *P. alpinum* (2n=4x=28). Using a hybrid sequence assembly approach including data from both long (Oxford nanopore sequence reads) and short (Illumina) sequence reads combined with Omni-C, we present the first assembly of the *P. pratense* genome. The *de novo* assembled genome consists of 41,151 scaffolds with a genome size of 4.41 Gb with the and the largest scaffold was 263 Mb. Quality of the genome sequence assessed with BUSCO scores is 97.25%. Further, RNAseq data was generated for different parts of the timothy plant (leaf, root, crown, leaf sheath, stem, inflorescence) to annotate the genome. The genome annotation has resulted in 53,593 genes wth average length around 918 bp. The Complete single copy and duplicated BUSCOs were 92%. A group at the Swedish University of Agricultural Sciences (SLU) is sequencing the parental genomes of *P. nodosum* and *P. alpinum*. We are combining the genome sequences of the three species to better understand the complex genomic constitution of *P. pratense*. The timothy genome sequence with complete annotation provides an important new resource for forage grass research and breeding.

Heritability and variability of the most important traits of meadow fescue synthetic cultivars

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Meadow fescue (*Festuca pratensis* Huds.) is one of the most important perennial grasses for animal feed production on grasslands. It is characterized by high and stable yield potential and good biomass quality. The aim of this study was to determine the broad and narrow sense heritability and genetic ($CV_G\%$) and phenotypic ($CV_F\%$) coefficient of variation of the most important traits of two synthetic cultivars (Kruševački 21 and Pradel). Individual plants for hybridization, in order to obtain progeny by the method of Comstock and Robinson, were selected completely randomly. In order 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 Kruševački 21, 60 full-sib progeny were studied in two sets, while in the Pradel a total of 39 full-sib progeny were studied. Within each full-sib progeny, 60 plants were analyzed.

Narrow sense heritability has great practical significance for breeders considering that it indicates how much the individual's phenotype is determined by the genes received from the parents. Narrow sense heritability represents the relationship between the additive component of genetic variance and phenotypic variance. The highest narrow sense heritability was determined for dry matter yield (0.841) in cv. Kruševački 21, while in the cv. Pradel high values (above 0.75) of this parameter were noted for most studied traits. High values for broad and narrow sense heritability means that these traits largely depend on genetic factors. The high heritability values indicate the great proportion of genetic and additive component in total phenotypic variability and suitability for breeding. The lowest narrow sense heritability is determined in cv. Kruševački 21 for the number of leaves per generative tiler (0.286) and for leaf length and width (0.217; 0.269) in cv. Pradel. These traits are quite variable, so significant variation in some individual genotypes can be expected depending on environmental factors and the year of investigation.

Genetic and phenotypic coefficients of variation as relative indicators of variability are used to compare the level of variability of traits expressed in different units of measure. The most variable traits in cv. Kruševački 21 are the number of vegetative tillers per plant and dry matter yield per plant. Cv. Pradel has the highest genetic and phenotypic coefficients of variation noted for the number of vegetative and generative tillers per plant and dry matter yield per plant. The lowest genetic and phenotypic coefficients of variation in cv. Kruševački 21 were determined for the number of leaves per generative tiler, and at cv. Pradel the least variable trait was leaf length.

Table 1: Narrow (h^2_n) and broad (h^2_b) sense heritability, genetic ($CV_G\%$) and phenotypic ($CV_F\%$) coefficient of variation in meadow fescue synthetic cultivars

Trait	Kruševački 21				Pradel			
	h^2_n	h^2_b	$CV_F\%$	$CV_g\%$	h^2_n	h^2_b	$CV_F\%$	$CV_g\%$
Heading date	0.468	0.934	9.48	9.17	0.776	0.786	7.35	6.52
Plant height	0.535	0.869	9.21	8.59	0.749	0.833	6.92	6.32
Leaf length	0.459	0.932	16.49	15.93	0.217	0.279	7.97	4.21
Leaf width	0.489	0.965	14.57	14.32	0.269	0.475	9.77	6.74
Number of leaves per gen. tiler	0.286	0.561	4.13	3.10	0.768	0.825	11.32	10.28
Panicle length	0.439	0.944	13.46	13.08	0.764	0.925	15.81	15.20
Number of veg. tillers per plant	0.531	0.742	32.16	27.71	0.799	0.861	46.36	43.02
Number of gen. tillers per plant	0.841	0.892	15.91	15.03	0.784	0.862	33.09	30.73
DMY per plant	0.443	0.983	25.65	25.19	0.750	0.839	24.39	22.17

Session II:
Advanced phenotyping and genotyping technologies

New phenotyping, genotyping and statistical tools for forage breeding

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Various high throughput technologies and the statistical methods needed to use them are now available for forage breeding. From testing in research programmes to implementation in breeding schemes, their implementation is gradually being extended. These technologies, in addition to requiring new equipment, also ask for skills in data management and statistical analyses.

New phenotyping tools

Throughout the last 50 years of forage breeding, new phenotyping tools have been set up to characterise forage yield and various quality components. Resistance to pests and diseases and to abiotic stresses have also been studied as a component of yield and yield stability over time and conditions. The list of traits and protocols, both in field or controlled conditions, continues to grow. In the development of these tools, cost-effectiveness has been and remains a constant concern. One of the most obvious examples of the last decade has seen major developments in image capture and analysis. By using unmaned aerial vehicles or Lidar-type machinery, non-destructive images are recorded. Various cameras (RGB in visible light, multispectral, etc) provide different views of the experiments. Images can be recorded several times, giving access to the dynamics of the process, whether in terms of yield development or plant height, drought onset, etc. Several publications on forage grasses and legumes, including at this conference, have demonstrated the potential of this tool. In short, after image capture, image analysis is carried out to extract plant traits such as height, biomass, drought tolerance, soil cover, disease attack, etc. A good correlation between observed traits and the traits calculated from images is often observed, even if some side effects can alter the correlation. For example, the occurrence of lodging in lucerne field plots, which is a heterogeneous event in the experiment, reduce the accuracy of yield and height prediction.

New genotyping tools

Understanding and elucidating the genetic mechanisms that underlie the traits of interest is essential in plant breeding. In this respect, the last decade has seen the development of high-throughput, cost-effective genotyping tools. For forage species, genotyping by sequencing (GBS) has been widely adopted in research programmes. In these heterozygous and sometimes polyploidy species, GBS markers can be revealed at the individual level, giving access to allele frequency and sometimes to allele dosage, as well as at the population level for which allele frequency is obtained for pooled leaf samples. Restriction enzymes, used to limit the genome complexity, are chosen so the reading depth matches the sequencing effort and missing data is reduced. With GBS, depending on the studies, 10K to 500K were obtained. Whole genome sequencing has been used in a few number of species, generating millions of SNP. Access to the genome sequence of species makes these new tools all the more useful and accurate. Reference genome sequences have been released for most of the major forage species, offering insights on genome structure, in comparison to model species and/or major (grain) species. For some species, several genomes have been released, offering the opportunity of pangenome analyses.

The reference genome sequences are also of major interest for mapping GBS reads and extracting SNP with their position on the sequence. Comparison of experiments is thus feasible.

Thanks to these genotyping tools which produce a dense SNP coverage across the genome, genome-wide association studies (GWAS) are conducted for a number of traits in dedicated populations with a high diversity. Genome prediction has been tested and promising predictive abilities have been reported. New insights on genetic diversity have been obtained, renewing the knowledge on genetic resources and their potential use in breeding.

Statistical studies

Geneticists make intensive use of statistics. Analysis of increasing amount of data requires computing efforts for phenotyping, genotyping and analysis of genetic determinism of traits.

Interpretation of images are based on analysis of pixel position and/or colour, with the test of various options, so that traits or trait response to a stress are estimated. Field experiments, when conducted on a large number of genotypes as required in genetic studies, often show uncontrolled variation due to soil heterogeneity. In these conditions, the integration of spatial effects that account for uncontrolled variation benefit to the estimation of corrected genotype values.

In genotyping pipelines, read sequences are mapped on reference genome sequences. Series of trimming, application of thresholds and tests are required to ensure the validity of SNP detection and allele frequency.

GWAS and genomic prediction are domains of innovation where new procedures are proposed. Most of them are integrated in dedicated software or packages but the user has to make the appropriate choice of options depending on the dataset and objectives. The risk of detecting false-positive QTL or over-fitted models must be reduced as much as possible.

What next for breeding?

Studies on new genetic tools have shown promising results for forage crops. The transfer from research to breeding is a current challenge. There are three main difficulties: cost-effectiveness, equipment and skills.

Cost-effectiveness is particularly important for genotyping. A genotyping cost close to that of phenotyping cost is needed to implement genotyping in breeding programmes. In addition, for a breeding company, genotyping method must be licence-free, or outsourced to a licenced service provider. GBS is therefore not appropriate. Genotyping methods based on sequencing of captured fragments or amplicons could be considered but the number of SNP are not as high as that of GBS. Whole genome sequencing does not require a licence but remains expensive.

New equipment is also needed: drone or vehicle, camera, computer, memory, computing capacity to process phenotype and genotype data. In addition, the staff must acquire additional skills and change the way they organise their work.

In the coming years, proof of concept will be required to test the effectiveness of breeding schemes that use all these new tools. This activity is included in the BELIS project (Breeding European Legumes for Increased Sustainability) supported by an Innovation-Action programme of Horizon Europe, which will be launched in October 2023 for five years.

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A nested association mapping population reveals candidate genes for stem rust resistance in Italian ryegrass

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Italian ryegrass (*Lolium multiflorum* Lam.) is valued for its high biomass yield and good forage quality and is, therefore, one of the most important forage grasses in Europe. Although Italian ryegrass was subject to intensive selection activities in different breeding programs during the last decades, stem rust disease, caused by the obligate biotroph pathogen *Puccinia graminis* subsp. *graminicola*, is still an unresolved issue. Recently, increasing daily average temperatures and decreasing precipitation due to climate change have further increased disease pressure. Stem rust infects leaf sheaths and inflorescences and consequently reduces seed yield, resulting in a severe problem in seed multiplication. Although the disease can be controlled by fungicides to a certain degree, a more sustainable approach is to breed for stem rust resistant cultivars. For this, knowledge on the inheritance of resistance is a prerequisite. We developed, for the first time, a nested association mapping (NAM) population for Italian ryegrass with the aim to identify potential genes conferring resistance to stem rust and to unravel the genetic architecture of this trait. The NAM population consisted of 728 F_2 individuals, derived from crossing 23 diverse founders with one common founder, followed by open pollination among the F_1 progeny. For genotyping, restriction site-associated DNA sequencing resulted in 4,762,767 single-nucleotide-polymorphism (SNP) markers at affordable costs. Stem rust resistance of the NAM plants was assessed in three field experiments (each with two replicates) at seed-harvesting time using a scale from 1 (no stem rust symptoms) to 9 (very heavy stem rust infection). A mixed linear model approach detected three highly significant loci associated with stem rust resistance, one located on chromosome 6 and two on chromosome 7. On chromosome 7, a SNP significantly associated with resistance caused a missense mutation within a gene with a predicted receptor like serine/threonine protein kinase function. This protein kinases family is known in other crops to be involved in resistance to fungal diseases. The missense mutation explained approximatively 6.34% of the phenotypic variation within the NAM population and had an effect size of $\beta = -0.97$. In general, all the SNPs highly associated

with stem rust resistance can be directly used for marker-assisted selection in our breeding program. Further, our study shows that a NAM population in Italian ryegrass is effective to find candidate genes for stem rust, which can be further used for functional validation.

Red clover's genetic playground: Leveraging low-coverage resequencing for enhanced breeding programs

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Red clover (*Trifolium pratense* L.) is a highly valued forage legume renowned for its exceptional fodder quality and ecological advantages. However, breeding red clover poses challenges due to its inherent heterozygosity resulting from cross-pollination. The breeding ideotype objectives aim to optimize yield and fodder parameters while incorporating important traits such as phytoestrogen content, adjusting polyphenol oxidase (PPO) activity, nitrogen fixation efficiency and resistance to biotic and abiotic stresses.

1To overcome these challenges and improve breeding efficiency, low-coverage resequencing (LCR) genotyping methods have emerged as a promising solution, particularly suitable for species with small genome sizes like red clover (approximately 420 megabase pairs). In our study, we resequenced a total of 470 red clover plants, which underwent phenotypic testing on clonally propagated individuals. The integration of whole-genome genotyping into breeding programs enables the identification of genetic markers associated with desired traits. An advantage of resequencing is its ability to identify alleles in candidate genes. However, resequencing requires computationally intensive and complex bioinformatic processing, as well as the availability of a high-quality reference sequence. Moreover, the use of short reads presents challenges in accurately distinguishing haplotypes.

We utilized LCR genotyping as a promising approach for red clover breeding. LCR genotyping involves resequencing the entire genome at relatively low coverage, typically around 5x for diploid and 10x for tetraploid plants. The high-density genotype information obtained facilitates the identification of genetic variations linked to specific traits of interest through genome-wide association studies (GWAS), as well as the prediction of breeding values using a genomic prediction model. By incorporating these results into marker-assisted selection and genomic selection, breeders can harness the data derived from resequencing to enhance the efficiency and precision of red clover breeding programs. This approach enables the identification and utilization of genetic markers associated with desired traits, enabling the selection of superior genotypes exhibiting improved yield, fodder quality, stress tolerance, and other important traits.

The application of low-coverage genotyping through resequencing offers an effective and informative approach for red clover breeding. By employing this method, breeders can expedite the development of improved red clover varieties with enhanced traits, thereby contributing to the sustainability and productivity of red clover cultivation in diverse agricultural and environmental systems. The integration of LCR genotyping into breeding programs opens up opportunities for significant advancements in red clover breeding, benefiting both farmers and the environment.

Implementation of multiple cycles of genome-wide selection for seasonal forage yield in perennial ryegrass.

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In production systems where animals are at pasture for up to 300 days per year it is important that forage yield meets feed demand throughout the year. The economic value of forage in spring, summer, and autumn in an Irish dairy system has already been established and can be utilised to determine the annual value of forage weighted by season. In this study we have evaluated genomic selection (GS) as a means of improving gains for the annual value of forage yield. A small population of tetraploid half-sib families were evaluated for forage yield in both simulated grazing and conservation management regimes over two years, and their maternal parents were genotyped. Predictive ability of GS models varied between 0.02 for summer forage yield and 0.27 for spring forage yield, with the annual value of forage yield having a predictive ability of 0.23. We carried out an initial cycle of among-family and within-family selection using Genomic Estimated Breeding Values (GEBVs) for the annual value of forage yield. This was followed by a further two cycles of GS within the new synthetic populations produced from the previous rounds of selection. The synthetics produced after each round of selection were established in a field trial alongside the starting population and seasonal forage yield was evaluated over two years. Greatest emphasis was placed on spring forage yield during selection and we observed an upwards trend in spring yield, with a significant difference between new synthetics after the second cycle of GS and the starting population.

Phenotyping our future crops: in-field, non-destructive, high-throughput phenotyping of above and belowground plant traits

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Continued improvements in crop performance are needed to cope with climate change and to keep pace with population growth. To tackle these global challenges, novel approaches are required that help to investigate the genetics of agriculturally important crop traits and to accelerate the development of superior plant varieties.

Plant phenotyping covers the description and characterization of complex plant traits using non-destructive tools in the context of crop research and breeding. As part of the Emphasis-Belgium (funded by FWO) and the FutureAdapt project (funded by Government of Flanders), a state-of-the-art High-Throughput Field Phenotyping (HTFP) facility has been constructed.

The facility is located in Melle, Belgium (50.99281°N, 3.78602°E) on loamy sand soil and enables investigating the response of crops to drought and salinity stress using mobile rain-out-shelters. Each of the six shelters consists of three subblocks (300 m^2 each), enabling a three-year crop rotation (Figure 1).

Aboveground phenotyping is based on unmanned aerial vehicles (UAVs) equipped with RGB, multispectral and thermal sensors (e.g., De Swaef et al. 2021), belowground phenotyping is performed based on electrical resistivity tomography (ERT) (e.g., Blanchy et al. 2020). Both provide high spatial resolution images of 0.2–4 cm and 10 cm, respectively. Monitoring the crop frequently throughout the entire growing season reveals plant responses to the stress applied, as well as their recovery in a realistic field environment. A control field with an identical layout, under rainfed conditions serves as reference. In a calibration pit, soil moisture, soil temperature and electrical resistivity sensors continuously monitor the water status in each soil horizon, with the aim of establishing robust field-specific pedophysical relationships. Finally, an on-site weather station is present to measure climatic conditions.

Growth curves per plant/genotype/plot (e.g., Borra-Serrano et al. 2020) are fitted to decipher plant responses and to extract the drought related traits, in

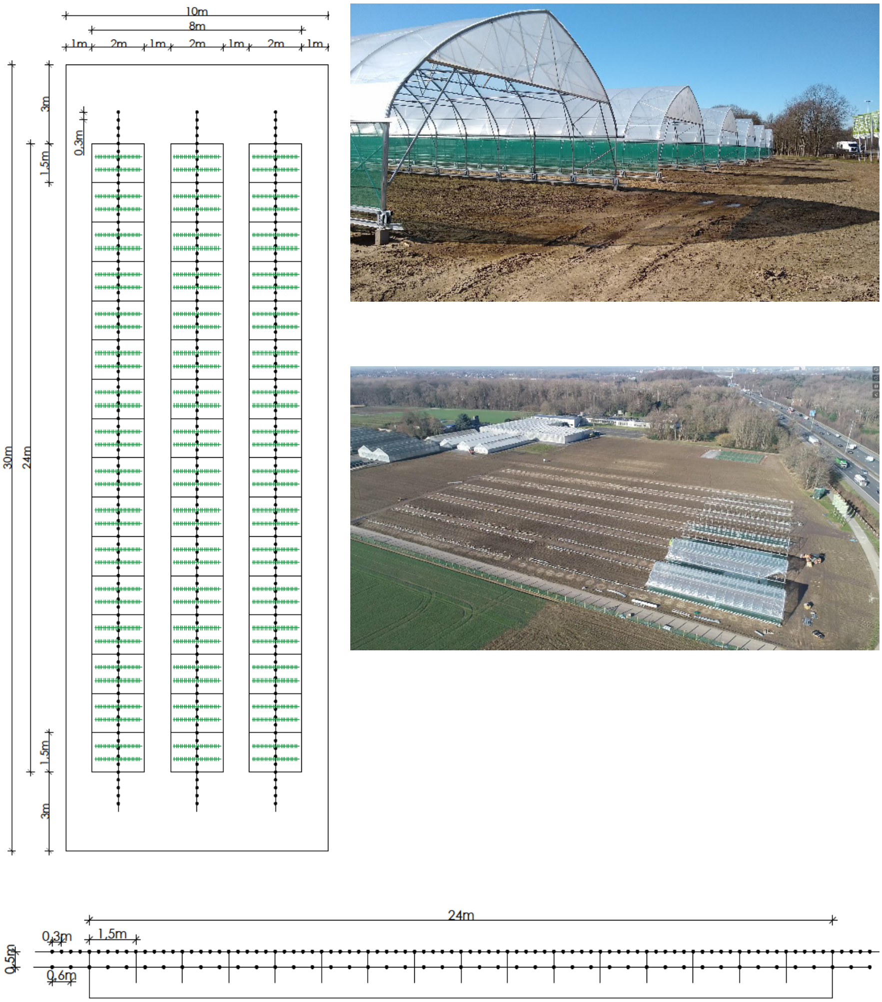

Figure 1: Top and side view of one subblock, showing the surface and buried electrical resistivity cables, with respective spacing and location, and areal overview of the infrastructure (February 2023).

a high-throughput manner. At maximum capacity 800 mini-plots can be evaluated simultaneously (e.g., Saleem et al. 2022).

