Minutes from 6th EUCARPIA Festulolium Working Group Workshop

6th EUCARPIA Festulolium Working Group Workshop was organized by Centre of Plant Structural and Functional Genetics and DLF Hladké Životice in Olomouc, April 5-6. In total, 30 grass researchers and breeders from 11 countries participated the meeting. During five sessions, 19 talks were presented including two invited speeches of Beat Boller and Hana Šimková. In the first day, the tour to DLF breeding station in Hladké Životice and to the native house of Gregor Mendel was realized. The last, but not least, part of the workshop was the general discussion. Six topics were discussed:

1, The publication of the multilocation Festulolium field trial.

The results on three-years field evaluation of various Festulolium cultivars and their genotyping will be published as soon as possible. The responsible person for manuscript preparation is Marc Ghesquiere with help of Vladimir Černoch, David Kopecky, Mike Humphreys and other people involved in Festulolium field trial.

2, New Festulolium field trial

New field trial was proposed. The proposition was broadly accepted and many representatives of European countries (including those not participated on the initial field trial) displayed their interest. There are two options:

1, to repeat the initial trial with another existing cultivars or genotypes

2, to evaluate mixtures (this idea was proposed by representatives of Germany (Peter Westermeier and Stephan Hartmann) and broaden by Vladimir Černoch. The idea is to use 6-7 mixtures (4-5 local mixtures and 2 standard used in all trials), where one variant will be a mixture without Festulolium, one variant will be a mixture, where one component is replaced by Festulolium (the cultivar proposed to by the most suitable for the country, based on results from initial trial) and the third variant will be Festulolium cultivar by itself. This means 3x(6-7) = 18-21 rows. Three replicates will be sown. All these should be silage mixtures.

The year of establishment of new trial is assumed to be 2017. The responsible person will be Vladimir Černoch (vc@dlf.cz) and the breeders and researchers interested in participation on the field trial are encouraged to email him and propose the plant material to be included in the new trial.

3, Development of genotyping platform for analyses on genome composition, genetic diversity, phylogenetic relationships and Festulolium **breeding**

The development of the genotyping platform using SNP markers has been proposed and will be the topic for later discussion in conjunction with the previous topic as far as possible. M. Ghesquière suggested continuing to exchange actively ideas on this currently in 2016 and early in 2017 before the next meeting of the Group in Lithuania by September 2017.

4, The European project proposals

The call for Horison2020 is too soon to be suitable for the Festulolium community and thus, there will be no proposition for the common project. The other options were pointed (such as COST) and will be the topics for later discussions.

5, The core group of EUCARPIA Festulolium Working Group

The head of EUCARPIA Festulolium Working Group Prof. Mike Humphreys stepped down. The election for new head of the group was realized. Dr. Marc Ghesquiere was proposed and accepted as a new head of the group. The group expressed many thanks to Prof. Humphreys for his work as a head since the establishment of the group in 2010. The core group will be working in the same constitution: Liv Østrem, Vladimir Černoch, Marc Ghesquiere, Mike Humphreys and Zbigniew Zwierzykowski.

6, Next EUCARPIA Festulolium Working Group Workshop

Next workshop will be held in the frame of EUCARPIA Fodder Crops and Amenity Grasses conference in Lithuania (September, 2017). The Festulolium Working Group would like to express many thanks to Dr. Gintaras Brazauskas (the head of organizing committee) for hosting next Festulolium Working Group Workshop.

David Kopecký (the head of organizing committee)

Marc Ghesquiere (the head of EUCARPIA Festulolium WG)

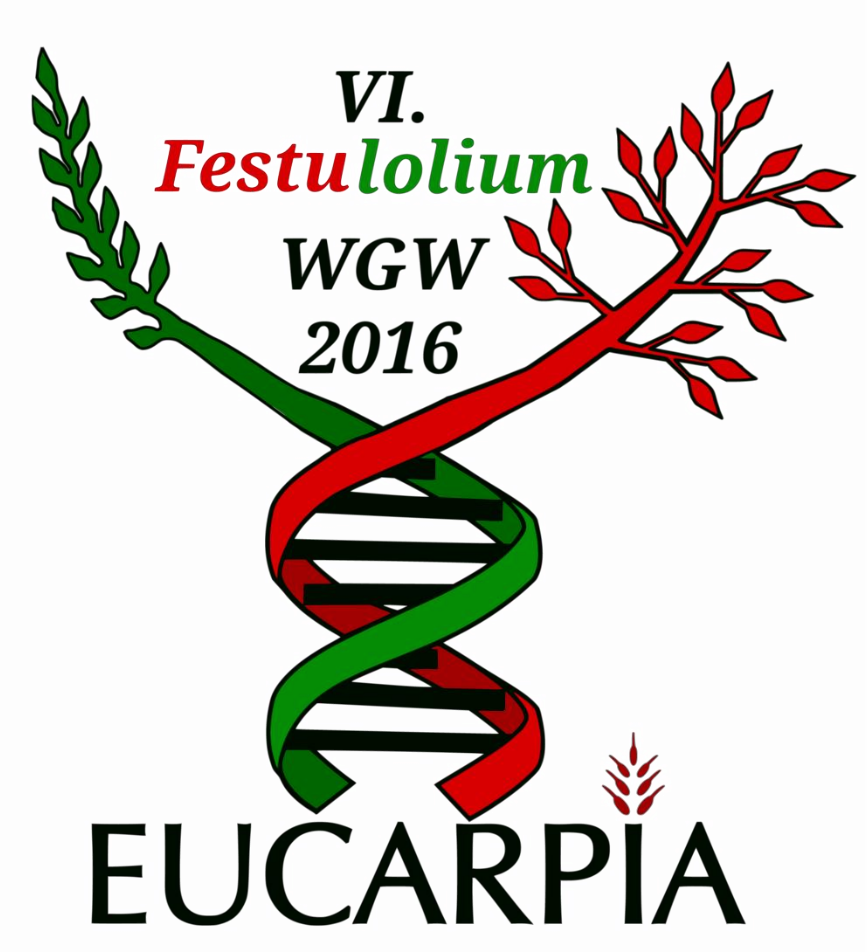
Mike Humphreys (the ex-head of EUCARPIA Festulolium WG and the member of core group)

VI.
Festulolium
WGW
2016

VI. Festulolium Working Group Workshop

5 - 6 April, 2016

Olomouc, Czech Republic



Editors: Štěpán Stočes and David Kopecký

Institute of Experimental Botany, Olomouc, 2016

Program of 6th Festulolium Working Group Workshop

Day 1: Tuesday April 5

8:30	Departure from Arigone hotel
9:00-9:20	Opening of Workshop
9:20-10:00 Invited talk:	**Beat Boller** (Agroscope, Switzerland): *Potential and limits of breeding hybrid ryegrass, a commercially successful forage species hybrid.*

Session I: Breeding, phenotyping and physiology (Chairman: Liv Østrem)

10:00-10:05	**Grazina Statkeviciute** (LRCAF Institute of Agriculture, Lithuania): *Festulolium breeding in Lithuania: an overview.*
10:05-10:10	**Joost Baert** (ILVO, Belgium): *Comparison of Festulolium as mid-term ley for silage with other cool season grasses.*
10:10-10:15	**Dejan Sokolovic** (Institute of Forage Crops, Serbia): *Summer persistency of different Festulolium hybrids in Serbian climate.*
10:15-10:20	**Stanislav Hejduk** (Mendel Univeristy, Czech Republic): *The differences among root systems of Festuloliums and the parent species.*
10:20-10:25	**Vilma Kemesyte** (LRCAF Institute of Agriculture, Lithuania): *Winter survival evaluation of the Lithuanian Festulolium cultivars.*
10:25-10:30	**Stephan Hartmann** (Bavarian State Research Center for Agriculture, Germany): *Widening the genetic diversity of Bavarian meadow fescue (Festuca pratensis HUDS.) genepool by introgression of perennial ryegrass (Lolium perenne L.)*
10:35-11:15	Coffee break

Session II: Ecology and breeding (Chairman: Vladimír Černoch)

11:15-11:40	**Mike Humphreys** (IBERS, Wales): Developing climate-smart grasslands
11:40-12:05	**John Harper** (IBERS, Wales): Festulolium hybrids in a changing climate, what can they offer? A story from the flood plains to the high mountains.
12:05-13:05	Lunch
13:05	Departure to DLF Hladké Životice
13:50	Arrival to Hladké Životice
13:50-15:15	Visit of DLF Hladké Životice
15:15-16:00	Coffee break
16:15	Departure to Hynčice
16:45	Arrival to Hynčice
16:45-17:45	Visit of birthplace of G. J. Mendel
18:00-21:00	Conference Dinner
21:15	Departure to Olomouc
22:00	Arrival to Olomouc

Day 2: Wednesday April 6

8:30	Departure from Arigone hotel
9:00-9:40 Invited talk:	**Hana Šimková** (Institute of Experimental Botany, Czech Republic): *BioNano optical mapping: an emerging technology to analyse complex genomes*

Session III: Molecular biology, cytogenetics and cytometry (Chairman: Zbigniew Zwierzykowski)

9:45-9:50	**Dimitra Loka** (IBERS, Wales) *How one stress might impact on another.*
9:50-9:55	**Veronika Burešová** (Institute of Experimental Botany, Czech Republic): *3D organization of plant nucleus in interspecific hybrids.*
9:55-10:00	**Laiane Corsini Rocha** (Federal University of Lavras, Brasil): *Particularities in the behavior of 45S rDNA sites of Lolium and Festuca.*
10:00-10:05	**Tomasz Książczyk** (Institute of Plant Genetics, Poland): *Obtaining and cytogenetic identification of reciprocal hybrids between Festuca glaucescens × Festuca pratensis.*
10:05-10:50	Coffee break

Session IV: Genomics and transcriptomics (Chairman: Marc Ghesquiere)