This facility will be accessible for universities, research institutes and industries. In spring 2023 a proof-of-concept experiment with contrasting varieties of soybean is set up for testing and calibrating the systems. The facility will be operational and accessible for collaborations, from autumn 2023 onwards.

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Use of NIR spectra to predict heading date in perennial ryegrass

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Genomic selection – the prediction of breeding values using DNA polymorphisms has become a transformative technique that is being embraced by both animal and plant breeders. Additional studies showed that other sources of molecular variation, such as transcriptomics and metabolomics, could be also used to predict complex traits.

Information captured with different omics approaches allows to acquire endophenotypic variations, which occur due to expression of genotype and the action of regulatory networks. Both genotyping and the omics approaches remain expensive for routine operations of breeding programs. However, it has been proposed that NIRS (near-infrared reflectance spectroscopy) could be used to capture the endophenotypes. The approach was named phenomic selection (PS), and comes with a number of advantages, as acquisition of spectra can be very economical, high- throughput upon automation, and non-invasive.

Plant material originates from a field trial on perennial ryegrass (Lolium perenne L.). The spaced plants field trial consisted of 30 diploid perennial ryegrass populations comprising of 60 genotypes each (1800 genotypes in total). Plants were grown in two replicates in a partially balanced incomplete block design, under two managements: frequent grazing (8 cuts per year) and conservation (4 cuts per year). Herbage sampling took place over 3 grazing periods (2015–2017). Heading date (HD) was recorded for all genotypes and plants were genotyped-by- sequencing. Principal component analysis (PCA) and t-SNE (t-distributed stochastic neighbor embedding) were used to compute similarity between spaced plants based on unprocessed NIR spectra from separate cuts. t-SNE or PCA results for each cut were used separately to build relationship matrices, or combined (all possible combinations of two, three, four cuts, as well as a matrix consisting of all five cuts data available). Calculation of the H matrix and H-BLUP model were then trained, and compared with GBLUP prediction using a genomic relationship matrix. Predictive accuracy (PA) was calculated as Pearson's correlation between observed and predicted phenotype using 5-k fold cross-validation.

Our results show that when all available NIRS measurements were used to build the H matrix, phenomic data could be successfully used in prediction of heading date in perennial ryegrass, with mean predictive accuracy of 0.77 as compared to prediction level of 0.81 obtained with molecular data.

Acknowledgments
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Exploring the genetic diversity of the IPK *Medicago* germplasm collection using GBS

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The Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) has been conserving *Medicago* genetic resources for several decades. Currently, they comprise 1,236 accessions from different parts of the world from more than 41 botanical species. Of these, 491 accessions are preserved as ecotypes or wild material (*e.g. M. polymorpha*, *M. falcata* and *M. truncatula*), while 745 accessions are varieties or breeding lines (*M. sativa* and *M.* × *varia*).

Medicago sativa L., commonly known as alfalfa or lucerne, is a perennial forage legume that has been cultivated globally for centuries. It is often referred to as the 'Queen of forages' due to its wide adaptability to various environments, high yield, and high fodder quality. The *Medicago* germplasm collection represents a valuable tool for population and evolutionary genetic studies and provides plant breeders with a critical resource of diverse genetic material to improve crop yield, quality, and adaptation to environmental stresses.

Genotyping-by-sequencing (GBS) with the *Pst* I/*Mse* I as restriction enzyme combination was used to explore the plant genetic diversity of the entire *Medicago* collection of the IPK on a genome wide scale. The GBS data were de-novo assembled resulting in approximately 10,000 loci and more than 122,000 SNPs. These SNPs were utilised to assess the genetic diversity within our *Medicago* germplasm, both on the intra- and on the interspecific level.

Our findings provide valuable information for future breeding programs, e.g. for the selection of genetically distant accessions. By using genome-wide association studies (GWAS) or marker-assisted selection (MAS), these could be used for the development of agronomically improved *Medicago* varieties regarding traits like yield, resistances or nutrient efficiency.

Acknowledgements
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Development and proof-of-concept application of genome-enabled selection for alfalfa biomass yield in Northern Italy: preliminary results

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In a previous study, alfalfa genomic selection (GS) for a Northern Italy genetic base displayed a predictive ability of 0.32 along with a predicted advantage over phenotypic selection (PS) (Annicchiarico et al. 2015), based on a training set of 124 parents undergoing genotyping-by-sequencing (GBS) and phenotyped according to 3-year biomass yield under limited supplemental irrigation of their half-sib progenies. This study provides a comparison of GS *vs* PS for the same genetic base in terms of actual genetic gain for biomass yield, envisaging three possible GS models, namely, rrBLUP and linear Support Vector Regression (SVRlin), which emerged as best statistical models in the earlier study, and rrBLUP restricted to 150 markers that displayed odd score > 1.5 in a GWAS. GS selected 18 parent genotypes out of 1536 belonging to the elite, widely-adapted landrace MSP017 from Northern Italy. Because of budget constraints, GS relied on GBS using half of the genotyping effort applied to the original training set, thereby reducing the available markers (from over 11000 to 6780) and the predictive ability of the actually adopted GS models. While rrBLUP and SVRlin issued correlated breeding values ($r = 0.89$) and shared 11 of the 18 selected parents, neither of them shared any selected parent with rrBLUP limited to GWAS-selected SNPs. PS implied the selection under limited supplemental irrigation of 18 parent genotypes out of 1776 plants of MSP017, using two stages of multi-year selection for biomass yield. The Syn-2 generation of the four synthetic varieties issued by GS or PS, the unselected landrace MSP017, and the recent reference cultivar Alfitalia were evaluated for two-year dry biomass yield in two growing environments represented by a rainfed (with 237 mm and 171 mm April-September rainfall in 2021 and 2022, respectively) and an irrigated (with additional 320 mm during the summer period of each year) water regime. We adopted a randomized complete block design with six replicates per environment, using 4.5 m^2 plots. Rainfed cropping halved the number of harvests relative to irrigated cropping (3 *vs* 6). However, genotype × environment interaction was not significant ($P > 0.10$), in agreement with the wide-adaptation target of producing phenotyping data for GS and performing PS under limited supplemental irrigation. GS based

Table 1: Two-year dry biomass yield across two water regimes of alfalfa experimental populations derived from three genomic selection (GS) models or phenotypic selection (PS), the reference landrace MSP017 (acting as genetic base), and the reference cultivar Alfitalia.

Material	Biomass yield (t/ha)
GS based on the rrBLUP model	5.72 a
GS based on SVRlin model	5.43 ab
Alfitalia	4.99 bc
Landrace MSP017 (genetic base)	4.97 bc
GS based on rrBLUP model using 150 SNPs from GWAS	4.97 bc
PS	4.47 c

Column means followed by different letters differ at $P < 0.05$ according to Duncan's test

on rrBLUP displayed a manifest yield advantage (>14.5%) over the unselected landrace, the elite cultivar Alfitalia, and GS based on rrBLUP limited to GWAS-selected SNPs (Table 1). GS based on SVRlin was somewhat less efficient but not significantly inferior to that based on rrBLUP. PS failed to improve biomass yield relative to the genetic base, despite its considerable investment in time and resources (Table 1).

Acknowledgement

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Haplotype-phased genomes of timothy grasses – *P. nodosum, P. alpinum P. pratense*

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Timothy (*Phleum pratense* L.) is a widely adapted and globally important cool season perennial forage grass. Timothy is hexaploid (2n = 6x = 42) and likely originated through natural hybridization between the diploid *P. nodosum* (2n = 2x = 14) and the tetraploid *P. alpinum* (2n = 4x = 28) species. In the efforts to dissect and understand the genetics and the origin of timothy and its progenitors, the PKI Lab at SLU Uppsala (https://pkilab.org) has sequenced the genomes of the three species using a combination of PacBio HiFi long-read system (DNA and isoform sequencing – Iso-Seq), DNase Hi-C data (Dovetail OmniC) and Illumina short-read RNA sequencing. The chromosome-scale haplotype-resolved *de novo* genomes of *P. nodosum* (82x coverage) and *P. alpinum* (184x coverage) have already been constructed using HiFi reads and proximity ligation data, whereas the *de novo* assembly of the timothy (95x coverage) is currently in progress. Over 93% of the *de novo* assembled sequences could be anchored to 7 chromosomes for *P. nodosum* (assembly size – 1.3 Gb), and to 14 chromosomes for *P. alpinum* (assembly size – 2.5 Gb across two subgenomes). The BUSCO scores showed that over 96%, 98% and 98% of *Poales*-conserved orthologs (n = 4,896) were fully captured by the *P. nodosum*, *P. alpinum* and *P. pratense* draft *de novo* assemblies, respectively. Around 75% of the anchored genomes of *P. nodosum* (970.4 Mb) and *P. alpinum* (1.87 Gb) were shown to be repetitive and the *Gypsy* family of long-terminal repeat retroelements were the most abundant repeats, accounting for ~ 35% of all repeat sequences in both progenitor species. Around 49,800 *P. nodosum* and 111,480 *P. alpinum* protein-coding genes models, supported by transcriptome datasets and proteins sequences from closely related grass species, were predicted. Of the 111,481 predicted genes in *P. alpinum*, 51% (56,861) were derived from the A and 49% (54,619) from B sub-genome. The transcriptome profiles of the two species were constructed using PacBio isoform sequencing (Iso-Seq) generated from spike, leaf and root tissues. After clustering Iso-Seq reads into high-quality non-redundant full-length unique transcripts, 235,652 *P. nodosum* and 245,720 *P. alpinum* high-confidence transcript models were predicted. The phased *de novo* assembly and annotation of the hexaploid timothy *P. pratense* will soon be finalized. The haplotype-phased reference genomes and high-quality transcriptomes of the three *Phleum* spp. will be valuable resources for evolutionary and conservation genetics research of the timothy grasses, and for developing novel genomic resources and tools for timothy breeding.

Conventional and UAV-based phenotyping to characterize a broad collection of European lucerne germplasm in a Nordic environment

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Lucerne may become an attractive crop for Nordic growing conditions as a consequence of the changing climate. This study assessed the variation for adaptation to such conditions (featuring large photoperiod variation, long winters, and short intensive growing seasons) within a genetically broad European semi-dormant genetic base using conventional and aerial phenotyping for trait characterization.

A field experiment was established in 2022 at Vollebekk, Ås, Norway, to evaluate a collection of 135 half-sib progenies of parents randomly sorted out of the European elite population described in Annicchiarico et al. (2016). All mother plants have been genotyped via genotyping-by-sequencing (GBS). Five Norwegian varieties were included in the trial for comparison. Seeds were sown in the greenhouse and six-week-old plants were transplanted to the field in June as semi-dense stands consisting of 25 plants per observation plot (~0.5 m^2) placed in three replicate blocks. We recorded flowering time, when at least three plants showed at least one open flower, in Julian calendar days starting from July 1st; dry matter yield (only one harvest, taken in August); and winter survival (plant were scored as live or dead, and the survival rate was calculated). Genetic parameters were estimated using DeltaGen (Jahufer & Luo, 2018) half-sib model. UAV (unmanned aerial vehicle) imaging with RGB (red, green and blue) and multispectral cameras were conducted at the same time as the observations of all traits.

Considerable phenotypic variation was observed in dry matter yield, flowering time and winter survival, which showed narrow-sense heritability (h^2) of 0.57, 0.81 and 0.61, respectively. The observed phenotypic means for dry matter yield ranged from 80 to 179 g/plot in European half-sib progenies versus from 88 to 117 g/plot in Norwegian varieties. The phenotypic means for flowering time ranged from 4.6 to 16.0 days in European half-sib progenies versus 9.0–11.3 days in Norwegian varieties. For winter survival rate, the means ranged from 63 to

100% in European half-sib progenies while Norwegian varieties ranged from 93 to 100%. A genetic correlation of 0.24 were found between winter survival and flowering time.

Several VIs (vegetation indices) that capture different plant characteristics will be obtained from the aerial imaging. These extracted features will be used as input data for ML (machine learning) algorithms (Shafiee et al. 2021). Models will be tested on the manually recorded traits to estimate the best hyperparameters using grid search for trait prediction. Image-predicted traits will be used to further investigate genetic architecture of the traits. Based on the GBS data, single nucleotide (SNP) variants will be called to produce either full dosage tetraploid SNPs or its approximation as provided by allele frequencies, and the two representations will be compared for their effect on genomic regression models predicting all considered traits.

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Unleashing the potential of drones: how high-throughput field phenotyping can assist forage grass breeding

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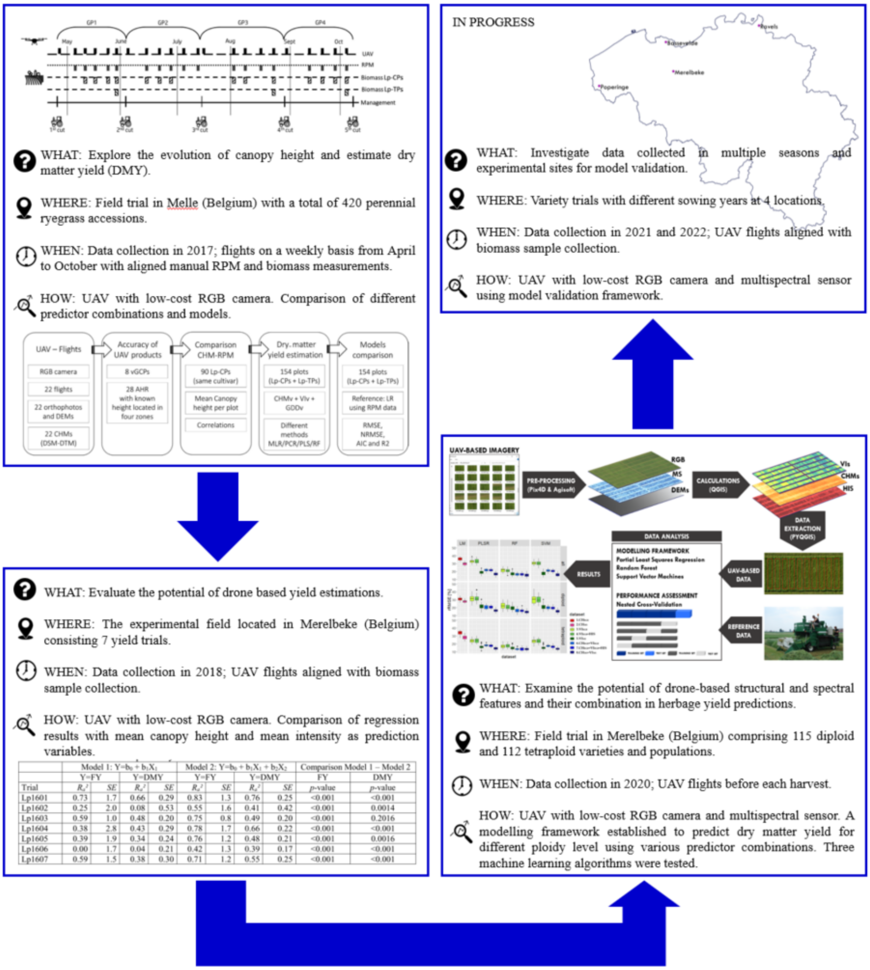
Forage grasses are a vital component of livestock diets, important for their grazing, as well as the production of hay and silage. Forage grasses offer a source of high-quality nutrition and are widely cultivated in a variety of environments across the world, including temperate regions of Europe. Breeding programmes for forage grasses are crucial for improving their yield and quality, while also enhancing resistance to stress conditions such as drought, frost, disease, etc. Plant phenotypic parameters are of special interest to breeders. However, their assessment remains a significant challenge as a large number of plots need to be monitored. To address this challenge, high-throughput field phenotyping (HTFP) methods are being developed. These technologies enable rapid and non-destructive evaluation of plant traits. This way, breeders can boost the efficiency and effectiveness of the breeding process.

With the advancement of HTFP technologies, a variety of novel sensors, airborne platforms and algorithms have been employed to gather information on phenotypic parameters. Among research conducted within this domain, ILVO (Belgium) aimed at creating HTFP protocols and procedures for perennial ryegrass breeding programme. It all started in 2017 when an unmanned aerial vehicle (UAV) with a low-cost RGB camera was used to perform weekly flights between April and October. The main objective of this study [1] was to examine the capacity of UAV imagery to determine canopy height (CH) and to explore how it changes over space and time. For this purpose, multiple ground control points and artificial height references were placed across the field trial and data from rising plate meters (RPM) was collected. With this approach, we were able to accurately estimate the canopy height (RMSE of 0.94 cm). All



WHAT: Explore the evolution of canopy height and estimate dry matter yield (DMY).

WHERE: Field trial in Melle (Belgium) with a total of 420 perennial ryegrass accessions.

WHEN: Data collection in 2017; flights on a weekly basis from April to October with aligned manual RPM and biomass measurements.

HOW: UAV with low-cost RGB camera. Comparison of different predictor combinations and models.

WHAT: Evaluate the potential of drone based yield estimations.

WHERE: The experimental field located in Merelbeke (Belgium) consisting 7 yield trials.

WHEN: Data collection in 2018; UAV flights aligned with biomass sample collection.

HOW: UAV with low-cost RGB camera. Comparison of regression results with mean canopy height and mean intensity as prediction variables.