10:50-11:15	**Eva Hřibová** (Institute of Experimental Botany, Czech Republic): *Comparative analysis of repetitive DNA in eight representatives of fescues and ryegrasses.*
11:15-11:40	**Mallikarjuna Rao Kovi** (Norwegian University of Life Sciences, Norway): *Festulolium transcriptome changes during cold acclimation, deacclimation and reacclimation.*
11:40-12:05	**Štěpán Stočes** (Institute of Experimental Botany, Czech Republic): *Gene expression in interspecific hybrids.*

12:05-13:00 Lunch	
13:00-13:45	Lab Tour

Session V: Festulolium common trial (Chairman: Mike Humphreys)

13:45-14:10	**David Kopecký** (Institute of Experimental Botany, Czech Republic): *Genotyping of Festulolium.*
14:10-14:35	**Marc Ghesquiere** (INRA, France): *From phenotype to genotype: the case-study of a common field trial initiated through Eucarpia for promoting Festulolium breeding.*
14:35-15:10	Coffee break
15:10-15:45	**Vladimir Černoch** (DLF Hladké Životice, Czech Republic): *Results of multilocation X Festulolium trials*
15:45-17:15	**General discussion on the Festulolium common field trial**
17:15-17:20	**General conclusions and meeting closure**
17:30	**Departure to Olomouc city center**
18:00-19:00	City Tour of Olomouc
19:00-22:00	Conference Dinner

Abstracts of Oral presentations

Potential and limits of breeding hybrid ryegrass, a commercially successful forage species hybrid

Beat Boller and Christoph Grieder

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Hybrid ryegrass, *Lolium x boucheanum* Kunth (syn. *Lolium x hybridum* Hausskn.) is an interspecific hybrid involving Italian ryegrass (*Lolium multiflorum ssp. italicum* Volkart ex Schinz et Keller) and perennial ryegrass (*Lolium perenne* L.). Hybrid ryegrass combines the characters of both parent species in different ways. The resulting varieties can range from almost completely Italian types, characterized by a strong tendency to form inflorescences in the summer months ("re-heading types"), broad leaves and the presence of long awns to *perenne* types which do not "re-head" more than perennial ryegrass, have narrow leaves and no or just very short awns. Most varieties however are classified as intermediate.

Hybrid ryegrass breeding has a long history. Reportedly, Dr John Garton, a pioneer of scientific based plant breeding in the United Kingdom of the late 19th century, deliberately crossed perennial and Italian ryegrass to produce unusual plant types and created a stable strain that exhibited desirable characters of both parent species. Modern variety breeding of hybrid ryegrass was first carried out in New Zealand, with "Grasslands Manawa" released in 1943, a variety which is still in use today. The first *perenne x multiflorum* crosses at our Agroscope breeding program were carried out in 1973, directly followed by colchicine treatment, and resulted in the release of 'Gazella' in 1985. Until today, we released 16 more varieties, mostly tetraploids. Breeding progress in DM yield of hybrid ryegrass over the years was similar to the two parent species (about 0.4 % / year).

An analysis of the results of plot trials with candidate varieties from our program over the past 20 years shows that in dry matter yield and forage quality (digestibility of organic matter DOM), on average, hybrid ryegrass behaves intermediate, but at the tetraploid (4x) level tends to perform closer to the better of the parent varieties. DM yield in both first and second main harvest years of tetraploid hybrid ryegrass was only slightly and insignificantly (-0.5 and -3.4 %) lower than Italian ryegrass but significantly higher (+3.9 and +9.6 %) than perennial ryegrass. DOM of tetraploid hybrid ryegrass was 13.2 g/kg lower than perennial but 14.7 g/kg higher than Italian ryegrass. When comparing hybrid ryegrass candidates with varying tendency to re-heading, there is a strong negative correlation

of re-heading with DOM (-0.78) but only a slight positive correlation with DM yield (+0.15 and +0.21 for H1 and H2). Therefore, it is possible to select high yielding and weakly re-heading varieties with high DOM content.
Commercial success of hybrid ryegrass is quite consistent over the years, but varies considerably among countries. In the European Union as a whole, seed production of hybrid ryegrass in 2014 and 2015 represented only 3 and 4 % of all ryegrass seed produced. However, for example in France, the respective figures were 14 and 27 %.

It appears that the easy crossing of *perenne* and *multiflorum* permits good breeding progress in hybrid ryegrass because the most advanced breeding material of both species can be used to enhance the breeding pool. However, commercial success does not readily follow breeding progress except where the species is well established in the market.

Festulolium breeding in Lithuania: an overview

Gražina Statkevičiūtė, Vilma Kemešytė, Gintaras Brazauskas

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The history of Festulolium breeding in Lithuania was initiated in 1979 when the first interspecific crosses were performed at the LRCAF Institute of Agriculture (former Lithuanian Institute of Agriculture). Over 100 breeding lines have since reached the final stages of field testing trials and 4 varieties, namely Punia DS, Puga, Vėtra and Lina DS, were registered in Common European Variety List in total. Various crossing combinations have been used in breeding programmes and/or research projects, including species of genus *Lolium*: *L. perenne, L. multiflorum* ssp *multiflorum* and *L. multiflorum* ssp *italicum*, and *Festuca*: *F. pratensis, F. arundinaceae* and *F. glaucescens*. *Lolium* x *Festuca* hybrid fertility restoration was achieved by treating immature embryos, shoot tips or immature inflorescences with colchicine solution *in vitro*, the maximum success rate was achieved in hybrid embryo treatment. Two of the registered varieties, Punia DS and Puga, were obtained from crosses between *F. pratensis* and *L. multiflorum* ssp *italicum*, Vėtra was developed from crossing *F. arundinaceae* and *L. multiflorum* ssp *italicum* and Lina DS resulted from crossing *F. pratensis* and *L. multiflorum* ssp *multiflorum*. All varieties were highly tolerant to diseases and had good overwintering, the latter being one of the main priorities in local breeding programmes, yet later focus was diverted to breeding of other perennial grasses. The last interspecific crosses were performed in 2000 and the latest variety (Lina DS) was registered in 2012. Considering growing interest from local seed production companies and farmers, future plans include re-opening of Festulolium breeding at the LRCAF Institute of Agriculture.

Yield and feeding value of Festulolium as mid-term ley compared to other cool season grasses

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The yield and feeding quality of two cultivars of each of the species *Festulolium*, *Festuca arundinacea*, *Festuca pratensis*, *Lolium perenne* and *Lolium hybridum* were compared under a cutting management in three successive years at two N-application levels.

Festulolium and hybrid ryegrass had the highest annual yield in the first year. From the second harvest year onwards tall fescue was the highest yielding species. Meadow fescue always had the lowest yield. Festulolium and hybrid ryegrass had on average the highest yield in the first cut.

Perennial ryegrass was the most rust susceptible species. Festulolium and hybrid ryegrass showed most reheading in summer.

Festulolium had a significantly higher digestibility and content of water soluble carbohydrates (WSC) than tall fescue but a generally lower digestibility and WSC content than perennial and hybrid ryegrass.

Summer persistency of different Festulolium hybrids in Serbian climate

Dejan Sokolović[1], Mike Humphreys[2], Marc Ghesquière[3], Vladimir Cernoch[4], Joost Baert[5], Susanne Barth[6], Liv Østrem[7], Zbigniew Zwierzykowski[8], Snežana Babić[1], Aleksandar Simić[9], Jasmina Radović[1]and Zoran Lugić[1]

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The idea of *Festulolium* hybrids creating originated from need to combine some characteristics from both *Festuca* and *Lolium* species. Initially, scheme was to incorporate *Festuca* tolerance to abiotic stresses in *L.perenne* and *L.multiflorum,* which represent high yielding and digestible fodder plants compatible to intensive grasslands management. All *Festulolium* cultivars of predominant contribution of *Lolium* genome (Lolioid type) are productive, with high quality and digestible forage. But, since their areal of cropping is somewhat limited according to *Lolium* drought susceptibility, are they more tolerant to abiotic stresses, especially to moisture reduction.

This investigation was part of the common *Festulolium* trial was performed on 8 locations across European continent. Productivity and persistency of 15 different Lolioid *Festulolium* cultivars was investigated in moderate continental climate in Kruševac, Serbia. Plots in the trial (7,5m^2) were completely randomised, each in 3 replications. Summer persistency was analyzed by evaluation of ground cover reduction after summer periods in second and third year. Concerning hybrid combination, best persistency (38.5% of coverage reduction in third year) was determined for *LmFg*, even thought there was only one cultivar of this type. Majority of cultivars were *L.multiflorum* and *F.pratensis* hybrids (8) and they showed residual coverage between 30 and 52%.

If we compare two different approaches in *Festulolium* breeding, better persistency had cultivars obtained by amphiploidisation (62.3% of coverage reduction). In the same time reduction of stand density of cultivars created by introgression was more than 77%.

General conclusion for the trail in Serbian droughty climate is that majority of cultivars suffered from severe stand density reduction after first year of cutting in comparison with *Festuca* control cultivars, but showed significantly better persistency in comparison with *Lolium* cultivars.

Evaluation of root system and aboveground biomass of *xFestulolium* grasses in early stages of their development

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xFestulolium hybrids are widely used for forage production in the Czech Republic both on arable land and in permanent grasslands. Their advantages concerning better persistence forage quality and stresses tolerance are well known. There are some uncertainties relating their root systems. Unlike the parent species *xFestulolium* hybrids are regarded as having deeper and thicker root system what enable them to increase water uptake capacity and improve infiltration rate of soil.

We established two trials where *xFestulolium* grasses and their parent species were grown in fine sand for 62 and 111 days respectively. Following species and cultivars were used for the trial: *Festuca pratensis* (cv. Pronela), *Festuca arundinacea* (cv. Kora), *Lolium multiflorum* (cv. Moravice), *xFestulolium pabulare* (cv. Hostyn), *xFestulolium krassanii* (cv. Hykor) and *xFestulolium loliaceum* (cv. Prior). The differences in the weight of below- and aboveground biomass and leaf area, root depth, total length and surface area were evaluated. The highest biomass (roots and shoots) was found for the cv. Hostýn and cv. Moravice while the lowest values had cv. Pronela. R/S ratio ranged from 0.3 to 0.6 and was not significantly different among the cultivars. The depth of roots ranged between 160 (cv. Pronela) to 350 mm (cv. Moravice) in the 1st trial (May to July) and between 56 and 178 mm for the same cultivars in the second trial conducted under cool weather (September to December). The differences in plants morphology were related mainly to the rate of the initial development of given species.