	Model 1: $Y=b_0+b_1X_1$				Model 2: $Y=b_0+b_1X_1+b_2X_2$				Comparison Model 1 – Model 2	
	Y=FY		Y=DMY		Y=FY		Y=DMY		FY	DMY
Trial	R_a^2	*SE*	R_a^2	*SE*	R_a^2	*SE*	R_a^2	*SE*	*p*-value	*p*-value
Lp1601	0.73	1.7	0.66	0.29	0.83	1.3	0.76	0.25	<0.001	<0.001
Lp1602	0.25	2.0	0.08	0.53	0.55	1.6	0.41	0.42	<0.001	0.0014
Lp1603	0.59	1.0	0.48	0.20	0.75	0.8	0.49	0.20	<0.001	0.2016
Lp1604	0.38	2.8	0.43	0.29	0.78	1.7	0.66	0.22	<0.001	<0.001
Lp1605	0.39	1.9	0.34	0.24	0.76	1.2	0.48	0.21	<0.001	0.0016
Lp1606	0.00	1.7	0.04	0.21	0.42	1.3	0.39	0.17	<0.001	<0.001
Lp1607	0.59	1.5	0.38	0.30	0.71	1.2	0.55	0.25	<0.001	<0.001

WHAT: Examine the potential of drone-based structural and spectral features and their combination in herbage yield predictions.

WHERE: Field trial in Merelbeke (Belgium) comprising 115 diploid and 112 tetraploid varieties and populations.

WHEN: Data collection in 2020; UAV flights before each harvest.

HOW: UAV with low-cost RGB camera and multispectral sensor. A modelling framework established to predict dry matter yield for different ploidy level using various predictor combinations. Three machine learning algorithms were tested.

IN PROGRESS

WHAT: Investigate data collected in multiple seasons and experimental sites for model validation.

WHERE: Variety trials with different sowing years at 4 locations.

WHEN: Data collection in 2021 and 2022; UAV flights aligned with biomass sample collection.

HOW: UAV with low-cost RGB camera and multispectral sensor using model validation framework.

Figure 1: Scheme summarizing the research of HTFP procedures for perennial ryegrass yield estimation for breeding at ILVO (Belgium).

models built to predict dry matter yield (DMY) performed better (lower RMSE) when UAV-based CH data was applied rather than the RPM data. In the follow-up study [2], the mean intensity (MI) parameter, treated as a proxy for bending leaves, was combined with CH information. The integration of MI resulted in improved yield estimations.

In the most recent work [3], we explored if the incorporation of multispectral (MS) imagery and machine learning techniques can further optimize the HTFP procedures. Findings indicated that combining structural and spectral features improved the yield prediction accuracy (with the lowest rRMSE being 13.1%). On average, MS-based predictors worked better than RGB ones. Also, random forest outperformed the other tested algorithms. The ultimate goal in yield predictions is to guarantee that established procedures are generally applicable. Thereby, new multi-seasonal data acquired in 2021 and 2022 from a few field trials will be used to validate and increase the robustness of developed models. The findings of these studies demonstrate the potential of drones for field phenotyping in breeding programmes of forage grasses.

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Rapid forage yield and growth rate measurement using a remote-controlled LIDAR sensor in perennial ryegrass field plots

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Improving dry matter yield (DMY) is a primary goal in pasture plant breeding. However, accurate and reliable measurement of this trait is costly and/or laborious for breeding companies, and current methods cannot measure growth rate accurately on frequent intervals. A rapid, remotely controlled digital system using LIDAR (light detection and ranging) in ambient light conditions was developed to measure biomass and DMY in real time at a ground speed of 2.7 km/hr. The system was tested on three sites in New Zealand during spring 2019 using perennial ryegrass monoculture plots in plant breeding and cultivar evaluation trials. Fresh weight and dry matter yield data were collected and compared with LIDAR scan outputs at three sites. High correlation between LIDAR and FW data were detected with $R^2 = 0.82$, 0.81 and 0.75 for sites 1, 2, and 3. Similarly, high correlation between LIDAR and DMY were detected with $R^2 = 0.80$ and 0.83 at sites 1 and 2, respectively. The system also provided data for growth rate measurement, enabling breeders to put selection pressure on a key determinant of DMY over time. While utilisation of such systems for farm management is recommended in cases where non-destructive, accurate and precise real-time pasture DMY and growth rate measurement is required we will review a few challenges that need attention as well.

Youth Session

Exploiting new discoveries on self-incompatibility for forage grass breeding

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Self-incompatibility (SI), a mechanism preventing self-pollination, has been intensively studied over the past 60 years. Many different SI systems exist among the angiosperms, with a great variety in their physiological mechanisms and their genetic controls. Only recently, we have identified the genes responsible for the recognition of self-pollen by the pistil in perennial ryegrass (*Lolium perenne*) (Rohner *et al*, 2023). This unique SI system in grass is governed by two multi-allelic loci, *S* and *Z*, each containing three genes. For each locus, two genes, annotated as containing a domain of unknown function (DUF) 247, are coding for small transmembrane proteins expressed in the pollen (*LpSDUF247-I* and *LpSDUF247-II* at the locus, *LpZDUF247-I* and *LpZDUF247-II* at the z-locus). The third gene is expressed in the female tissue and is coding for a small protein, predicted to be secreted (*LpsS* and *LpsZ*).

The discovery of the genetic control of SI in grasses is also enabling new possibilities for practical breeding. Using targeted sequencing technologies, we can now predict cross-compatibility and test these predictions using semi *in-vivo* pollination. This validation of the alleles via a large phenotyping assay of different cross-/self-pollinations will help to identify the polymorphisms determining allele specificity. Markers designed to target these polymorphisms will not only enable breeders to predict the compatibility of their crosses but also to assess the allelic diversity of SI genes in their breeding material. Indeed, when designing population breeding schemes, breeders may face cross-compatibility problems leading to unbalanced allele segregation in their populations. This potential bias of allele segregation can now be avoided by characterizing the SI alleles present in the parents of polycrosses. Knowing the SI allelic composition of breeding germplasm could therefore not only lead to optimized polycross design but also bring grass breeding closer to F1 and population-based hybrid breeding.

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Establishing multi-trait genomic selection for forage improvement

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Genomic selection (GS) has the potential to accelerate genetic gain for multiple traits in perennial ryegrass breeding programs. This first requires the development of accurate genomic prediction models for all traits enabling the calculation of Genomic Estimated Breeding Values (GEBVs) on selection candidates during routine breeding. Accuracy of GS models depends on trait heritability, training population size, relatedness between training and selection population, statistical models used, and marker density (De Los Campos et al. 2015).

We have been developing an experimental population in perennial ryegrass to empirically evaluate multi-trait genomic selection. Our starting germplasm consisted of 60 genotypes from each of 30 diploid families/cultivars/ecotypes (1800 genotypes in total). This parental population was established in a spaced plant field trial under two managements (frequent grazing and conservation) and data collected for heading date (HD), crown rust resistance (CR), Organic Matter Digestibility (OMD), Neutral Detergent Fiber (NDF), and Crude protein (CP). Parental plants were cross pollinated to produce F1 and subsequently F2 families ($n = 266$), and these were evaluated for seasonal forage yield in simulated grazing and conservation management. An SNP database was established using genotyping-by-sequencing (GBS) data from the population. Using these data we were able to build statistical models to predict all traits using genome-wide marker data.

Our results showed that the predictive ability of models was encouraging for all traits currently evaluated in national list trials. Unsurprisingly, predictive ability depended on a close relationship between selection candidates and training material; however, there are clear opportunities to accelerate genetic gain in forage breeding using GS for multi-trait among-and-within-family selection.

Acknowledgments
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Evaluation of silage and grain yield of different maize (*Zea mays* L.) genotypes in ecological and conventional conditions

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The world's population has grown rapidly in recent decades. As a result, global demand for agricultural products is rising. However, the intensification of agriculture is closely linked to high emissions of greenhouse gases. Keeping up with these climate changes in agriculture, while protecting the planet, has become a major issue in recent years. To address these challenges, the European Commission published the European Green Deal in 2019. The main goal of the program is to achieve net zero greenhouse gas emissions by 2050, making us the world's first climate-neutral continent. In order to achieve all of this, multi-step regulations covering different sectors are necessary. Criteria in agriculture are also defined: increase the share of organically farmed land to 25%, reduce the use of fertilisers and pesticides.

The benefits of organic farming are widely debated. In one hand, some promote it as a solution to sustainable food security challenges. According to these views, organic agriculture is a production system that maintains the health of the ecosystem and people. It is based on the processes and cycles of biodiversity adapted to local conditions. On the other hand, others criticise it for being underdeveloped. Traditional agriculture uses a diverse set of technologies and the best available knowledge, with the ultimate aim of providing an abundant food supply at the lowest cost. Moreover, several studies have confirmed that organic farming yields are on average 20% lower than conventional farming.

The aim of our study was to compare the yield of maize (*Zea mays* L.) genotypes bred in Martonvásár in three different cropping environments. The silage and grain yields of different maize hybrids and parental lines were evaluated in a three replicate small plot experiment in an organic field and an adjacent conventional field (irrigated and non-irrigated).

The average green mass yield of the hybrids in the organic agriculture was more than 20% lower compared to the traditional cultivation. We have established a bigger difference in the grain yield between the cropping systems. The results of the inbreed lines were similar with smaller yield gap.

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Optimized tetraploidization strategies in tissue culture for *Lolium* and *Festuca*

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Festulolium, an intergeneric cross between *Lolium* and *Festuca*, combines fodder grass qualities like digestibility from *Lolium* with resilience to abiotic stress from *Festuca*. Despite the promising combination, *Festulolium* hybrids suffer from genomic instability, leading to reduced seed production (BAERT et al., 2020). Here, we describe our approach to generate diploids and tetraploids with identical genomic constitution, creating a well-defined framework to study the effect of polyploidization on genome elimination and genome dominance and thus better understand the genomic instabilities of *Festulolium*.

To obtain *Festulolium* hybrids at the tetraploid level, four selected parents of *Lolium multiflorum* (*Lm*), *Lolium perenne* (*Lp*) and *Festuca pratensis* (*Fp*) were mitotically polyploidized during clonal multiplication. This preserves the genomic constitution of the diploid, in contrast to the traditional polyploidization methods applying antimitotic treatment on seeds.

Based on the initial evaluation of antimitotic agents oryzalin, trifluraline or colchicine at three different concentrations (1 µM, 3 µM, 10 µM oryzalin or trifluraline; 10 µM, 30 µM, 100 µM colchicine), colchicine was chosen as antimitotic agent in subsequent genome doubling experiments. The first set-up, where the antimitotic agent was added to the falcon with the isolated meristems that were later planted in potting soil, only resulted in mixoploids. Next, three methods were applied on isolated meristems *in vitro*: i) multiplication on media supplemented with two different concentrations of colchicine (30 µM, 100 µM) during at least 4 weeks; ii) a short shock treatment (24h) in liquid medium with three different doses of colchicine (0.5 mM, 1 mM, 2 mM); iii) combination of both former methods, with the shock treatment (24h in 2 mM colchicine) followed by multiplication on media supplemented with colchicine. Grown plantlets were evaluated by flow cytometry to confirm their polyploid status. Inspecting the number of obtained tetraploids revealed a positive effect of the combination of both techniques (method iii) (Table 1). Mortality in the meristems and numbers of mixo- and tetraploids found further indicate genotype-specific responses. These findings are in line with observations during regular tissue culture propagation, where growth during different conditions vary based on the plant genotype. Finally, the obtained tetraploid plants will be used in future hybridizations to create tetraploid *Festulolium* hybrids. Additionally, the same procedure of tetraploidization will be applied on diploid hybrids obtained from the *Lp x Fp* and *Lm x Fp* crosses at the diploid level. Comparing these distinct *Festulolium* tetraploids allows to investigate the effect of polyploidy on genomic constitution and to study the processes driving chromosome elimination and genome dominance in the first few generations of interspecific hybrids, in function of ploidy and the genetic distance between the subgenomes.

Table 1: Overview of the three in vitro polyploidization methods evaluated in this study. Meristems cultured on media supplemented with colchicine (i) or undergoing a shock treatment for 24h with antimitotic agents prior to culture on medium (ii) only resulted in mixoploids, while the combination of treatments (iii) generated tetraploids confirmed by flow cytometry. 'Plantlets' represent the number of growing meristems that survived the treatments, while 'confirmed 4x' represent the number of tetraploids confirmed by flow cytometry. Table 1 summarizes experiments on different genotypes of Fp (2), Lp (4) and Lm (3).

shock	medium	# meristem	# plantlets	mixo	confirmed 4x	% plantles	% mixo	% 4x
i. Media supplemented with colchicine								
/	0 µM	326	102	7	0	31	7	0
/	30 µM	249	190	10	0	76	5	0
/	100 µM	133	91	14	5	68	15	5
ii. Shock treatment in liquid culture								
2 mM	0 µM	30	9	5	0	30	56	0
1 mM	0 µM	30	19	15	0	63	79	0
0,5 mM	0 µM	30	10	3	0	33	30	0
0 mM	0 µM	15	10	0	0	67	0	0
iii. Shock treatment + media with colchicine								
2 mM	0 µM	118	60	5	4	51	8	7
2 mM	30 µM	144	87	14	12	60	16	14
2 mM	100 µM	162	12	3	1	7	25	8

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Optimized *Lolium perenne* L. protoplasts isolation and transformation for CRISPR-Cas9 downstream applications.

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The project "Improving adaptability and resilience of perennial ryegrass for safe and sustainable food systems through CRISPR-Cas9 technology – EditGrass4Food" aims to improve adaptability and resilience of perennial ryegrass (*Lolium perenne* L.) for safe and sustainable food systems through gene editing. The three Baltic countries and Norway are members of the consortium that is coordinated by the University of Latvia.

In Europe, *Lolium perenne* L. is the most widely distributed and grown forage grass used to feed livestock. However, this species does not grow well under freezing or drought conditions, which implies a problem for expanding its cultivation towards northern-eastern European regions. Genome editing with CRISPR-Cas9 can help obtain resilient genotypes and identify mechanisms underlying stress tolerance. Since perennial ryegrass is not a model organism, there is a lack of available experimental data regarding standardized transformation and regeneration procedures. Moreover, *Lolium perenne* L. is a highly heterogenous species, which creates an additional challenge when performing genetic studies. For example, forward-genetics approaches used in crops to identify abiotic stress resistance genes cannot be easily applied in *Lolium* studies.

We aimed to create a platform for screening the efficiency of different guide RNAs (gRNAs) knocking out genes potentially involved in drought and frost stress tolerances. Therefore, we started by establishing protocols to generate, in an asexual manner, plant material that could be used for gene editing. For this, we set up the *in vitro* culture of tillers. Tillers grown in solid media supplemented with growth promoters were processed to generate protoplasts. We tested different protocols for both the isolation and transformation of protoplasts. For the isolation of protoplasts, we optimized the following variables: enzymes' concentrations, mannitol pretreatment, enzymatic treatment duration, and vacuum infiltration. The optimized protocol yields, on average, 1.5×10^6 protoplasts per ml of enzyme solution, which is sufficient for downstream applications such as transformation assays. Later, we compared two different protoplasts transformation methods: PEG and electroporation.

The gRNAs tested in protoplasts can be used for agrobacterium-mediated transformation. In our case, we focus on the co-cultivation of either meristematic calli or meristems with agrobacterium strains carrying the previously tested gRNAs.

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Session III:
Qualitative and quantitative traits

Thirty years of alfalfa genetic markers

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The impetus for this presentation was the publication in 1993 of the first two genetic maps of alfalfa; I was the senior author on one of them. Thirty years on, what has been the impact of genetic markers on alfalfa genetic research and, more importantly, on cultivar development? The early alfalfa genetic maps were (in retrospect) painstakingly built using restriction fragment length polymorphisms (RFLP); at the time, a map with 100 markers was a near miracle of technology. But the reality of these markers and the maps constructed from them was that they were not particularly easy to apply to problems in an autotetraploid perennial. Further iterations of genetic markers became cheaper and easier to assay, but they were still not particularly useful until the relatively recent development of genome-wide single nucleotide markers, which could be assayed using arrays, technologies like genotyping-by-sequencing, or target capture-based sequencing. The upshot is that although markers have been used for 30 years to study genetic diversity and to map qualitative and quantitative traits, the direct impact of marker technologies has been insignificant, aside from trivial uses such as tracking transgenes. Quantitative trait loci have been mapped in many studies, but resolution is usually rather limited and the development of targeted markers for routine use in breeding programs has not been undertaken. Perhaps I sound like a broken record, but today's marker and sequencing technologies seem to finally offer applications in alfalfa, as in other crops, that can be, and that will be, used to directly impact cultivar development.

I want to discuss several areas where I think markers can have the most impact in alfalfa improvement. First, markers can be useful in assessing genetic relationships within breeding populations, resulting in the simplest case in better estimations of breeding values. Second, and following from this point, genomic prediction of breeding values and the use of marker-only selection to more rapidly turn cycles of selection for traits such as yield, forage nutritive value, or persistence seems to have merit. Third, marker technologies are no longer time or cost limiting for the genetic dissection of traits; assuming our phenotyping capabilities are up to the task – and this, perhaps, is the key difficulty we face in alfalfa improvement – we should be able to do a better job at not only mapping the locations of large effect QTL, but in developing usable markers for practical cultivar development. Finally, the lack of yield progress over the past 50 years is a major concern for alfalfa breeders, and I will argue that dissecting the physiological basis of yield has never been easier and that a dedicated effort to functionally understand alfalfa phenology across years, morphological development, and yield components could help us reimagine alfalfa for the coming decades, keeping it a competitive crop in cropping systems. Genetic markers will play a key role in that investigation.

GWAS for drought tolerance in red clover (*Trifolium pratense* L.)

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Red clover (*Trifolium pratense* L.) is an important forage legume of temperate regions in the world. In the light of climate change, selection for improved drought tolerance is becoming increasingly important in red clover. However, the genetic control of drought tolerance in red clover is poorly understood. A better understanding of the genetic control may facilitate the selection for drought tolerance in red clover. The present study was performed in the framework of the H2020 EUCLEG project (grant agreement n° 727312), and is elaborated in Vleugels et al. (2023b).

In previous work, a diverse set of 396 red clover accessions was phenotyped for drought tolerance in a two-year drought experiment at ILVO (Melle, Belgium). Canopy cover (CC) and canopy height (CH) were identified as suitable traits to phenotype drought tolerance when observed at the end of the drought period or in the recovery period after the drought period (Vleugels et al. 2023a). The same collection of accessions was genotyped using a pooled genotyping-by-sequencing approach with 200 plants per accession: 12,765 SNPs (63.4%) were mapped on the seven chromosomes, while a remaining 7372 SNPs were located on scaffolds with unassigned chromosomal position (Frey et al. 2022).

In this study, we performed genome-wide association studies (GWAS) for drought tolerance, using the phenotypic and genotypic data available from previous studies. Drought tolerance was expressed as the relative performance [(control – drought)/control] for CC and CH. We used phenotypic observations from key time points during the drought periods and the recovery periods from both trial years 2019 and 2020. GWAS were performed using the multi-locus mixed model (MLMM) approach. SNPs were integrated as cofactors into a mixed model regression approach, as explained in Frey et al. (2022).

Various QTLs for drought tolerance were found (Table 1). Three strong QTLs were located on LG3 and LG6. The SNPs ‘LG3_3530421’ and ‘LG3_11998225’ on LG3 respectively explained 31.4% and 22.3% of the phenotypic variation for CH in 2019 and CC in 2020. Two closely linked QTLs on LG6, probably referring to the same target gene, explained 31.9% and 27.5% of the variation for CH in 2019. An additional, weaker QTL ‘LG1_22496879’ explained 15% of the

Table 1: Single nucleotide polymorphisms associated with drought tolerance in red clover

SNP ID	Chr	Position (bp)	Variance explained (%)	Trait	Year	Time point
LG1_22496879	1	22,496,879	15.0	CC	2020	61d after drought start
LG3_3530421	3	3,530,421	31.4	CH	2019	44d after drought start
LG3_11998225	3	11,998,225	22.3	CC	2020	61d after drought start
LG4_2357602	4	2,357,602	5.8	CH	2019	31d after drought start
LG5_4354015	5	4,354,015	5.9	CC	2019	44d after drought start
LG5_4354093	5	4,354,093	5.7	CC	2019	44d after drought start
LG5_13575628	5	13,575,628	9.5	CH	2019	41d after drought relief
LG6_9239088	6	9,239,088	31.9	CH	2019	41d after drought relief
LG6_9239175	6	9,239,175	27.5	CH	2019	44d after drought start
scaf_298_215478	NA	215,478	7.0	CC	2019	21d after drought relief
scaf_654_21060	NA	21,060	7.3	CH	2020	8d after drought relief
scaf_1046_34671	NA	34,671	7.3	CC	2019	44d after drought start
scaf_1836_11203	NA	11,203	11.3	CH	2019	41d after drought relief
scaf_21186_400	NA	400	14.2	CH	2019	15d after drought start
scaf_21186_400	NA	400	10.8	CH	2019	31d after drought start

Column headers: SNP identifier, chromosome, SNP position on the chromosome, phenotypic variance explained by that SNP, trait (CC: canopy cover; CH: canopy height), year of observation, and time point of observation of the trait

phenotypic variation for CC in 2020. Two QTLs were located on scaffolds: the SNP ‘scaf_1836_11203’ which explained 11.3% of variation for CH in 2019, and ‘scaf_21186_400’ which explained 14.2% and 10.8% of variation for CH at two time points in 2019.

In a next step, SNP positions and sequences will be compared to the red clover reference genome sequence to allocated possible target genes. Subsequently, these sequences will be compared to the *M. truncatula* genome to provide information on the gene function. This information could shed light on the genetic control of drought tolerance and be the basis for the development of molecular markers, which could speed up selection for drought tolerance in red clover.

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Breeding Soft-seeded Hairy Vetch

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Hairy vetch (*Vicia villosa* Roth.) is a major global legume cover crop. Hairy vetch has good survival and excellent spring biomass production. One weakness of hairy vetch is that it has a high incidence of physiological seed dormancy (PY), also known as hard seed. Overcoming this weakness by developing soft seeded varieties would result in greater adoption of hairy vetch in cover crop systems because producers would not have to worry about hairy vetch becoming a weed issue in the seed bank. We report here on progress made in developing soft seeded hairy vetch varieties. A recurrent phenotypic selection program was initiated to develop soft seeded hairy vetch populations. As expected, PY was very dependent on seed production environment. Despite this variability, genetic variation for the trait was determined with an estimated narrow sense heritability estimated at $h^2 = 0.40$ (Kucek et al., 2020). After two years of selection, soft seeded lines have improved from 74% PY to 67% hard seed, with the most advanced soft-seeded synthetic at 61% PY. We used a genome wide association (GWAS) approach to determine if we could find genomic regions or loci associated with PY. Our intent is that markers associated with PY loci could be used in marker assisted selection (MAS) to accelerate selection gains. As part of the GWAS effort, the first hairy vetch reference assembly was created (Fuller et al., 2023). For phenotypic data in autumn of 2018, two space-plant seed production nurseries were established in Knox City, TX, USA and Corvallis, OR, USA. At each site, 800 seed from 40 half-sibling families were planted for a total of 1600 plants. During the 2019 growing season, plants were allowed to go to flower and produce seed. Ripe unopened seed pods were collected from each plant. Pods were gently opened without disturbing the seed coat of seed within. Germination tests were conducted on processed seed from each plant. Genomic DNA was extracted from each plant followed by Illumina sequencing.

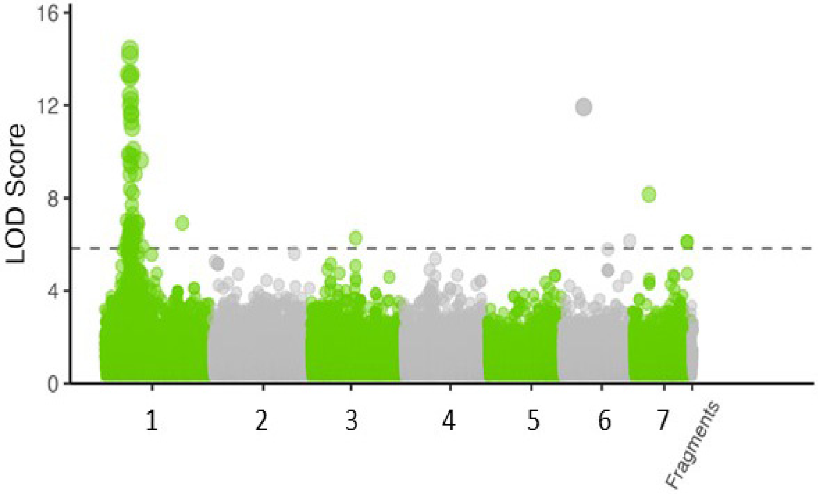

Figure 1: Hardseed Manhattan Plot

SNP GWAS analysis revealed a highly significant association peak (LOD 15) and a secondary smaller peak (LOD 12) (Fig. 1). The major locus had a 23% PY marker effect per allele dose. This result somewhat confirmed our hypothesis that the trait is controlled by a few major genes and/or genomic regions. Based on these results we are currently developing targeted markers for these two genomic regions for use in MAS schemes.

Differences between di- and tetraploid red clover cultivars. A review.

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Red clover (*Trifolium pratense* L.) has been the most important forage legume cultivated in Europe since 16th century. It is the second largest forage legume crop in the world in terms of production area, after lucerne. This species is cultivated not only in crop rotation, but also in permanent grasslands, where it substantially increases both, the forage yield and quality. It occurs naturally as diploid ($2n = 14$) and tetraploid red clover genotype was first created in 1939 using colchicine treatment on germinating seeds. The first commercial cultivars were released in 1950's.

Tetraploid cultivars exceed diploid counterparts in fresh forage yield (up to 20 %), plant height, leaves and seed size (by 50 %). A number of authors report also a higher tolerance to abiotic stresses (drought). The drawback of tetraploids is considerable lower seed yield and higher water content in its forage. Based on statistical data from the period 2012–2021 presented by authorities in the Czech Republic, tetraploid cultivars produced just 65% seed yield compared diploids, resulting higher seed price of tetraploids. Higher water content extends the period of forage wilting before silage making, resulting in higher risk of nutrients loss due to respiration and/or rain. These are the main reasons, why diploid cultivars make up more than 71 % of red clover multiplying areas in the Czech Republic and the seed of diploids prevails also in the world market.

Higher seed yield through targeted selection for reduced seed shattering in Italian ryegrass (*Lolium multiflorum* Lam.)

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Seed yield is a crucial factor for economic seed production of Italian ryegrass (*Lolium multiflorum* Lam.) cultivars. A preliminary experiment showed that seed shattering is a main factor limiting seed yield in Agroscope breeding material. Therefore, the goal of this study was to i) evaluate the potential of reducing seed shattering through recurrent phenotypic selection in spaced plants and ii) test whether seed yield in swards can be improved using this selection method. Starting from a base population of 300 plants, spaced plants were selected negatively for high shattering and positively for low shattering over two generations. This resulted in four differently selected (positive, double positive, negative and double negative) populations and an unselected reference population. In 2022, these five populations were compared in a trial with spaced plants at one location and a trial with sown rows (simulating a sward) at three locations. Spaced plants were harvested according to physiological maturity based on temperature sum after begin of flowering, whereby an early and late harvesting (difference between them of approx. 5 days) was applied for rows. In both trials, seed shattering at harvest was calculated as the ratio of shattered to total (shattered and non-shattered) seed weight.

In the spaced plant trial, the double positive selection showed lowest seed shattering (15.3%), followed by the positive (22.5%), neutral (38.7%), negative (41.9%) and double negative (47.2%) selection. In sown rows, seed shattering was significantly affected by selection, harvest time, as well as location-by-harvest time and selection-by-harvest time interaction. Thereby, seed shattering was lower for early compared to late harvesting and ranking of the selections was the same as in spaced plants for both harvest times (double positive = 2.41% and 9.31%, positive = 4.13% and 16.73%, neutral = 6.12% and 20.22%, negative = 7.34% and 21.56%, double negative = 7.92% and 28.44% for early and late harvest time, respectively). Lower shattering in rows corresponded with higher harvestable yield (non-shattered seeds), whereby positive selection reduced the yield-gap between early and late harvest.

Our results show that phenotypic selection on spaced plants for low seed shattering is very efficient to increase seed yield in Italian ryegrass. Furthermore, vigor and earliness were not influenced by selection of spaced plants, meaning that these traits can be selected independently. Early harvesting strongly reduces seed shattering in swards and would be a first measure to increase seed yield in practice. However, early harvesting is not always possible e.g., due to restricting weather conditions. Therefore, selection for low seed shattering is an efficient method to ensure high yields under various conditions.

Genetic selection for nonfiber carbohydrates in alfalfa (*Medicago sativa* L.) stem

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A large proportion of alfalfa proteins escapes microbial digestion leading to urea excretion into the environment. An inflow of carbohydrates must be provided to rumen microbes to ensure a better N use efficiency. The genetic selection of alfalfa for a high nonfiber carbohydrate (NFC, sum of soluble sugars, starch and pectin) concentration is a sustainable approach to reduce N losses. As NFC concentration in alfalfa forage is strongly influenced by daily fluctuations while stem NFC is more stable, our method of recurrent selection for high NFC in alfalfa was based on stem NFC concentration. Our objective were to test in the field alfalfa populations obtained after 4 cycles of selection for high stem NFC.

Fifty genotypes from four genetic backgrounds were selected based on their winter survival, yield and high stem NFC concentrations and intercrossed to develop the NFC1 population. A recurrent selection for vigor and stem NFC concentration was then performed to develop the populations NFC2, NFC3 and NFC4. A control population (NFC0) was also developed by intercrossing 40 alfalfa plants randomly selected within the four genetic backgrounds. The populations developed were then evaluated in a field trial. Populations were transplanted in row of 25 plants and arranged in a randomised complete block design with six repetitions. The field trial was established at Saint-Augustin-de-Desmaures (QC, Canada) and harvested for the two first post-seeding years. Annual yields were evaluated (3–4 cuts per year) and samples from the first and second cuts were collected to estimate nutritive value attributes by visible and near-infrared reflectance spectroscopy.

We observed an increase in NFC concentration of 3.7 g/100 g DM in the NFC4 population compared to the NFC0 population (Table 1), which represents an increase of 19% of NFC. Furthermore, although numerically lower, crude protein concentration for NFC4 was not statistically different of that of NFC0 population (Table 1). Then, the energy-to-protein ratio stepped from 1.17 in the NFC0 to 1.46 in the NFC4 population (Table 1). The acid detergent and neutral detergent fibre concentrations were respectively 3.1 and 3.7 g/100 g DM lower in the NFC4 population compared to the NFC0 population (Table 1). The increase in energy concentration thus was obtained at the expense of the fiber concentrations, resulting in a 2.8 % increase of *in vitro* dry matter digestibility in the NFC4

Table 1: Annual yield and nutritive attributes (whole plant) of the initial alfalfa population (NFC0) and the population resulting of four cycles of recurrent selection for high non-fiber carbohydrate concentration in stems (NFC4) evaluated at St-Augustin-de-Desmaures (QC, Canada) in first and second post-seeding years

	Populations			*P*-value		
Attributes	NFC0	NFC4	SEM	Year (y)	Pop (P)	Y × P
Annual yield (g DM/plant)	202	209	10.3	<0.001	0.525	0.352
Acid detergent fiber (ADF; g/100 g DM)	33.6	30.5	0.48	<0.001	**0.006**	0.868
Neutral detergent fiber (aNDF; g/100 g DM)	39.2	35.5	0.46	<0.001	**0.002**	0.685
In vitro dry matter digestibility (g/100 g DM)	79.8	82.0	0.40	<0.001	**0.011**	0.354
NDF digestibility (g/100 g aNDF)	49.2	50.1	0.52	<0.001	0.302	0.664
Non-fiber carbohydrate (NFC; g/100 g DM)	19.0	22.7	0.48	<0.001	**0.003**	0.602
Soluble sugars + Starch (g/100 g DM)	12.5	16.3	0.47	<0.001	**0.002**	0.574
Crude protein (CP; g/100 g DM)	16.5	15.8	0.22	0.346	0.061	0.435
NFC/CP ratio	1.17	1.46	0.046	<0.001	**0.006**	0.220

Notes. Nutritive attributes were weighted for yield measured at each cut as a proportion of the sum of two cuts yield**.** SEM, standard error of the mean; aNDF, neutral detergent fiber assayed with a heat-stable amylase and sodium sulfite.

population compared to the NFC0 population (Table 1). The annual yield of the NFC4 population was similar to the NFC0 population (Table 1).

The selection method used to increase NFC concentrations in alfalfa stems is an effective approach to improve the energy-to-protein balance in alfalfa, while maintaining yields and improving its digestibility. Using this selection method to produce high-NFC alfalfa could improve the environmental footprint of alfalfa through improved N use efficiency of ruminants fed with this alfalfa.

Session IV:
Biotic and abiotic stresses

Differential growth of a panel of perennial ryegrass accessions following excess water treatment over the winter under field conditions

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Excess water is in general considered an abiotic stress in plants, but the level how much water is considered an excess varies widely between plant species. An over two growing seasons replicated excess flooding experiment was planted with 24 accessions of perennial ryegrass which had been vegetatively propagated to ensure equal representation of genotypes within an acession, both varieties and ecotypes, from various geographical origins. The excess water treatment received over the winter periods excess water with irrigation. Yields increased in the winter-flooded treatment in contrast to the non-artificial watered control treatment. Differences in composition of macro- and micronutrient profiles were observed too by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). In conclusion perennial ryegrass is coping extremely well with excess water supplied over the winter period and can utilise it effectively in spring. This is in contrast to winter barley which is severly yield depressed under excess water treatment in winter.

Phenotypic characterisation of drought tolerance in red clover (*Trifolium pratense* L.)

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Forage production is increasingly affected by drought periods, which have become more frequent and more severe in recent years. In red clover (*Trifolium pratense* L.), it is unclear how drought affects plant growth, and which observations at which time points are suitable to distinguish drought-sensitive from drought-tolerant material. This study was performed in the framework of the H2020 EUCLEG project (grant agreement n°727312), and is elaborated in Vleugels et al. (2023).

A trial was conducted in Melle, Belgium (50.991N, 3.783E, 23 m asl) using a diverse set of 396 red clover accessions described in Frey et al. (2022). Each accession was planted in a one-meter row under retractable rain-out shelters. An adjacent parcel with the same design and treatment, but without the drought treatment, was established as a control. The trial was monitored during two growing seasons: 2019 and 2020. Four cuts were taken per year, and drought periods lasting 6 to 8 weeks were imposed in both years between cuts 1 and 2. Plant growth was monitored through UAV (unmanned aerial vehicle) observations for canopy height (CH) and canopy cover (CC). UAV measurements for canopy temperature obtained shortly after the drought treatments allowed the calculation of CWSI (crop water stress index).

Drought caused substantial reductions in CC and CH in both years (Table 1). Reductions in CC appeared 2 weeks after the onset of drought, and were maximal towards the end of the drought period (44% and 46% reduction in year 1 and year 2, respectively). Congruently, reductions in CH were maximal at the end of the drought periods (37% and 9% in years 1 and 2, respectively). However, CH displayed smaller relative reductions than CC, possibly due to lodging in the control trial and more intensive flowering in the drought trial. During most of the remaining growth season after the drought period, drought-treated plots lagged behind in terms of CC and CH, indicating a legacy effect of drought. Average CWSI values for control and drought-treated plots were 0.39 vs. 0.67 in year 1, and 0.16 vs. 0.37 in year 2, respectively, indicating clearly higher stress levels in drought-treated plots compared to control plots.

In conclusion, thermal data (CWSI) and CC are superior indicators of drought tolerance. CH can also be used, but care must be taken to correct for the effects of lodging and/or flowering. The most suitable period to phenotype drought tolerance is at the end of the drought period, and/or in the recovery period three to four weeks after drought relief. These results should help breeders to improve drought tolerance in red clover in the light of climate change. Future perspectives include a GWAS analysis that will reveal genomic regions associated with drought tolerance with the aim to reveal the genetic control of drought tolerance in red clover.

Table 1: Average canopy cover (CC) and canopy height (CH) observed over all accessions in the control and drought trials in both trial years 2019 and 2020

Year	DOY	CC (% coverage)		CH (cm)		
		Control μ ± CV	Drought μ ± CV	Control μ ± CV	Drought μ ± CV	
2019	133	97 .6 ± 0.0	98 .6 ± 0.0	47 ± 10	40 ± 10	
2019	149	55 .1 ± 0 .2	51 .1 ± 0 .1	22 ± 21	14 ± 19	✂
2019	165	99 .1 ± 0.0	80 .4 ± 0.0	46 ± 20	31 ± 19	
2019	178	96.7 ± 0.0	54.4 ± 0.2	55 ± 3	34 ± 15	
2019	198	36.0 ± 0.2	24.2 ± 0.2	18 ± 12	11 ± 10	✂
2019	205	58.6 ± 0.2	41.5 ± 0.2	27 ± 24	11 ± 26	
2019	225	88.3 ± 0.1	68.4 ± 0.1	39 ± 24	35 ± 17	
2019	238	40.3 ± 0.2	35.2 ± 0.2	5 ± 20	8 ± 8	✂
2019	256	77.8 ± 0.1	66.1 ± 0.1	27 ± 15	21 ± 17	
2019	273	87.5 ± 0.1	79.0 ± 0.1	23 ± 17	21 ± 14	
2019	280	18.1 ± 0.3	23.5 ± 0.2	9 ± 1	6 ± 0	✂
2019	309	66.4 ± 0.1	66.1 ± 0.1	11 ± 18	10 ± 13	
2020	127	99.0 ± 0.0	98.2 ± 0.0	39 ± 13	35 ± 12	
2020	139	4.5 ± 0.5	16.0 ± 0.1	9 ± 9	8 ± 9	✂
2020	161	80.4 ± 0.1	64.2 ± 0.1	25 ± 24	25 ± 23	
2020	195	91.3 ± 0.0	49.1 ± 0.2	44 ± 4	34 ± 14	
2020	204	8.2 ± 0.3	3.4 ± 0.2	6 ± 5	6 ± 6	✂
2020	220	63.9 ± 0.1	45.0 ± 0.1	20 ± 21	14 ± 18	
2020	245	91.0 ± 0.0	89.9 ± 0.0	31 ± 18	33 ± 17	
2020	258	39.8 ± 0.1	37.2 ± 0.1	9 ± 7	NA	✂
2020	266	47.1 ± 0.1	46.0 ± 0.1	12 ± 13	12 ± 10	
2020	288	61.8 ± 0.1	64.8 ± 0.1	10 ± 13	13 ± 13	
2020	311	51.4 ± 0.1	44.7 ± 0.1	5 ± 11	4 ± 7	✂
2020	329	43.1 ± 0.1	38.1 ± 0.1	7 ± 13	7 ± 9	

DOY: day of the year; CC: canopy cover; CH: canopy height; CV: coefficient of variation. CC is represented as the average percentage of ground cover. CH is represented as the percentage of the maximal observed height in the trial. Cuts are indicated with dashed horizontal lines and scissors. Drought periods are shaded.