This study has been supported by the Ministry of Agriculture of the Czech Republic, project No. QJ1330189.

Winter survival evaluation of the Lithuanian Festulolium cultivars

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The higher tolerance to unfavourable winter conditions (low temperature, unstable snow cover and snow mould attack) is important for persistence of grasses in the Nemoral environmental zone which covers Southern Scandinavia and Baltic states.

The aim of this study was to evaluate winter survival of four Festulolium cultivars developed at the Lithuanian Research Centre for Agriculture and Forestry: Lina DS (*Lolium multiflorum* subsp. *multiflorum* x *Festuca pratensis*), Punia DS (*Festuca pratensis* x *Lolium multiflorum* subsp. *italicum*), Puga (*Lolium multiflorum* subsp. *italicum* x *Festuca pratensis*) and Vėtra (*Lolium multiflorum* subsp. *italicum* x *Festuca arundinacea*). The field experiments were carried out in the Central part of Lithuania during 2007–2015. One cycle of experiment was three growing years.

During the study period the overwintering conditions differed from mild (in 2008 and 2015) to harsh (in 2010, 2013 and 2014). The mild winters were characterized by short (2–6 weeks) period of lower than -10 °C temperatures and no permanent snow cover. Plant survival rate at these conditions ranged from 93 to 100 %, whereas only 34–63 % of the plants survived during the harsh winters with permanent, however thin snow cover and permanent deep and long frost. Although the lowest damages after winters were estimated for cultivar Punia DS, while Lina DS showed poorest overwintering in all years, the dry matter yield of both cultivars were similar (11.6–11.8 t ha^{-1}). Such result could be decided by early spring growth of the cultivar Lina DS.

This work was funded through long-term research program "Genetics and purposeful change of genotypes of agricultural and forest plants" implemented by Lithuanian Research Centre for Agriculture and Forestry.

Widening the genetic diversity of Bavarian meadow fescue (*Festuca pratensis* HUDS.) genepool by introgression of perennial ryegrass (*Lolium perenne* L.)

Tatjana Lunenberg, Peter Westermeier, Stephan Hartmann

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Meadow fescue (*Festuca pratensis* HUDS.; Fp) is a grass species with high nutritional value and is well adapted to various abiotic stresses, such as frost or drought. Its use in intensive managed grassland with a high proportion of perennial ryegrass is amongst others limited due to a slow juvenile development and a low production of side shoots and thus Fp is repressed in mixed pastures with high cutting frequencies. As in Fp there is only little natural genetic variation for the traits juvenile development and competitiveness available, introgression of perennial ryegrass (*Lolium perenne* L.; Lp) was used to broaden the genetic basis of these traits. To achieve the high proportions of Fp in former Bavarian grassland before intensification, improved meadow fescue is one important component.

As a starting point diploid Lp from the Bavarian gene pool with a good winter hardiness and fast mass growth after cutting was crossed by hand pollination to diploid plants from the Bavarian Fp gene pool, followed by an embryo rescue step. The resulting F_1-*Festulolium* generation was characterized by a dramatically decreased male fertility. Manual backcrossing with Fp (BC_1) was again followed by an embryo rescue step. Within BC_1 generation fertility was restored, so further backcrosses (BC_2 to BC_4) could be done open pollinated in isolation plots.

Different traits were used for selection of progenies with the desired characteristics. In F_1 generation AFLP markers or GISH method were used to verify crossing products. In BC_1 and BC_2 generations the most feasible trait for selecting Fp types was the leaf morphology (rolled shape of emerging leaves). BC plants were also tested in controlled environments (greenhouse/climate chamber) for juvenile development and tillering (competitiveness) as well as frost tolerance and root development (abiotic stress tolerance). BC_1 and BC_2 showed a wide variation in trait characteristics and promising candidates could be selected for further studies.

This work has been supported by the Bavarian State Ministry for Food, Agriculture and Forestry (grant No. A/13/08).

Developing climate-smart grasslands

Humphreys M[1], Loka D[1], Gwynn-Jones D[1], Scullion J[1], Doonan J[1], Gasior D[1], Harper J[1], Farrell M[1], Kingston-Smith A[1], Dodd R[2], Chadwick D[2], Hill P[2], Mills G[2], Hayes F[2], Robinson D[2], Jones D[2]

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We report a new and innovative research project funded by the Wales National Research Network for Low Carbon Energy and the Environment and focused on improved grasslands. These dominate the Welsh landscape occupying 43% of Welsh lowlands and 26% of Welsh uplands, where they sustain livestock farming. Against a backdrop of increasing atmospheric CO_2, ground- level O_3 and temperature, the UK and many areas of Europe have recently witnessed unprecedented extreme weather patterns impacting on the capacity of agricultural grasslands to deliver a range of ecosystem services (e.g. food security, carbon and water storage, biodiversity). These atypical weather events include extreme floods, high levels of ground-level O_3, wildfires, prolonged periods of heat stress and drought. To future-proof the ecological, socioeconomic and cultural aspects of agricultural landscapes, land users will need to adapt management practices to meet this challenge. Central to achieving this goal is the adoption and implementation of holistic and novel climate-smart plant breeding programmes with soil- and animal-based management strategies to provide landscape resilience. Critically, we need to develop regimes which provide resistance to more than one stress (multi-stress resilience). The project aims to develop and validate new regimes specific to the protection of lowland productive grasslands.