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Vleugels T, Saleem A, Dubey R, Muylle H, Borra-Serrano I, Lootens P, De Swaef T, Roldán-Ruiz I (2023) Phenotypic characterisation of drought tolerance in red clover (*Trifolium pratense* L.). Front Plant Sci. *Under revision*

Cold stress study during emergence of maize (*Zea mays* L.) inbred lines

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Thanks to its excellent adaptability the cultivation of maize covers a wide area, from the tropics to the temperate zone. In the beginning, its spread was prevented by its sensitivity to cold as 30°C is the optimum temperature for the development of this tropical plant, and a short day cycle is required. Climate change has made it necessary to study the adaptation of maize to cooler temperature. Improved cold tolerance at germination is one of the most important conditions for early sowing. The advantage of cold tolerant maize hybrids is that they can be sown earlier, allowing longer growing seasons and higher yields, due to the fact that the most sensitive period in terms of water requirements, flowering, takes place earlier, i.e. before the onset of summer drought and heat. This will result in higher yields and lower grain moisture at harvest.

At the Agricultural Institute of Centre for Agricultural Research in Martonvasar continuous research is carried out to improve the cold tolerance of maize. In the present experiment, the cold tolerance of 60 genetically different maize inbred lines was investigated in a Phytotron climate chamber (PGV-36). The aim of our research is to identify cold tolerant lines that can be used as parental components to produce good cold tolerant hybrids and/or as sources of starting materials for new cold tolerant inbred lines. After observing and evaluating changes in phenological traits under cold-test (8°C for 10 days followed by 13.5°C for 30 days), the results of the cold-tolerance traits of interest: percentage of emergence, number of days from sowing to emergence, cold-test index results, have been used to highlight several inbred lines that could be good starting materials for further research on genetic selection for cold tolerance.

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Morpho-biochemical response perennial ryegrass (*Lolium perenne* L.) populations to water shortage

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Drought is one of the most significant effects of global climate change. It is expected to be more intense in some parts of southern Europe and the Balkan Peninsula in the next decade. Drought stress is the most important restriction factor for food and feed productivity. Perennial ryegrass (*Lolium perenne* L.) forms the basis of grassland production in temperate pastures and represents one of the most important forage grasses globally. Although perennial ryegrass has a high herbage yield, good quality, and high digestibility in ruminant nutrition, it relies on high levels of water to maintain optimal growth rates. The best way to overcome this issue could be to breed new varieties with increased drought tolerance. The aim of this research was to determine the genetic variability of the most important morphological and biochemical traits as well as dry matter yield at different watering levels. Morpho-biochemical responses to drought stress were investigated in three different perennial ryegrass populations (cultivars Shandon and K-11, and the experimental breeding population) at four different levels of watering. Plants were grown in mortar sand in plastic tubes 7.5 cm in diameter and 90 cm in length under a rain shelter. Ten plants per population in three replications (clones) from each watering treatment were analysed. Shoot dry matter yield and traits of the root system in deeper layers were measured at the end of the experiment after three months of reduced watering treatment. In addition, the activity of the antioxidant enzymes catalase (CAT) and guaiacol peroxidase (GPx), total antioxidant capacity (TAC), total phenolic content (TAC), and proline were used to evaluate the effects of drought stress and plant responses. Statistical analysis of the data (ANOVA) revealed significant between-population variability for all properties analised in the study under different watering treatments. The highest average yield and root percentage in the deeper layer of the whole experiment was determined in the experimental breeding population, which was predominant in stressed plants. On the other hand, cultivar Shandon had the highest production under control conditions. There wasn't a general trend in antioxidant activities. While CAT activity was increased in stressed plants, especially in K-11 and experimental breeding population, GPx activity

Table 1: Summarized morphological and biochemical results of drought-stressed perennial ryegrass populations. Abbervations: % Root – % of plants with a root length more than 80 cm; CAT – catalase; GRx – guaiacol peroxidase; TAC – total antioxidant capacity; TPC – total phenolic content.

		Yeald	% Root	CAT	GRx	TAC	TPC	Proline
K11	K	1.17	0.93	21.7	208.4	0.34	1452	4.25
	R1	0.83	1	19.5	188.3	0.38	1948	4.95
	R2	0.57	1	36.1	195.0	0.42	2047	8.19
	R3	0.54	0.8	39.0	193.7	0.37	2132	22.83
Experim. population	K	1.51	1	26.4	214.8	0.58	1457	4.60
	R1	1.02	1	22.0	207.2	0.58	1661	6.91
	R2	0.85	1	24.3	200.6	0.57	1897	16.16
	R3	0.71	0.93	38.6	199.8	0.66	2138	44.70
Shandon	K	1.55	0.87	27.8	245.1	0.58	1854	7.12
	R1	1.03	0.86	17.9	247.9	0.53	2148	7.10
	R2	0.68	0.87	26.2	226.2	0.65	2177	11.44
	R3	0.55	0.67	30.9	186	0.59	2460	44.49
$\bar{X}$K11		0.76^{b}	0.93^{a}	29.1^{a}	196.4^{b}	0.38^{b}	1895^{b}	10.06^{b}
$\bar{X}$Eks		1.02^{a}	0.98^{a}	27.8^{ab}	205.4^{b}	0.60^{a}	1788^{c}	18.09^{a}
$\bar{X}$Sh		0.95^{a}	0.82^{b}	25.7^{b}	226.3^{a}	0.59^{a}	2160^{a}	17.54^{a}
$\bar{X}$ irrigation	$\bar{X}$K	1.39^{a}	0.93^{a}	25.3^{c}	222.8^{a}	0.50^{b}	1588^{d}	5.33^{c}
	$\bar{X}$R1	0.96^{b}	0.95^{a}	19.8^{d}	214.5^{ab}	0.50^{b}	1919^{c}	6.32^{c}
	$\bar{X}$R2	0.70^{c}	0.96^{a}	28.8^{b}	207.3^{b}	0.54^{a}	2040^{b}	11.93^{b}
	$\bar{X}$R3	0.60^{c}	0.8^{b}	36.1^{a}	193.2^{c}	0.54^{a}	2244^{a}	37.34^{a}

significantly dropped, particularly in Shandon. This decreased GPx activity could indicate that his capacity to scavenge rective oxygen species was weakened by severe drought stress. High levels of proline and phenolic compounds were detected in all drought stressed plants. This was expected due to their properties to stabilize subcellular structures, scavenge free radicals, and buffer cellular redox potential. Based on these results, the experimental breeding population has the highest potential for drought resilience and biomass production in drought field conditions, which should be tested in the future.

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Fv:Fm and RWC measurements as indicators of drought stress response in perennial ryegrass

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Drought induced yield losses can be mitigated by growing drought-tolerant cultivars, which in turn requires reliable screening methods during the breeding process. There are multiple ways to phenotype plant drought sensitivity, each with their own advantages and limitations. Maximum quantum efficiency of photosystem II (Fv:Fm) is reproducable, non-destructive and fast method, especially when hand-held tools are used. However Fv:Fm might not show any signifficant changes untill the stress becomes severe. Leaf relative water content (RWC) has also been widely used as a reliable indicator of plant water status. It is very simple and does not require any expensive equipment other than the scale, however it is laborious and destructive. The aim of this experiment was to compare these two drought stress evaluation methods in perennial ryegrass in controlled environment.

Perennial ryegrass genotypes (n = 48) were propagated into vegetative clones and grown in the plastic pots commercial potting substrate. Plants were transferred into a climate chamber (PlantMaster, Germany) after 4–6 weeks, with a light/dark photoperiod of 16/8 h, day/night temperature 20 °C. To induce a water deficit stress, plants were deprived of water for 9 days. Fv:Fm was measured daily. The plants were dark adapted for 15 min and three to five leaves were measured with fluorometer LI-600 (LI-COR Biosciences, USA). RWC was calculated right after the final watering and later on the 2nd, 4th, 6th and finally 8th or 9th day (depending on soil moisture content). At least 3 leaves per plant were taken for RWC evaluation. Statistical analysis of the results was implemented with the open sourceR statistical environment (version 4.3.0; R Development CoreTeam, 2023).

The Fv:Fm values started to drop only on the 6th day of treatment, signifficant change was estimated on the 7th and final day (Fig 1 A). RWC remained stable for the first 4 days, then sharp decline was estimated on the 6th and final days of treatment (Fig 1 B).

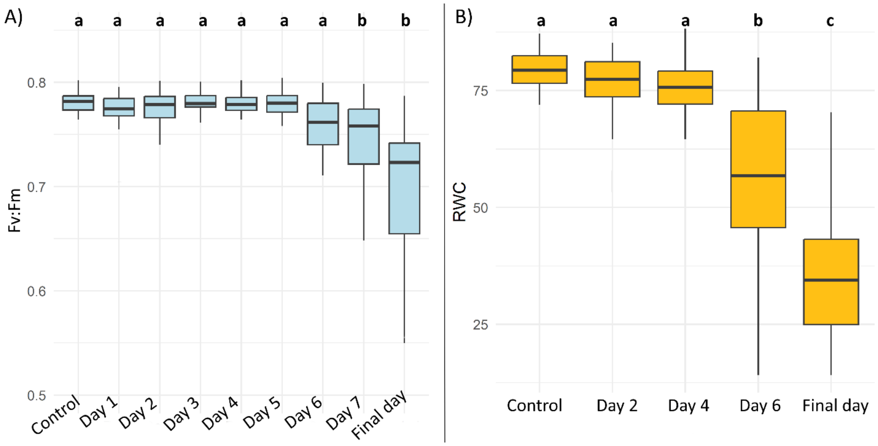

Figure 1: Perennial ryegrass drought stress response based on the maximum photochemical efficiency of photosystem II (Fv:Fm) (A) and leaf relative water content (RWC) (B). The letters indicate difference among group means (Tukey's HSD test, $p < 0.005$).

Strong correlation was estimated between Fv:Fm and RWC on the 6th ($r = 0.69$, $p < 0.001$) and final day of treatment ($r = 0.72$, $p < 0.001$) and but there was no correlation between 2nd and 4th day measurements. To sum up, RWC is a more sensitive indicator of plant stress level compared to Fv:Fm, however it is laborious and time consuming, especially when large number of plants has to be screened.

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Towards an efficient detection of genetic diversity in multispecies grassland

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Natural and semi-natural permanent grasslands are an important component of sustainable ruminant livestock production in many regions of Europe. They are usually composed of many different species and the species richness of these pastures and meadows substantially contributes to ecosystem services such as nutrient cycling, carbon sequestration or the prevention of soil erosion. In addition, plant genetic diversity, i.e., the diversity within individual species, has been shown to contribute to ecosystem stability and resilience, and, most importantly, provides an invaluable reservoir of genetic resources for the breeding of forage grasses and legumes. In order to efficiently manage and utilise genetic diversity in grasslands, an efficient method for its detection is urgently needed.

We developed a cost-effective, amplicon sequencing method that has the potential for large-scale detection of genetic diversity in multispecies grasslands. The method is based on the targeted sequencing of genomic loci that are conserved among the most important forage grass and legume species but are diverse within each individual species. The nucleotide diversity of the selected amplicons ranged from 5.19×10^{-3} to 1.29×10^{-2} in five key species (*Lolium perenne, L. multiflorum, Dactylis glomerata, Trifolium repens* and *T. pratense*). This is comparable to the range observed in some flowering or disease resistance genes. In a first proof of concept, the method was successfully used to separate pure and mixed samples of six perennial ryegrass (*L. perenne*) cultivars, indicating its potential for detecting differences in genetic diversity in this species. In a second step, we simulated genetic changes by overseeding seminatural grassland and analysed genetic diversity of multispecies samples collected at four different timepoints and using two different sampling strategies. Preliminary analyses showed that we were able to assign 76.5% of the sequencing reads to the correct species. We also observed SNP-level allele frequency changes between the samples harvested before and after overseeding.

The method presented here has the potential to investigate within-species diversity in mixed-species samples and presents an important step towards efficient, large-scale monitoring of genetic diversity in pastures and meadows.

Surviving under ice: Insights into gene expression changes during ice-encasement in perennial grasses

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The increase in global temperatures due the climate change is projected to create variable weather patterns across the globe (IPCC, 2014). Reports indicate that there was an increase in the frequency and duration of winter warming episodes (WWE) over the past few decades, a trend which is expected to amplify in the future. Snowmelt from these WWEs could expose the plants to ambient air temperatures or lead to the formation of nonpermeable ice cover (ice encasement) causing hypoxic or anoxic conditions for plants, therefore increasing the risk of winter kill. The current study aim to identify genes involved in ice-encasement tolerance and study gene expression differentiation during ice-encasement in survivor plants of timothy. The surviving plants of cultivars Engmo, Noreng, Grindstad and Snorri collected from the field trials (4 years) at Tromsø and Vesterålen were subjected to ice-encasement tests along with the young plants raised from the original seed lots used to establish the field trials. LD_{50} varied across cultivars and material. The surviving plants from both locations had lower LD_{50} compared to plants from their respective original seed lots. Differential expression analysis identified genes involved in freezing stress (ERF, dehydrins, DREB), detoxification (peroxidase, glutathione transferase), anaerobic respiration (alcohol dehydrogenase) etc as differentially expressed. Functional enrichment analysis revealed that genes associated with glycolysis/gluconeogenesis, pyruvate metabolism, fructose and mannose metabolism and circadian rhythm-plant pathways etc. are highly enriched under ice-encasement. Comparative transcriptome analysis identified genes associated with glycolysis/gluconeogenesis, glyoxylate and dicarboxylate metabolism, pyruvate metabolism and TCA cycle pathways are differentially expressed between the field survivors (4 years) and the young seedlings during ice encasement. We speculate that the observed gene expression differentiation between field-surviving plants and young seedlings might be due to selection in the field or an effect of juvenility.

Wilt of red clover caused by *Fusarium oxysporum*

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Red clover (*Trifolium pratense* L.) is an important forage legume in Serbia, grown in many regions, especially due to its ability to grow on acid soils. Red clover plants with symptoms of stunting, slight chlorosis and necrosis of leaves, discoloration and partial root rot were sampled in the field where red clover is grown in monoculture in Ćuprija, Serbia, in July, 2021. Several isolates were obtained from the roots and one (F16A) was further identified and characterized based on morphological, molecular and pathogenic characteristics. On potato dextrose agar (PDA) after 7 days at 25°C the isolate formed colonies, 74–78 mm in diameter. Aerial mycelium was abundant, cottony, and pale in color. On carnation leaf agar (CLA), the isolate formed macroconidia, microconidia and chlamydospores. Numerous macroconidia in sporodochia were formed on conidiophores or on monophialides arising directly from hyphae. Macroconidia were slightly falcate, with a foot-shaped basal cell and a curved apical cell, thin walled and with three (14.0–24.9x3.4–5.4 µm), four (19.9–30.5x3.4–5.2 µm) or five (27.4–37.5x3.6–4.5 µm) septa. Microconidia were produced in false heads that form directly on hyphae. Microconidia had roundish to ellipsoidal shape, with one septa (11.9–16.5x2.6–4.0 µm) or with no septa (5.9–13.4x2.1–4.1 µm). Chlamydospores were globose to spherical with thick and smooth walls (5.6–10.9x6.3–12.9 µm). Described morphological features were in accordance with Burgess et al. (1994) for *F. oxysporum*. Species level identification was confirmed by isolating genomic DNA followed by PCR amplification of *tef1* region (Translation elongation factor 1-α) using EF1 and EF2 primers. BLAST analysis of the nucleotide sequence revealed that isolate F16A was identical with several reference sequences of *F. oxysporum* deposited in NCBI GenBank. Pathogenicity of the isolate F16A was tested on detached leaves, stem fragments and roots. For inoculation 7 day old cultures of the isolate grown on PDA were used. Leaves were inoculated with mycelial plugs and after 7 days necrotic lesions developed (average diameter 4.1 mm). The stem fragments, 40 mm in length, were stuck in fungal colony, and after 7 days necrosis developed (average length 20.1 mm). Roots of young red clover plants (92 days) were inoculated using toothpick inoculation method (Chambers, 1988). After 40 days of incubation on the cross section of the roots, tissue around the inoculation site and in the cortex exhibited rot. Control leaves, stems and plants were inoculated with sterile PDA or sterile toothpicks and following inoculation remained symptomless. The fungus was re-isolated from infected tissue. *F. oxysporum* produces a wide range of mycotoxins such as trichothecenes, zearalenones, fumonisin B1, fumonisin B2 and fusaric acid which can impact animal and human health (Ohberg, 2008). In Serbia *F. oxysporum* has been previously identified and described on cereals, industrial crops, forage crops and on vegetables.

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Early morphological traits condition the performance of lucerne plants in different competitive situations

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The fate of individual plants in a dense stand, whether mono- or multispecific, conditions the persistency and level of production of cultivated species. Growth in monoculture is not comparable to that in mixture, due to the complex interactions (complementarity, competition, facilitation) between neighbouring plants. These interactions, together with the genotype of the plant, influence the final yield of individuals and their status of dominant or subordinate plants.

The present study aimed at analyzing the morphological and genetic evolution of a phenotypically diverse lucerne population. A F1 was grown under different competition conditions: intraspecific competition (pure lucerne), and interspecific competition represented by the mixture with red clover or tall fescue. Our hypothesis was that the future of a plant in a dense stand is influenced by its early morphological traits which condition future interactions with neighbouring plants. We detected genetic markers (QTL) involved in early morphological traits and compared the allele frequency of all markers between the 30% of plants with high biomass yield and the 30% with low yield, in order to determine whether the QTL played a role in the fate of the plants.

Experimental design

Pure lucerne, lucerne-red clover, and lucerne-fescue treatments were studied from May 2020 to October 2021, in 1.2 m^2 trays with a planting density of 400 plants.m^{-2}, in two replications. Early morphological measurements were made at the beginning of the experiments, before any competition between plants was established. The initial rate of leaf emission presented by the number of leaves emitted in the first 12 days, as well as the length and width of leaf number 3 were collected. Dry biomass yield was then collected for the next two years, obtained by drying at 60 °C for 72 h the shoots of each individual plant on all treatments.

QTL detection

With GBS genotyping (genotyping by sequencing), 20 000 SNP markers were obtained, covering all chromosomes. The detection of QTL associated with early morphological traits was carried out with all plants from the three treatments. For leaf emission rate, only one QTL was identified. For leaf length, 5 QTL were detected. For leaf width, 4 QTL were detected. Several QTL are common between length and width of leaf 3, which may indicate that both traits are controlled by the same genetic determinants.

Effect of QTL on plant fate

The frequency of each marker was compared between dominant (30% highest biomass yield) and dominated (30% lowest biomass yield) plants in the three different treatments ("pure lucerne", "lucerne-red clover" and "lucerne-fescue"). This comparison revealed 1081, 1601 and 997 markers involved in the biomass difference between the two groups of plants in the three treatments, respectively. These markers were located on all chromosomes and were close to or superimposed to the QTL controlling the three early phenotypic traits. This result is consistent with our hypothesis of the role of early growth traits in shaping the fate and size hierarchy of plants in dense stands.

Recurrent molecular selection for improved field resistance to crown rot (*Sclerotinia trifoliorum*) in red clover (*Trifolium pratense*)

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Red clover (*Trifolium pratense* L.) is a high yielding perennial forage legume, widely grown in temperate areas, primarily for conservation uses. It has a number of qualities that make it attractive as a sustainable forage species, but its use is limited in part due to a lack of resistance to the potent soil borne pathogen *Sclerotinia trifoliorum* Erikss. This necrotrophic ascomycete cannot be controlled well in a sward by use of fungicides and thus it limits persistency of red clover. Furthermore, best practice is to avoid short cycle rotations, with typical recommendations of a five- to seven-year break between red clover crops.

In this paper we describe the improvement of resistance to *S. trifoliorum* in *T. pratense* through a recurrent selection breeding methodology incorporating a founder population consisting of diverse germplasm. After four rounds of selection and crossing this methodology has yielded a number of half sib families where partial resistance (infection occurs but fails to progress) exists in more than 55% of the population. Furthermore, full resistance (infection does not establish) has been achieved in these families in more than 30% of the population. This has promising implications for the development of cultivars with greater persistency.

The genetic basis of *S. trifoliorum* resistance was investigated by comparing the 96 most resistant *T. pratense* genotypes with the 96 that were most susceptible to the pathogen in the F4 families. Leaf material was infected by placing a plug of agar containing fungal hyphae on the leaf surface *in vitro*. The infection was scored daily for 14 days, and the cumulative score taken as the measure of resistance. Double digest GBS (LGC, Germany) produced 104,723 reliable SNP variants across the seven chromosomes of red clover. The SNP were interrogated using R to initially produce principal components which were used as covariates in a GWAS analysis using rrBLUP, to fine map markers potentially involved in pathogen resistance. 20 significant SNP were found on chromosomes 1 to 6, with 2 extremely significant SNP on chromosomes 4 and 5. The most resistant half sib lines have been taken forward for varietal development.

Evaluating root characteristics under field conditions in perennial ryegrass for potential application in commercial breeding programmes

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Introduction

Over the past 100 years, breeding for perennial ryegrass has largely focused on improvements in economic production traits, such as yield, digestibility and disease resistance, leaving a huge gulf in our understanding of the genetics underlying other traits that could be improved to combine the highly successful traits of perennial ryegrass with those more negative traits that have remained unimproved. Breeding for improved root traits such as depth and volume has the potential to improve key traits such as nitrogen use efficiency, resilience to abiotic stresses such as drought to extend the usable life swards and carbon sequestration (1). Deeper penetrating roots can also improve soil structure by improving aeration and drainage, ultimately improving soil fertility and reducing erosion (2,3). This study aims to evaluate the feasibility of root-based evaluation methods under field conditions that can be combined with above-ground trait measurement under commercial grass breeding conditions.

Methods

54 samples were taken from an established intermediate tetraploid perennial ryegrass trial (sown October 2021) at AFBI, Loughgall NI, during October 2022. The trial included 18 varieties replicated 3 times in an incomplete block design (6 m^2 plots), and included 1 commercial *Festulolium* control, as well as 7 of the top-performing commercial varieties on the Recommended List for England and Wales in 2021. A digger was used to take 30 cm soil cores (one from each trial plot) which were stored at 4°C until washing. For washing, each sample was submerged in water for 1 hour before being washed through a 3.5 mm sieve. The roots were then carefully cut at the collar and washed again through a 2.5 mm and 1.8 mm sieve. The clean roots were stored in water at 4°C until scanning. Five intact typical roots from each sample were scanned, (submerged in water in an acrylic tray) using an Epsom flatbed scanner with a mounted LED light above. A sheet of paper was placed over the scanning tray to diffuse the light. Each image was saved before being analysed using the open-access root scanning

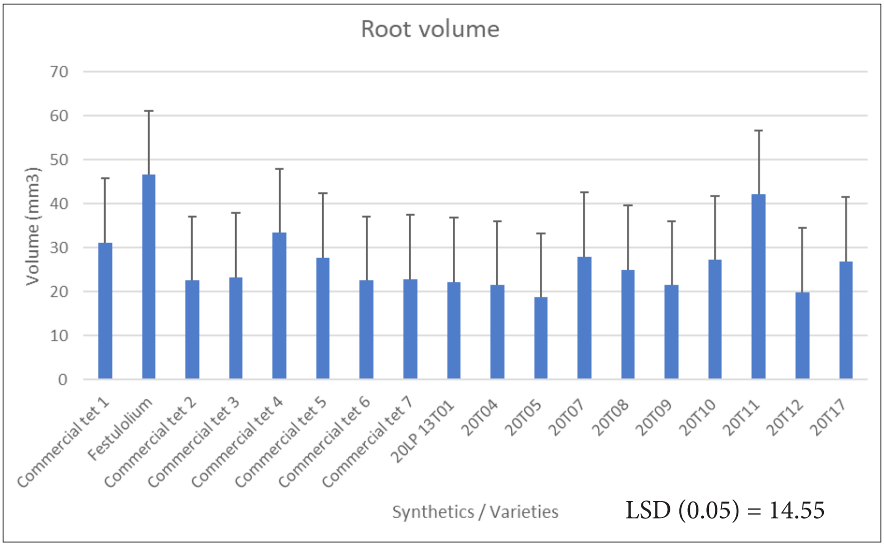

Figure 1: Mean root volume from intermediate tetraploid trial.

Table 1: Volume, network area and surface area averages of intermediate tetraploid synthetics

Variety	Network area (mm^2)	Surface area (mm^2)	Volume (mm^3)
20LP13T01	39.83ab	141.0ab	22.31ab
20T04	40.96ab	143.9ab	21.52ab
20T05	37.00a	130.1a	18.79a
20T07	51.81abc	182.4abc	27.98abc
20T08	46.60ab	165.2ab	25.02ab
20T09	40.14ab	143.4ab	21.52ab
20T10	50.93ab	176.9ab	27.30ab
20T11	72.51cd	255.0cd	42.17cd
20T12	39.37ab	140.0ab	19.92ab
20T17	46.24ab	164.0ab	26.91ab
Commercial tet 1	56.32abc	198.5abc	31.22abc
Commercial *Festulolium*	81.01d	285.3d	46.65d
Commercial tet 2	44.45ab	155.5ab	22.54ab
Commercial tet 3	45.23ab	159.0ab	23.30ab
Commercial tet 4	59.31bc	210.6bcd	33.45bcd
Commercial tet 5	48.41ab	171.5ab	27.84abc
Commercial tet 6	46.05ab	163.4ab	22.63ab
Commercial tet 7	44.06ab	152.3ab	22.93ab
P-value	0.015	0.021	0.025

platform Rhizovision (4). The data were analysed using a linear mixed model methodology (REML estimation method) followed by Fisher's Least Significant Difference Test to compare pairwise differences between varieties.

Results

Total root length, diameter and number of root tips were analysed but not statistically significant. Root volume, network area and surface area were statistically significantly different ($P < 0.05$) and similar trends were observed, with the commercial *Festulolium* producing higher values compared with all other varieties (Figure 1; root volume only presented). One high-performing synthetic was identified (20T11), which produced significantly greater root volume, network area and surface area than most other synthetics in the trial (Table 1). However, this synthetic was not significantly different to other synthetics within the trials despite clear differences in mean, due to high variability within the synthetics tested. This would be expected given that only one sampling is presented here. Further data will be collected to test the validity of this trend.

Conclusion

The commercial *Festulolium* used in this study is known to have deep rooting traits. This was reflected in the data and sets a benchmark for comparing the perennial ryegrass varieties. 20T11 stands out as a possible deep-rooting perennial ryegrass which showed promising results in volume, surface area and network area. For more accurate conclusions further data will be analysed at different points in the growing season and over multiple seasons. Dry matter and root mass measurements will also be taken. Overall, this method showed potential to identify the best parent material for the initiation of crossing for root traits in perennial ryegrass using only a single core per sample, while also screening for important above-ground traits, including yield and digestibility.

Acknowledgements

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Red clover root microbiota and interaction of root rot and clover rot pathogens

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Red clover (*Trifolium pratense*), a crop constrained by weak persistence, is the number one forage legume in Northern Europe. Whether plant loss is a sign of an increasing number of known or new pathogens, a decreasing occurrence of beneficial soil microorganisms or other factors is unclear. We collected red clover root samples from Swedish conventional and organic farming sites located between latitudes 55°N and 66°N and analyzed their prokaryotic and eukaryotic communities using amplicon sequencing (Jambagi et al. 2023). We found that microbial biodiversity was lowest in samples from southern Sweden and on conventional farms. *Rhizobium* was the most prevalent bacterial genus, followed by *Sphingomonas*, *Mucilaginibacter*, *Flavobacterium* and the unclassified *Chloroflexi* group KD4-96. The *Leptodontidium*, *Cladosporium*, *Clonostachys* and *Tetracladium* fungal genera known for endophytic, saprotrophic and mycoparasitic lifestyles were also frequently observed in all samples. Sixty-two potential pathogenic fungi were identified with a bias toward grass pathogens and a higher abundance in samples from conventional farms and southern region of Sweden. Co-occurrence networks revealed that the *Rhizobium leguminosarum* bv. *trifolii* is a keystone species in samples from the northern region and Swedish organic farms and is negatively associated with all fungal pathogenic taxa recognized in this study. We have established a hydroponics-based system for screening red clover for interaction to several fungal species. Next step was to run RNAseq analysis using red clover genotypes with somewhat contrasting responses to root rot and clover rot fungal pathogens. Validation of selected genes and their sequences is in progress.

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Divergent responses of perennial ryegrass and tall fescue to drought stress

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Forage grasses such as perennial ryegrass (*Lolium perenne* L.) and tall fescue (*Festuca arundinacea* Schreb.) are widely cultivated in Europe. In contrast to perennial ryegrass, tall fescue is known to withstand water deficit and maintain biomass yield production even under severe drought stress. Yet, the reasons for tall fescue's drought tolerance are unknown. In this study, we examined the drought responses of eight forage grass populations (four perennial ryegrass and four tall fescue) using field and lab-based phenotyping methods. Field studies showed that tall fescue was more tolerant to severe drought stress, with 62% of its genotypes surviving, compared to only 12% of the perennial ryegrass genotypes. Lab-based high throughput phenotyping revealed tall fescue limited transpiration when the mean soil moisture content was 46% (standard error [SE] = 1.5) and reduced its leaf elongation rate at a mean soil moisture content of 19% (SE = 1.0). In contrast, perennial ryegrass reduced its transpiration and leaf elongation at mean soil moisture contents of 33% (SE = 1.2) and 28% (SE = 0.008), respectively. Although preliminary examinations of the leaf surface revealed a higher density of stomata in tall fescue compared to perennial ryegrass, carbon isotope analysis indicated a higher water use efficiency of tall fescue. Our results suggest that tall fescue can maintain leaf elongation by reducing its transpiration rate under water deficit conditions, while perennial ryegrass continues to transpire and grow under mild drought stress but fails under severe stress. Tall fescue's physiological and anatomical traits described here can be harnessed for the future development of drought-tolerant forage grasses.

Response to waterlogging and drought in wild and domesticated accessions of timothy (*Phleum pratense*) and its wild relatives *P. alpinum* and *P. nodosum*

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Timothy (*Phleum pratense*) is a cool-season perennial forage grass widely grown for silage and hay production in northern regions. Due to the rapid climate change, there is a need for new varieties that are adapted to new growing conditions. Based on climate change scenarios, extreme weather with fluctuating periods of high precipitation and periods of drought will increase in Sweden and northern Europe. In this study, we evaluated the response of 19 wild and domesticated accessions of timothy and its presumed parental species *P. nodosum* and *P. alpinum* from different locations in northern Europe. The same accessions were exposed to waterlogging and drought in a greenhouse for three and four weeks, respectively. We also studied their recovery after drought during 18 days of re-watering. Under waterlogging, there was no significant difference in shoot dry weight between plants of the three species grown in waterlogged and non-waterlogged conditions. However, the root dry weight was significantly lower in most accessions, except for one wild timothy and one wild *P. nodosum* accession under waterlogged conditions. We found a large formation of aerenchyma in the adventitious roots of wild and domesticated *Phleum* accessions exposed to waterlogging. This may explain the large shoot growth observed in the exposed plants. Under drought conditions, all wild and domesticated accessions of the three species had significantly lower shoot dry weight than unexposed plants of the same accessions. However, these accessions differed in root dry weight, with some wild timothy accessions showing a higher production of roots during drought than in unexposed conditions. All accessions that were exposed to drought showed regrowth during re-watering. This regrowth was highest in the timothy accessions. Among the accessions studied, we identified several wild timothy accessions that were tolerant to both waterlogging and drought. These accessions are therefore interesting genetic resources and candidates for the development of climate-resilient timothy varieties.

Festulolium
Working Group
Workshop

History of *Festulolium* breeding in Czech Republic and future prospects

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Goal for *Festulolium* breeding is a combination of positive agricultural properties from two genera in a single variety. *Festulolium*, as a natural grass genera, is developed by intergeneric crossing of fescue and ryegrass species due to the close genetic phylogenies of these two genera. According to some phylogenetic analyses, current ryegrass and wide leaved fescue species evolved from the same ancestor. Natural crossing of both genera is quite often, however, diploid *Festulolium* hybrids are mostly sterile. On contrary, utilization of autotetraploid parents in plant breeding enables the development of fertile genotypes.

In Czech Republic, Mr. Fojtik (famous forage breeder) started with *Festulolium* breeding in the 1960s at breeding station in Hladke Zivotice. His vision about *Festulolium* was based on the first experiences about intergeneric crossing from UK and USA. Grass breeding in the 1960s and 1970s was based on phenotype selection, due to the general absence of molecular tools. Traditional breeding methods were employed including intensive selection, backcrosses and polyploidisation. Overcoming problems with heterogeneity and sterility of the first hybrid generations took a long time. *Festulolium* varieties reach homogeneity acceptable for registration only later in 6th to 7th generation after original crossing (i.e. after over 20 years of breeding). This was the reason why the first *Festulolium* varieties Becva, Felina, Hykor and Perun were listed in the end of 1980s and beginning of 1990s.

Recently, five types of *Festulolium* hybrids are recognized in agricultural practice:

Lolium multiflorum × *Festuca pratensis*	*Festulolium braunii* (K. Richter) A. Camus
Lolium perenne × *Festuca pratensis*	*Festulolium loliaceum* (Huds.) P. Fourn.
Lolium multiflorum × *Festuca arundinacea*	*Festulolium krasanii* Jirásek
Lolium multiflorum × *Festuca glaucescens**	
Lolium perenne × *Festuca mairei**	

*) Varieties of the last two crosses were developed newly in UK and hybrid species have no scientific name, yet.

Official registration of *Festulolium* varieties is essential requirement for their agricultural exploitation. Poland, former East Germany and Czechoslovakia became the first countries in Europe, where *Festulolium* was accepted as a new genera. In these countries the first *Festulolium* varieties were registered in the end of 1980s and beginning of 1990s. In other countries, the first varieties were registered as species used as controls during registration process, e.g. cv. Hykor has been registered as tall fescue in Germany. Later on, *Festulolium* was approved as a new (agricultural) genera within EU by Commission Directive 2004/55/EC in April 20, 2004.

In 1990s, breeding Station in Hladke Zivotice initiated close collaboration with the Institute of Experimental Botany in Olomouc. The main goal was the integration of molecular methods as integral parts of *Festulolium* breeding. Methods like flow cytometry (FC), genomic in situ hybridization (GISH) and Diversity Arrays Technology (DArT) allowed faster identification of hybrids and more efficient genotype selection.

In total, 28 *Festulolium* varieties from three seed companies are recently on the list in Czech Republic, including 26 varieties developed in Hladke Zivotice. All varieties were developed either from original crossing of Italian ryegrass with tall fescue followed by backcross with one of the other parent (varieties of both fescue and ryegrass phenotype) or by intergeneric hybridization of Italian ryegrass with meadow fescue (ryegrass phenotype). There is a high number of *Festulolium* varieties widespread on the grass seed market including Felina, Hykor, Fojtan, Hipast, Lukida (introgression type, close to tall fescue phenotype) and Perun, Perseus, Achilles, Hostyn and Helus (amphiploid type with phenotype close to Italian ryegrass).

Originally, breeding of *Festulolium* in Czech Republic targeted to the development of varieties for Central European climatic conditions and for agricultural management on arable land and grasslands. In 1990s, breeding station at Hladke Zivotice merged with global seed company DLF Trifolium. This merge initiated breeding of varieties for different climatic conditions and agriculture managements in Europe and other continents. Wide adaptability of current *Festulolium* varieties allowed their utilization in Central and East Europe, Germany and partly in Scandinavia, USA and Canada. On contrary, West and South Europe agriculture and parts of North and South America have required the development of varieties with different agricultural attributes. Thus, crossings and selection in local climatic conditions and under local agricultural management have been incorporated into the *Festulolium* breeding practice.