Festulolium hybrids in a changing climate, what can they offer? A story from the flood plains to the high mountains

John Harper, Dagmara Gasior, Ian Armstead, Mike Humphreys

IBERS, Aberystwyth University, Aberystwyth, Wales, UK, SY23 3EE

We are living in a period of climate change, it is still unclear to what extent our climate is changing and the effects these changes will have. One effect, observed in recent years has been extreme rainfall events causing widespread flooding in many areas of Europe. Grassland in Europe represents an important crop with flooding being a major stress factor leading too loss of production.

In this study a total of 48 diverse accessions were included: - *L.perenne* varieties, *L.perenne* ecotypes collected from areas prone to flooding, a range of Fescue species and Festulolium hybrids collected from natural flood meadows. The plants were subjected to two temperature regimes:- 10^0 C and 20^0 C respectively and three water conditions:- 1) normal watering, 2) crown of plant continually submerged and 3) whole plant fully submerged. The experimental conditions were maintained for 3 and 6 weeks respectively. The growth and survival of these plants under different conditions were monitored and the possibility of producing new hybrids having resilience to flooding events discussed.

A constraint in producing commercial Festulolium hybrids whether it be for resilience to flooding events or other abiotic stresses is their unstable nature, creating stable hybrids is a major goal for grass breeders. We have studied a Fescue species found in the high Alpine mountains which naturally forms hybrids with other fescue species and may help in forming stable hybrid combinations. In this paper we show the cytology of this species and discuss its hybrid forms and how it may be used in the production of Festulolium hybrids.

This work has been supported by the Biotechnology and Biological Sciences Research Council (BBSRC).

Recent advances in genomics – time for a forage grass genome initiative?

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For almost half of a century, intergeneric hybrids between *Lolium* and *Festuca* species (referred to as Festulolium) have been anticipated as an opportunity to combine the agronomic advantages of both genera. Meanwhile, we have learned much about the potential and limitations of Festulolium breeding.

Due to recent technological advantages, progress has been made to establish genetic and genomic tools that could help us to understand the biological system of Festuloliums. These tools include *in situ* hybridisation and image analysis techniques, excessive marker resources, flow-sorted chromosomes, extensive transcriptome data and synteny-based draft genome assemblies. One of the major challenges remaining is the exploitation those resources for Festulolium breeding and to efficiently transfer the tools available into the breeding nursery.

In this presentation, I will briefly touch upon the established resources and propose to collect those resources in a user-friendly platform in order to assist the breeders' daily work. Moreover, the most recent developments in genomics will be discussed with a view to stimulate concerted efforts to promote further genetic and genomic research into Festuloliums.

How one stress might impact on another

Loka D[1], Humphreys M[1],, Gwynn-Jones D[1], Scullion J[1], Doonan J[1], Gasior D[1], Harper J[1], Farrell M[1], Kingston-Smith A[1], Dodd R[2], Chadwick D[2], Hill P[2], Mills G[2], Hayes F[2], Robinson D[2], Jones D[2]

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According to the latest climate reports, global warming is projected to increase the intensity and frequency of extreme weather events resulting in severe abiotic stresses in plants and affecting ecosystem functioning. Therefore, new grass varieties, more tolerant to one or combined stresses, are required in order to maintain grassland production and function under adverse environmental conditions. Water-deficit, flooding and ozone are three of the main abiotic stresses affecting Welsh grasslands and during the course of one or more growing seasons will impact on grassland production and persistency. The impact from one stress may reduce resilience to a subsequent stress. Understanding the "tipping-points" which endanger crop perpetuity is an essential prerequisite to ensure future grassland resilience. The objectives of my research are to: 1. Select from diverse and novel *Festulolium* hybrid grass populations those that provide the greatest resilience to multiple stress conditions and 2. Identify morphological responses associated with increased resilience to multiple stress conditions. The grasses are: the *Festulolium* variety Prior *(L. perenne x F. pratensis*), shown to alter soil structure and hydrology to mitigate run-off and flooding; two advanced breeding populations of diploid *L. perenne* with genes for drought tolerance derived from the Mediterranean fescue species *F. arundinacea* and *F. glaucescens;* two tetraploid hybrid populations involving *L. perenne* in combination with *F. glaucescens* and *F. mairei* (from North Africa), respectively. As controls, we will use *Festulolium* variety AberNiche, the only *Festulolium* currently included on UK National Recommended lists, and AberWolf, IBERS' latest productive high sugar *L. perenne* variety. Treatments consist of: A) **Control**, plants maintained at optimum conditions, B) **Flood**, plants will be flooded for a maximum of 6 weeks followed by a 4-week recovery, C) **Drought**, plants will receive limited quantity of water for 12 weeks followed by a 4-week recovery, D) **Flood + Drought** combination for a total of 18 weeks followed by a 4-week recovery and E) **(A,B,C,D) + ozone treatment** for a total of 4 weeks followed by a 4-week recovery. Foliar and root changes are going to be monitored and recorded at the National Plant Phenomics Centre at IBERS, Aberystwyth University.