Nowadays, molecular and sequencing methods are more and more often used in breeding practice, including *Festulolium* breeding. In the last two decades, breeders and scientists in Europe focused on the identification of markers for drought tolerance (IGER Aberystwyth, UEB Olomouc). Drought tolerance is a polygenically inherited trait, which makes identification of closely associated molecular markers difficult. However, several candidate markers have been identified and used in selection of suitable genotypes (IGER). Other research direction is to identify other sources of drought tolerance beyond the widely utilized parental species for intergeneric hybridization. Only recently, extraordinary drought tolerance has been identified in the wild fescues including *Festuca glaucescens* and *Festuca mairei*. Targeted introgression of drought tolerance from these fescues into ryegrass species or existing *Festuloliums* could represent an efficient way to develop new varieties adaptable for ongoing climate changes. Besides drought tolerance, other traits including fast spring growth, disease tolerance/resistance and feeding quality are the other targets of such introgressions. With employment of molecular tools recently available, such development could be much faster and more efficient than previously thought.

Genome dominance in interspecific and intergeneric hybrids of *Lolium*, *Festuca* and *Festulolium*

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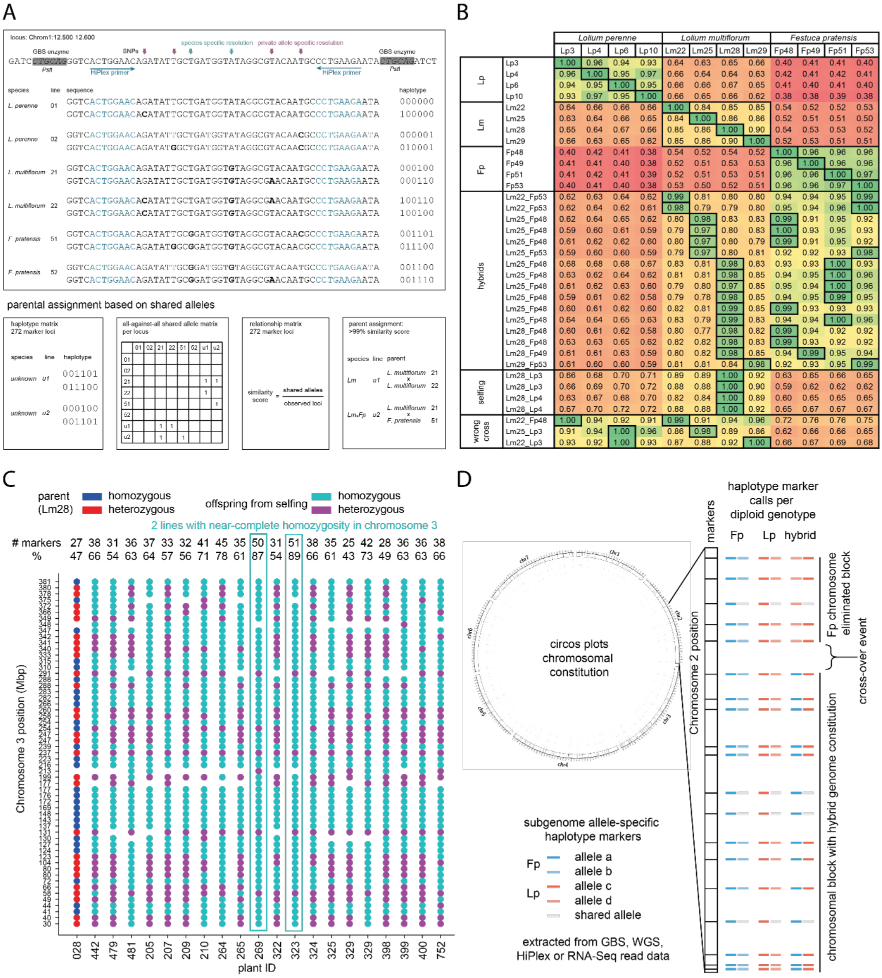
Interspecific hybrids bring the genomes of two species together within one nucleus, thus combining the genetic blueprints controlling agronomically interesting traits from both progenitor species in a novel organism. Whole Genome Duplication (WGD), the process whereby genomes are doubled and lead to polyploids, and interspecific hybridization are major contributors to genome diversity and evolution. In this study, we generate a set of hybrids covering a range of *within-* and *between-*species genetic diversity at haploid, diploid, and tetraploid level, including *Festuca-Lolium* hybrids, also called *Festulolium*. The *Festulolium* genome is known to be unstable and the *Festuca* subgenome tends to be progressively eliminated. We aim to study the effects of polyploidy and hybrid genome diversity on genome dosage, genome stability, allelic diversity, and transcriptional networks in the first few generations after the hybridization event. We will implement cytogenetic, genomic, and bioinformatics tools and use haplotype-based molecular markers to examine chromosome constitution and transcriptome profiling. In subsequent generations, we will investigate genome dominance at the level of chromosomal constitution to study the process of partial and gradual genome elimination; allele-specific expression to study species-specific regulation of expression modules and gene silencing; and the cellular and molecular genetic processes underlying genome dominance in polyploids and genomic shock after merging of divergent genomes.

We first fingerprinted four parental genotypes per species (*L. perenne* (*Lp*), *L. multiflorum* (*Lm*), *F. pratensis* (*Fp*)) using GBS, and identified a set of multi-allelic loci that discriminated between the parental lines by haplotyping GBS data with the SMAP software (Schaumont *et al.*, 2022) (**Figure 1A**). Next, two primers per discriminatory locus were designed to amplify the polymorphic region in a single HiPlex PCR reaction for 272 loci. Hybrid progeny plants were genotyped and all alleles were compared to the parental alleles across all loci to calculate a pairwise similarity score. The allele similarity score varied within species, be-



		Lolium perenne				*Lolium multiflorum*				*Festuca pratensis*			
		Lp3	Lp4	Lp6	Lp10	Lm22	Lm25	Lm28	Lm29	Fp48	Fp49	Fp51	Fp53
Lp	Lp3	1.00	0.96	0.94	0.93	0.64	0.63	0.65	0.66	0.40	0.41	0.41	0.40
	Lp4	0.96	1.00	0.95	0.97	0.66	0.64	0.64	0.63	0.42	0.41	0.42	0.41
	Lp6	0.94	0.95	1.00	0.95	0.66	0.66	0.67	0.65	0.41	0.40	0.41	0.40
	Lp10	0.93	0.97	0.95	1.00	0.66	0.65	0.66	0.62	0.38	0.38	0.39	0.38
Lm	Lm22	0.64	0.66	0.66	0.66	1.00	0.84	0.85	0.85	0.54	0.52	0.52	0.53
	Lm25	0.63	0.64	0.66	0.65	0.84	1.00	0.86	0.86	0.52	0.51	0.51	0.50
	Lm28	0.65	0.64	0.67	0.66	0.85	0.86	1.00	0.90	0.54	0.53	0.53	0.52
	Lm29	0.66	0.63	0.65	0.62	0.85	0.86	0.90	1.00	0.52	0.53	0.51	0.51
Fp	Fp48	0.40	0.42	0.41	0.38	0.54	0.52	0.54	0.52	1.00	0.96	0.96	0.96
	Fp49	0.41	0.41	0.40	0.38	0.52	0.51	0.53	0.53	0.96	1.00	0.96	0.96
	Fp51	0.41	0.42	0.41	0.39	0.52	0.51	0.53	0.51	0.96	0.96	1.00	0.97
	Fp53	0.40	0.41	0.40	0.38	0.53	0.50	0.52	0.51	0.96	0.96	0.97	1.00
hybrids	Lm22_Fp53	0.62	0.63	0.64	0.62	0.99	0.81	0.80	0.80	0.94	0.94	0.95	0.99
	Lm22_Fp53	0.62	0.64	0.62	0.61	0.98	0.79	0.79	0.80	0.95	0.94	0.96	1.00
	Lm25_Fp48	0.62	0.64	0.65	0.62	0.80	0.98	0.83	0.83	0.99	0.91	0.95	0.92
	Lm25_Fp48	0.59	0.60	0.61	0.59	0.79	0.97	0.80	0.83	1.00	0.93	0.95	0.93
	Lm25_Fp48	0.61	0.62	0.62	0.60	0.80	0.97	0.80	0.79	0.99	0.91	0.95	0.92
	Lm25_Fp53	0.59	0.60	0.60	0.59	0.79	0.97	0.80	0.81	0.92	0.92	0.93	0.98
	Lm25_Fp48	0.68	0.68	0.68	0.67	0.83	0.81	0.98	0.83	0.93	0.93	1.00	0.93
	Lm25_Fp48	0.63	0.62	0.64	0.62	0.81	0.81	0.98	0.85	0.94	0.95	1.00	0.96
	Lm25_Fp48	0.61	0.62	0.63	0.60	0.79	0.80	0.97	0.85	0.95	0.94	1.00	0.96
	Lm25_Fp48	0.59	0.61	0.62	0.58	0.79	0.78	0.98	0.85	0.94	0.95	0.99	0.95
	Lm25_Fp48	0.60	0.61	0.62	0.60	0.82	0.81	0.99	0.85	0.99	0.93	0.93	0.93
	Lm25_Fp48	0.64	0.64	0.65	0.63	0.83	0.79	0.99	0.83	0.94	0.94	1.00	0.96
	Lm28_Fp48	0.60	0.61	0.62	0.58	0.80	0.77	0.98	0.82	0.99	0.94	0.92	0.92
	Lm28_Fp48	0.63	0.63	0.65	0.62	0.82	0.82	0.98	0.83	0.99	0.93	0.92	0.92
	Lm28_Fp49	0.61	0.61	0.63	0.59	0.80	0.82	0.98	0.85	0.94	0.99	0.95	0.94
	Lm29_Fp53	0.61	0.60	0.60	0.59	0.81	0.81	0.84	0.98	0.92	0.93	0.95	0.99
selfing	Lm28_Lp3	0.66	0.68	0.70	0.71	0.89	0.89	1.00	0.92	0.63	0.65	0.66	0.65
	Lm28_Lp3	0.66	0.69	0.70	0.72	0.88	0.88	1.00	0.90	0.59	0.62	0.62	0.62
	Lm28_Lp4	0.63	0.67	0.68	0.70	0.82	0.84	1.00	0.89	0.60	0.65	0.65	0.65
	Lm28_Lp4	0.67	0.70	0.72	0.72	0.88	0.88	1.00	0.92	0.65	0.67	0.67	0.67
wrong cross	Lm22_Fp48	1.00	0.94	0.92	0.91	0.99	0.91	0.94	0.96	0.72	0.76	0.76	0.75
	Lm25_Lp3	0.91	0.94	1.00	0.96	0.86	0.98	0.89	0.86	0.62	0.66	0.66	0.65
	Lm22_Lp3	0.93	0.92	1.00	0.93	0.87	0.88	0.92	1.00	0.66	0.67	0.69	0.68

Figure 1. The versatile use of discriminatory multi-allelic haplotype markers in *Festulolium* hybrids. A. Per locus, neighboring SNPs are joined into haplotypes to increase genetic resolution. The fraction of loci with shared alleles is used to score Jaccard genetic similarity in pairwise comparisons. **B**. Haplotyping facilitates parental assignment based on allele similarity at 272 discriminatory HiPlex loci spread across the 7 chromosomes. **C**. Haplotype dosage plots reveal homozygous segments per chromosome in selfings. **D**. Circus plots show parent-of-origin per haplotype marker locus and delineate chromosomal segments by shared ancestral state of neighboring loci.

tween species, and allows to identify crosses between species (hybrids), as well as crosses within species, and selfings (**Figure 1B**). Haplotype dosage plots were used to identify individuals with homozygous chromosomes which can be used for genome sequence assembly without multiple generations of inbreeding or creation of doubled haploid lines (**Figure 1C**). Haplotypes are also used to create genome-wide chromosome constitution profiles that show parent-of-origin assignment of chromosomal segments. Projection of the gene space in the segments between adjacent markers lists gene-sets at the allele level, per individual. It can also be used to locate and quantify cross-over events and genome shuffling (**Figure 1D**). We are now cataloguing transcriptome-wide haplotype markers using WGS shotgun data and sliding frames in predicted genes to simulate the possibilities of using haplotype markers in transcripts to quantify allele-specific expression in pairwise combinations of interspecific hybrids. This will be applied to study expression level dominance, where genes of one subgenome take over control of gene networks, while chromosome elimination gradually shifts the chromosome constitution towards *Lolium* chromosomes, thus perturbing gene network composition.

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Deciphering genome dominance in *Festulolium*

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Allopolyploidization entailing the merger of two or more distinct genomes in a single hybrid organism is an important process in plant evolution and a valuable tool in breeding programs. However, non-equal segregation of parental chromosomes to the successive generations can occur in some interspecific hybrids. The mechanism of biased transmission is still not well elucidated but differential behavior of parental chromosomes during meiosis seem to be crucial. Our study encompassed analysis of chromosome composition of the F_2 generation of *Festuca* × *Lolium* hybrids and reciprocal backcrosses to elucidate effects of male and female meiosis on the shift in parental genome composition. We found that *Lolium* and *Festuca* homoeologues were transmitted differently to the F_2 generation. Female meiosis led to the replacement of *Festuca* chromosomes by their *Lolium* counterparts. In male meiosis, centromeres/kinetochores of *Festuca* univalents were attached to microtubules of spindle less frequently than those of *Lolium* univalents. They lagged in divisions and formed micronuclei, which were subsequently eliminated. Genome sequence analysis revealed a difference between parental (*Lolium* and *Festuca*) forms of kinetochore genes; there was a number of non-synonymous mutations between parental copies. Further, we found that half of the *Festuca* chromosome 7, a region were two key outer kinetochore proteins NDC80 and NNF1 are located, is heavily silenced prior and during meiosis. This resulted in the less efficient kinetochore assembly of the *Festuca* univalents compare to *Lolium* ones, a phenomenon confirmed by immunulocalization experiments. We hypothesize that silencing of *Festuca* alleles results in improper attachment of *Festuca* chromosomes to karyokinetic spindle and subsequently their gradual elimination.

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New insights of interspecific genetic variability within and between 4x *Festulolium* hybrid cvs by using KasPar markers

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In this study, we aimed to give evidence of selection possibly acting in the course of *Festulolium* breeding through the quantification of *Festuca* introgression both between and within chromosomes. For this, a set of forty-one SNPs was sampled from the library of 295 highly genome-specific SNPs developed by Neau E. et al. (2021). The chromosome location of the SNPs was defined by blasting the 100-bases sequence embedding each SNP onto the *L. perenne* sequence resulting in a chromosome covering of 4 SNPs (chr. 3) to 8 (chr. 2 and 7). (https://plants.ensembl.org/Lolium_perenne/Tools/Blast/).

Six tetraploid *Festulolium* cvs were genotyped: 4 cvs derived straightforwardly from *L. multiflorum* × *F. pratensis* amphiploids (cvs AberNiche, Achilles, Hostyn and Lifema); one cv, LoFa, comes from first *L. multiflorum* × *F. arundinacea* hybridization followed by one generation of back-cross into *L. multiflorum*. A new *Festulolium* cv, AberRoot, recently released as a *L. perenne* × *F. mairei* hybrid was also used. Thirty-six individuals per cv were assayed with 6 individuals per parent species (Lm, Fp and Fg) as controls together with 6 samples mixing the same 6 Lm and Fp pure DNA at a rate of 3:1, 1:1 and 1:3. The *Festuca* genome introgression was compared across cvs by computing the mean frequency of the *Festuca* allele, per cv and per SNP/cv, and implementing ANOVA according to the model: Cultivar + Chromosome + SNP nested in Chromosome and relevant interactions.

The mean rate of introgression was found to highly differ between all cvs, in good agreement with previous genome estimates from chromosome painting (fig. 1). Thus, Kopecký et al. (2006) observed that the mean number of translocated chromosome ranges, on average, from 11 (cv AberNiche), 16 (cv Achilles), 23 (cv Hostyn) to less than one in the cv LoFa.

Between chromosomes, variability across cvs appears to be low at high mean introgression rate as in cv Achilles, Hostyn and Lifema displaying a similar profile over all the 7 homeologous chromosome (fig. 2). On the other hand, the cv AberNiche seems to have retained more SNPs of *Festuca* origin on the chromosome 1, 2 and partly 7 than on the others. At very low frequency, the mapping of

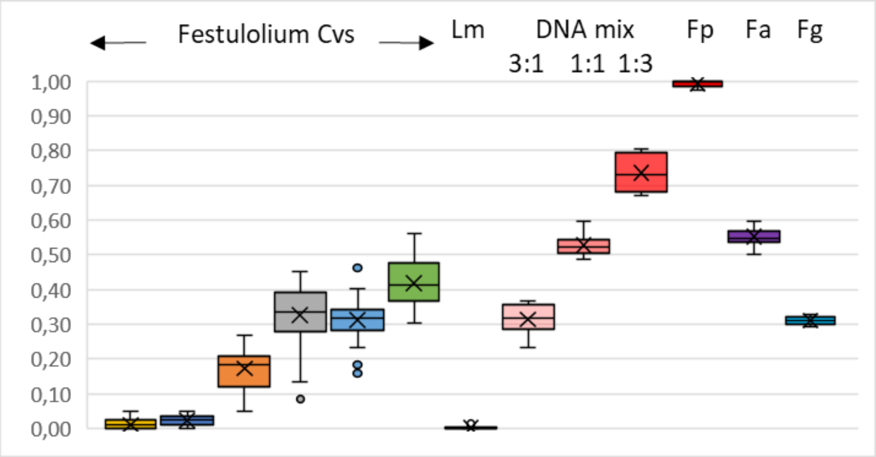

Fig. 1. Boxplots of the mean individual frequency of the *Festuca* allele across 41 SNPs by KasPAr chemistry. From left to right, the *Festulolium* cvs range from LoFa, AberRoot, AberNiche, Achilles, Hostyn and Lifema. In addition to pure DNA of *F. glaucescens* (Fg) and *F. arundinacea* (Fa), the controls include DNA mixtures in 3:1, 1:1, 1:3 ratio of pure *L. multiflorum* (Lm) with *F. pratensis* (Fp).

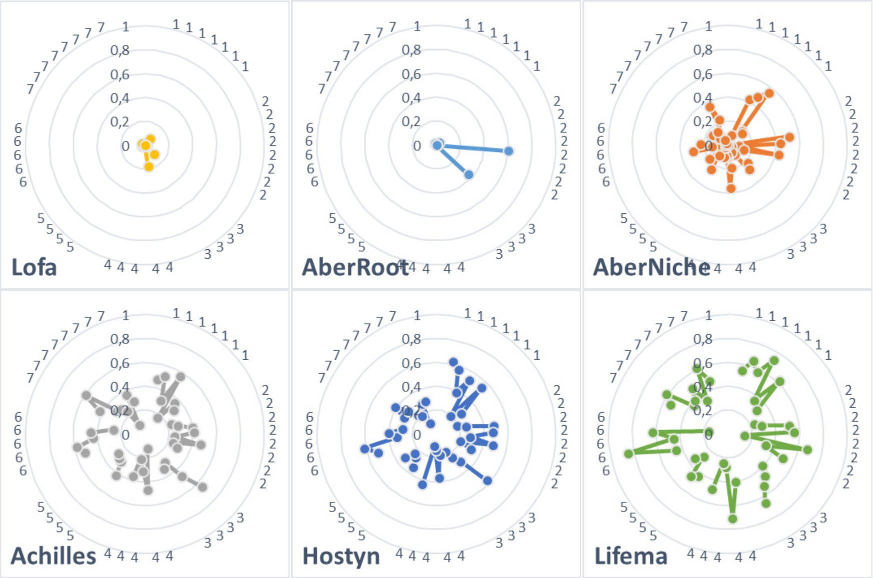

Fig. 2. Mean frequency of the *Festuca* allele at 41 SNPs and chromosome distribution in 6 *Festulolium* cvs. The SNPs are ordered clockwise between chromosomes and ranked along the DNA sequence of *L. perenne* within the chromosome.