Nuclear organization in interspecific plant hybrids

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The plant cell nucleus is enclosed within the nuclear envelope harbouring chromosome territories (CTs) and various nuclear bodies. It seems that the spatial organization of chromosomes is non-random and is characterized by many local and long-range contacts among genes and regulatory elements. Thus, it is evident that the architecture of interphase chromosomes plays a role in the regulation of gene expression. The introduction of sophisticated high-resolution microscopy and state-of-the-art genomics enables the complementary strategies to study CTs with high resolution. The aim of the project is to characterize spatial nuclear organization in interspecific hybrids and provide an insight into the positioning of chromatin from both parents using 3D-FISH.

In our presentation, we will present the results of our pilot experiment on the positioning of chromosome domains in interspecific hybrids (rye-wheat disomic chromosome arm substitution lines). The combination of flow sorting, *in situ* hybridization and high resolution confocal microscopy accompanied with spatial software module allowed us to achieve high resolution and avoid potential bias caused by the evaluation of nuclei in various cell cycle stages. Our next goal will be to analyse Festuca-Lolium hybrids with various proportions of parental genomes.

Particularities in the behavior of 45S rDNA sites of *Lolium* and *Festuca*

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Chromosomal fragile sites (FSs) are specific heritable regions on chromosomes that frequently form gaps or constrictions and may tend to break. FSs are widely studied in humans, mammals and yeast. They can be either spontaneous or induced by chemical inhibitors of transcription or replication. In plants, the first description of FSs was in 45S rDNA sites of *Lolium perenne* and *Lolium multiflorum* with the spontaneous occurrence of breaks and gaps. As consequences, changes were observed in the number, position and activity of 45S rDNA and presumed chromosomal fragments were described. Recently, FSs were also observed in *Festuca* and Festulolium hybrids. Despite breakages, micronuclei and DNA content analysis indicate no elimination of DNA. Neocentromeres have not been observed in the presumed fragments nor in any other chromosomal region. FISH and YOYO staining proved that chromosomes have extended NOR-regions, showing thin chromatin fibers that connect the presumed 'fragment' to chromosomal parts with centromeres, therefore, chromosomal fragments are not generated. This behavior is due to the high decondensation at 45S rDNA site that is correlated with the highly transcriptionally active regions evidenced by intense Ag-NOR staining. Apparently, these fibers are efficiently condensed during the subsequent stages of cell cycle, supporting the genomic stability in *Lolium,Festuca* and Festulolium. In another assay, we investigated whether 45S rDNA sites in *Lolium*, characterized as FSs, are hotspots for chromosomal breakages induced by ionizing irradiation followed by the formation of chromosomal fragments as well as whether they are targets of the repairing mechanism via adding telomeric sequences. De novo telomeric activity in *L. multiflorum* has not been detected. The 45S rDNA sites are not hotspots for the formation of chromosomal breakages induced by 50Gy X-ray and, thus, they should not be considered as fragile sites in the genome of *L. multiflorum*.

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Obtaining and cytogenetic identification of reciprocal hybrids between *Festuca glaucescens* and *Festuca pratensis*

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Polyploidy has played a major evolutionary role in the formation of plant species, whereas, interspecific crosses contributed significantly to plant evolution by generating new species, enabling gene exchanges between species. In the *Festuca-Lolium* complex, intergeneric hybrids may be obtained by conventional crossings, but in more cross combinations *in vitro* cultures of immature embryos are used to generate hybrid plants. Different experimental approaches have been applied in investigation of the mechanisms implied in speciation of polyploid species. The most important is the production of synthetic allopolyploids and comparison to natural forms using chromosome mapping and cytogenetic analysis. Phylogenetic studies revealed that the allohexaploid tall fescue (*F. arundinacea* Schreb.) evolved as a product of the natural hybridization of tetraploid *F. glaucescens* and diploid *F. pratensis* followed by spontaneous polyploidization. However, nothing is known about potential chromosome rearrangements of homoeologous genomes in tall fescue comparing to synthetic *F. glaucescens* ($4x$) × *F. pratensis* ($2x$) hybrids and their allohexaploid derivatives. The main aims of this study were: (*i*) obtaining intergeneric, reciprocal hybrids of tetraploid ($2n=4x=28$) forms of *F. glaucescens* and diploid ($2n=4x=14$) forms of *F. pratensis*, (*ii*) cytogenetic identification of obtained hybrids and discrimination of *F. glaucescens*- and *F. pratensis*-originated chromosomes in the hybrid genomes using GISH, and (*iii*) detection of number and location of rDNA sequences (5S and 35S rDNA) as well as identification of rDNA-bearing chromosomes using FISH. To produce reciprocal, triploid F_1 hybrids of *F. glaucescens* and *F. pratenis*, immature embryos (excised 14-18 days after pollination) were cultured on Gamborg's B_5 medium. Totally, 139 F_1 hybrids were obtained, including: *F. glaucescens* × *F. pratensis* 'Fure' – 29 hybrids, *F. glaucescens* × *F. pratensis* 'Skra' –57 hybrids and *F. pratensis* 'Fure' × *F. glaucescens* –53 hybrids. The dynamics of genome modification that occurred from the initial hybridization event to the stabilization of allopolyploid species remains largely unexplored. In this work, preliminary cytogenetic analyses of selected F_1 hybrids are presented.