SNPs enabled to state that introgression in cv LoFa involves two chromosomes (3 and 4) at least, by 6 and 7 individuals off 36. Introgression was even more emphasized in cv AberRoot by 25 and 20 individuals carrying the *Festuca* allele on chromosome 2 and 3 (resp.).

Within chromosome, however, the distribution of introgression rate did not show simple pattern suggesting that genetic recombination ability and chromosome pairing could primarily control *Festuca* introgression through location of the SNPs on the chromosome sequence.

Thus, it is concluded that loss of *Festuca* chromosomes through *Festulolium* breeding could be more driven by chance than by selection. In this context, SNP polymorphism gives new opportunities to state how single locus *Festuca* introgression may impact the phenotype through association studies and enhance new breeding.

Development and characterization of productive and resilient *Festulolium* hybrids for future climate

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In recent years, the observed weather anomalies left no doubts about climate change and its effect on crop productivity in Europe and worldwide. Rising temperatures, altered amounts and pattern of precipitation, shifts in the length of growing season affect overwintering, growth and development of crops at the cultivar or even species level resulting in frequent losses of yield (Jaškūnė et al. 2022). Moreover, climate change may impact grassland composition and grassland quality, having consequences on feed production for livestock (Ergon et al. 2018). In order to meet these challenges and to improve the production of perennial herbage plants both breeders and farmers should carefully rethink the cultivated species. *Festulolium* varieties are widely utilized in Lithuania because they are persistent under abiotic stresses and high yielding. However, changing climate challenge the existing *Festulolium* varieties to adapt to new growing conditions and still maintain the yield. Therefore, the breeding program of *Festulolium* was reopened in 2018 aiming at development of interspecific hybrids distinguishing by combination of superior agricultural traits, coming from fescue (*Festuca*) and ryegrass (*Lolium*) parental plants. We have crossed three species of ryegrass – perennial ryegrass (*Lolium perenne* L.), Italian ryegrass (*Lolium multiflorum* Lam. ssp. *italicum*) and Westerwolths ryegrass (*Lolium multiflorum* Lam. ssp. *multiflorum*) – and five species of fescue – meadow fescue (*Festuca pratensis* Huds.), red fescue (*Festuca rubra* L.), sheep fescue (*Festuca ovina* L.), hard fescue (*Festuca trachyphylla Krajina*) and tall fescue (*Festuca arundinacea* Schreb.). The genotypes obtained by crossing Italian ryegrass × tall fescue (cv. Ugne × cv. Monas) and perennial ryegrass × meadow fescue (cv. Veja DS × cv. Alanta and cv. Veja DS × cv. Raskila) were the most resistant to abiotic stresses as well as produced the highest dry matter and seed yield. However, the leafiness of the hybrid was lower, compared to the standard *Festulolium* varieties Lina DS and Punia DS, respectively 26–30 % and 42–37 %. The cross between Italian ryegrass and red fescue resulted in an ornamental, dwarf, fescue-type hybrid, which is suitable for the development of a lawn-type variety. The F2 generation of superior hybrids were planted in the field for evaluating their performance under natural conditions. The results of the first-year experiments revealed superior winter hardiness, in particular winter kill, of perennial ryegrass × meadow fescue and perennial ryegrass × tall fescue compared to the standard varieties (Figure 1). The developed productive, tolerant to stressful environmental conditions and

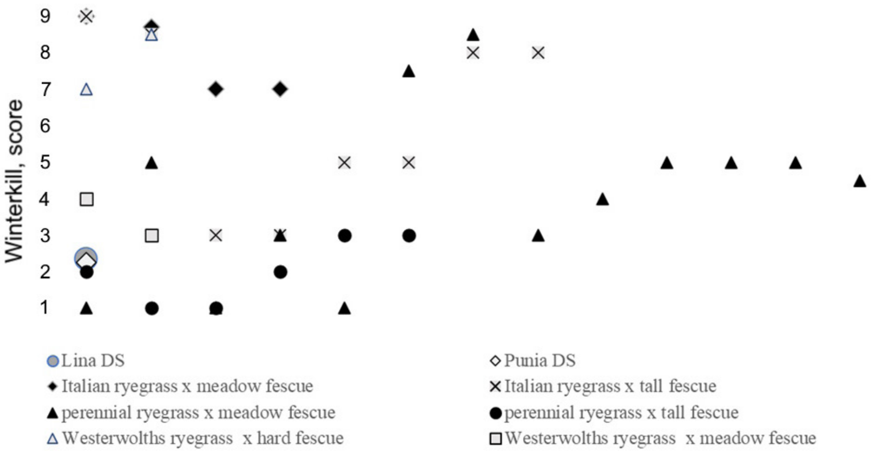

Figure 1. The mean score of winterkill of six *Festulolium* hybrids and two standard cultivars "Lina DS" and "Punia DS" over the winter of 2020/2021 and 2021/2022. Means *n = 6*.

good forage quality *Festulolium* hybrids are valuable breeding material for the development of new and commercially attractive varieties.

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Physiological and molecular indicators of winter-hardiness and frost tolerance in *Lolium perenne* × *Festuca pratensis* hybrids

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To survive winter period, plants have to sense the appropriate environmental signals (low temperature and short days) during the autumn, and respond by activating the mechanism of cold acclimation (CA), increasing their frost tolerance. In turn, upon exposure to warmer temperatures, plants resume growth and development. These warmer conditions trigger de-acclimation (DA) and consequently loss of frost tolerance. 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. However, the molecular mechanisms governing these processes, closely associated with plant winter-hardiness, are still largely unknown in forage grasses.

The *Lolium-Festuca* complex involves numerous species and hybrids crucial for grassland production in temperate regions. Some of them, have been also successfully applied as excellent plant models into the research to decipher mechanisms of tolerance to a wide range of environmental stress conditions in forage grasses.

Here, *L. perenne* (perennial ryegrass) × *F. pratensis* (meadow fescue) diploid and tetraploid hybrids were selected with respect to their winter-hardiness in the field conditions. Then, the hybrids with high and low winter-hardiness were compared analyzing the physiological and molecular indicators of frost tolerance in the sequence of cold acclimation (21 days in 4/2 °C day/night), de-acclimation (7days in 12 °C), and re-acclimation (7 days in 4/2 °C) processes in the simulated conditions in a phytotron. The study involved the analysis of: temperature causing 50% electrolyte leakage, numerous parameters of chlorophyll fluorescence, and expression of *cor14b* gene at transcript and protein levels.

The values of physiological and molecular parameters revealed differences between particular experimental time-points (CA, DA, and RA) for the analysed grass genotypes. Thus, it was proven that majority of these parameters were closely associated with the temperature conditions. Furthermore, clear differences in the values of physiological and molecular parameters were also observed at the analysed time-points between more and less winter-hardy hybrids.

Although, at this stage of work, the complete mechanism of winter-hardiness in the analysed *L. perenne* × *F. pratensis* hybrids has not been fully recognized, its crucial components have been indicated.

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Programme

Sunday 10. 9. 2023

14:00–20:00 registration and accommodation in the Hotel Avanti
14:00–18:00 cycling tour
20:00–21:00 Miroslav Trnka: Future agroclimatic conditions and implications for European grasslands

Monday, 11. 9. 2023

08:00–18:00 registration and accommodation in the Hotel Avanti

09:00–09:20 Welcome talks (David Kopecký, Tomáš Vymyslický, Jan Nedělník)
09:20–10:05 Bohumír Cagaš: Seed production of grasses and legumes in the Czech Republic and its impact on the agro and environmental spheres

10:05–10:35 coffee break

10:35–12:35 ***Session I: Genetic resources and natural diversity***
Moderators: Gillian Young and Josef Hakl

10:35–11:15 Odd Arne Rognli: Securing adaptation of timothy cultivars under climate change and during seed multiplication
11:15–11:35 Michelle M. Nay: Home sweet home – the genetic and phenotypic adaptation of red clover to European growing environments
11:35–11:55 Marie Pégard: Assessment of vulnerability to climate change in natural populations of perennial ryegrass across Europe from allele frequencies at adaptive loci
11:55–12:15 Tomáš Vymyslický: Grass and forage legume genetic resources in the Czech Republic and their practical utilization
12:15–12:35 Beat Boller: Triploid hybrids *Festuca apennina* × *Lolium* occur rarely in nature but show high colonizing potential without dominating the sward excessively

12:35–12:55 common photo

12:55–14:00 lunch

14:00–14:20 Bill Biligetu: Genetic variation and genome-environment association of alfalfa (*Medicago sativa* L.) populations originating from long-term grazing sites
14:20–14:40 Philip Greenspoon: Genetic gain from simulated forage breeding programs with genomic selection

14:40–15:00	Mareike Kavka: Shattering resistance in genetic resources of the fodder grasses *Lolium perenne* L. and *Festuca pratensis* Huds.
15:00–15:05	Monica Alexandrina Tod: Allelopathic effect of herbage water extracts on seed germination and seedling development of some perennial grassland species
15:05–15:10	Ana Uhlarik: Agronomical traits, seed colour and protein content of protein pea (*Pisum sativum* L.) cultivars grown in European conditions
15:10–15:15	Sanja Vasiljevic: Isoflavones profiles of some diploid and tetraploid red clover cultivars (*Trifolium pratense* L.) at flowering stage
15:15–15:20	Tomáš Vymyslický: Grass and forage legume genetic resources in the Czech Republic and their practical utilization
15:20–15:25	Yutang Chen: Assembling forage grass genomes in the long-read sequencing era: a case study with *Lolium perenne* L. and *L. multiflorum* Lam.
15:25–15:30	Mirjana Petrović: An initial metabolomic study on Hungarian clover
15:30–15:35	Annick Bertrand: Evaluation of alfalfa populations selected for reduced fall dormancy in a frequent cutting trial
15:35–15:40	Josef Hakl: Variability of sapogenins among *Medicago falcata* entries
15:40–15:45	Mallikarjuna Rao Kovi: Genome assembly and annotation of timothy (*Phleum pratense* L.)

15:45–16:20	coffee break

16:20–18:10 ***Festulolium Working Group Workshop***
Moderator: David Kopecký

16:20–17:00	Vladimír Černoch: History of *Festulolium* breeding in the Czech Republic and future prospects
17:00–17:20	Tom Ruttink: Genome dominance in interspecific and intergeneric hybrids of *Lolium*, *Festuca* and *Festulolium*
17:20–17:40	Joanna Majka: Deciphering genome dominance in *Festulolium*
17:40–17:45	Marie Pegard: New insights of interspecific genetic variability within and between 4x *Festulolium* hybrid cvs by using KasPar markers
17:45–17:50	Kristina Jaskune: Development and characterization of productive and resilient *Festulolium* hybrids for future climate
17:50–17:55	David Perlikowski: Physiological and molecular indicators of winter-hardiness and frost tolerance in *Lolium perenne* × *Festuca pratensis* hybrids
17:55–18:10	general discussion
19:00–22:00	welcome party at Mendel museum
20:00–21:00	Tour to the Mendel Museum (optional)

Tuesday, 12. 9. 2023

09:00–10:40 ***Session II: Advanced phenotyping and genotyping technologies***
Moderators: Mathieu Wident and Oldřich Trněný

09:00–09:40 Bernadette Julier: New phenotyping, genotyping and statistical tools for forage breeding
09:40–10:00 Jenny Kiesbauer: A nested association mapping population reveals candidate genes for stem rust resistance in Italian ryegrass
10:00–10:20 Oldřich Trněný: Red clover's genetic playground: Leveraging low-coverage resequencing for enhanced breeding programs
10:20–10:25 Stephen Byrne: Implementation of multiple cycles of genome-wide selection for seasonal forage yield in perennial ryegrass.
10:25–10:30 Joanna Pranga: Phenotyping our future crops: in-field, non-destructive, high-throughput phenotyping of above and belowground plant traits
10:30–10:35 Danny Milbourne: Use of NIR spectra to predict heading date in perennial ryegrass
10:35–10:40 Nagarjun Devabhakthini: Exploring the genetic diversity of the IPK *Medicago* germplasm collection using GBS

10:40–11:10 coffee break

11:10–11:30 Luciano Pecetti: Development and proof-of-concept application of genome-enabled selection for alfalfa biomass yield in Northern Italy: preliminary results
11:30–11:50 Girma Bedada: Haplotype-phased genomes of timothy grasses – *P. nodosum*, *P. alpinum, P. pratense*
11:50–12:10 Harkingto Harkingto: Conventional and UAV-based phenotyping to characterize a broad collection of European lucerne germplasm in a Nordic environment
12:10–12:30 Joanna Pranga: Unleashing the potential of drones: how high-throughput field phenotyping can assist forage grass breeding
12:30–12:50 Kioumars Ghamkhar: Rapid forage yield and growth rate measurement using a remote-controlled LIDAR sensor in perennial ryegrass field plots

12:50–14:00 lunch

14:00–15:15 ***Youth Session***
Moderators: Joanna Majka and Agniezska Konkolewska

14:00–14:40 Chloe Manzanares: Exploiting new discoveries on self-incompatibility for forage grass breeding

14:40–15:00 Agnieszka Konkolewska: Establishing multi-trait genomic selection for forage improvement
15:00–15:05 Ágnes Áldott-Sipos: Evaluation of silage and grain yield of different maize (*Zea mays* L.) genotypes in ecological and conventional conditions
15:05–15:10 Marlies Peeters: Optimized tetraploidisation strategies in tissue culture for *Lolium* and *Festuca*
15:10–15:15 Ferenz Sustek-Sánchez: Optimized *Lolium perenne* L. protoplasts isolation and transformation for CRISPR-Cas9 downstream applications.

15:15–15:45 coffee break

15:45–17:35 ***Session III: Qualitative and quantitative traits***
Moderators: Piet Arts and Libor Jalůvka

15:45–16:25 E. Charlie Brummer: Thirty years of alfalfa genetic markers
16:25–16:45 Hilde Muylle: GWAS for drought tolerance in red clover (*Trifolium pratense* L.)
16:45–17:05 Heathcliffe Riday: Breeding Soft-seeded Hairy Vetch
17:05–17:25 Stanislav Hejduk: Differences between di- and tetraploid red clover cultivars. A review.
17:25–17:30 Christoph Grieder: Higher seed yield through targeted selection for reduced seed shattering in Italian ryegrass (*Lolium multiflorum* Lam.)
17:30–17:35 Annie Claessens: Genetic selection for non-fiber carbohydrates in alfalfa (*Medicago sativa* L.) stem

17:40–19:00 Board meeting

Wednesday, 13. 9. 2023

08:00–18:30 Mid conference tour (Hladké Životice, Olomouc/Zubří, Troubsko)
19:30 Conference dinner – hotel Avanti (+short cultural programme)

Thursday, 14. 9. 2023

09:00–10:35 ***Session IV: Biotic and abiotic stresses***
Moderators: Beat Boller and Stanislav Hejduk

09:00–09:40 Susanne Barth: Differential growth of a panel of perennial ryegrass accessions following excess water treatment over the winter under field conditions

09:40–10:00	Tim Vleugels: Phenotypic characterisation of drought tolerance in red clover (*Trifolium pratense* L.)
10:00–10:05	Eszter Csepregi-Heilmann: Cold stress study during emergence of maize (Zea mays L.) inbred lines
10:05–10:10	Mladen Prijovic: Morpho-biochemical response perennial ryegrass (*Lolium perenne* L.) populations to water shortage
10:10–10:15	Grazina Statkeviciute: Fv:Fm and RWC measurements as indicators of drought stress response in perennial ryegrass
10:15–10:20	Roland Kölliker: Towards an efficient detection of genetic diversity in multispecies grassland
10:20–10:25	Akhil Reddy Pashapu: Surviving under ice: Insights into gene expression changes during ice-encasement in perennial grasses
10:25–10:30	Filip Bekčić: Wilt of red clover caused by *Fusarium oxysporum*
10:30–10:35	Bernadette Julier: Early morphological traits condition the performance of lucerne plants in different competitive situations
10:35–11:05	coffee break
11:05–11:25	Charlotte Jones: Recurrent molecular selection for improved field resistance to crown rot (*Sclerotinia trifoliorum*) in red clover (*Trifolium pratense*)
11:25–11:45	Gillian K. Young: Evaluating root characteristics under field conditions in perennial ryegrass for potential application in commercial breeding programmes
11:45–12:05	Shridhar Jambagi: Red clover root microbiota and interaction of root rot and clover rot pathogens
12:05–12:25	Reah Gonzales: Divergent responses of perennial ryegrass and tall fescue to drought stress
12:25–12:45	Silvana Moreno-Vallejo: Response to water logging and drought in wild and domesticated accessions of timothy (*Phleum pratense*) and its wild relatives *P. alpinum* and *P. nodosum*
12:45–13:30	***Business meeting (info from board, overview), prize giving ceremony, take-home messages***
13:30	lunch
15:00–18:00	Post-conference excursion – guided tour in Brno

Novel technologies, strategies and crops to sustain forage production in future climate

Abstracts of the 35th Meeting of the EUCARPIA Fodder Crops and Amenity Grasses Section in cooperation with the EUCARPIA *Festulolium* Working Group

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The Book of Abstracts of the 35th International Conference of the Fodder Crops and Amenity Grasses Section of EUCARPIA 'Novel technologies, strategies and crops to sustain forage production in future climate' summarizes the latest findings in breeding, ecology, physiology and genetics of forage grasses, clovers and amenity grass species. The book contains over sixty contributions from authors from Europe, America, Australia and New Zealand. We are living in a time of increased climate change, with more frequent occurrences of severe climate catastrophic events, as well as less visible minor climate changes, both of them leading to reduced sustainability of crop, and downstream livestock production. The central theme of the conference is therefore the timely topic of adaptation to climate change. This includes ecological adaptations of plants, but also modifications of breeding approaches, as well as the introduction of new technologies and strategies to successfully combat climate change. The aim of the conference and of the papers summarized in this book of abstracts is therefore to provide the latest information regarding research and breeding of forage and amenity grasses for future climatic conditions.

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