Comparative analysis of repetitive DNA in eight representatives of fescues and ryegrasses

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Next generation sequencing (NGS) technologies enable high throughput production of enormous amount of DNA sequence data in a short time. Due to their affordable costs, NGS methods have been used in whole genome studies without a need for reduction of genome complexity. Fescues and ryegrasses are species with large nuclear genomes (1C ranging from about 3,000 Mb to 10,000 Mb), which consist mainly from repetitive DNA. This class of DNA sequences evolves more rapidly than coding sequences and can be used to analyze genetic diversity and study processes accompanying speciation and genome evolution.

In this work we studied the contribution of repetitive DNA sequences to nuclear genome size variability of seven fescue (*Festuca*) and ryegrass (*Lolium*) species (*L. multiflorum, L. perenne*, *F. arundinaceae*, *F. gigantea*, *F. glaucescens*, *F. mairei* and two cultivars of *F. pratensis*). To do this, we have obtained partial genome sequencing data produced by illumina platform and used them for all-to-all comparisons. Genome-wide comparative analyses were done using RepeatExplorer pipeline to identify genomic abundance and orthologous repeat families as well as to estimate inter-genomic differences. Retrotransposons were the most abundant repeat types identified in all species. Out of them, Ty3/*Gypsy* elements were the most frequent and more abundant than Ty1/*Copia* and DNA transposons, while LINE elements were less frequent in nuclear genomes of all species. In general, high number of similarity hits was found between the species of *Festuca* and *Lolium*. Within the fescues, *F. mairei* and *F. glaucescens* showed the lowest similarity of DNA repeats as compared to other species of *Festuca*. Additionally to DNA transposons, Dotter analysis identified tandem organized repeats, which were detected in relatively high number in all studied species, with some of them being species-specific. This work provides detailed insights into the genome composition and its variation in fescues and ryegrasses. The data obtained will be a useful resource for repeat masking during genome sequence analyses and facilitate development of new cytogenetic markers suitable for studying the genome organization by FISH.

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Festulolium transcriptome changes during cold acclimation, deacclimation and reacclimation

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Festulolium (*F. pratensis* x *L. perenne*) is one of the main forage grass species utilized in temperate agricultural regions due to its high quality ryegrass and resistance to hostile environments. However, during late winter and early spring, plants are particularly vulnerable to winter injury. De-hardening resistance and re-hardening capacity plays a significant role in determining winter survival. Understanding these mechanisms is important for developing cultivars with improved winter survival. We have studied global transcriptome changes during a cycle of hardening, de-hardening and re-hardening, the relationship with freezing tolerance and interactions with vernalization in two genotypes from *Festulolium* candivar FuRs9806; one flower without vernalization (V-) and one need vernalization to flower (V+). The LT_{50} results showed that vernalization at +6 °C led to higher frost tolerance than pre-treatment at +15 °C. The V+ genotype vernalized at +6 °C showed the largest capacity to re-harden (3-6 °C) thus showing a significant difference between the two genotypes possibly linked to different vernalization requirements. Illumina paired end sequencing yielded 296 and 242 million reads for the V- and V+ genotypes, respectively. These reads were assembled into 332059 and 262382 transcripts with average contig lengths of 1570 and 1414 bp for the V- and V+ genotypes, respectively. In total, 9730 and 3530 significantly differentially expressed genes with FDR<0.01 were detected in V- and V+ genotypes, respectively. These genes include the vernalization genes, stress responsive genes involved in cold-response, cold sensing, signal transduction and osmo-sensing. Detailed global transcriptome changes affected by vernalization, hardening and de-hardening, will be presented. In addition, *De Novo* sequencing of *Festuca pratensis* genome performed by Illumina mate pair sequencing will be presented along with Festuca genome and synteny browsers. Furthermore, we used BLAST to search sequence similarities between our Trinity assembled Festulolium contigs to the *Lolium perenne* and *Festuca pratensis* genome sequences.

Gene expression in interspecific hybrids

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Interspecific hybrids result from the cross of two different, but usually closely related species and thus, carry two or even sometimes more subgenomes. Interspecific hybridization frequently followed by polyploidization play a key role in plant evolution. Moreover, artificial interspecific hybrids (such as Triticale, hybrid ryegrass and many others) made in breeding programs became popular among farmers and are frequently used in recent agriculture. However, only little is known about the mechanisms underlying interactions of two parental genomes present in hybrid individual.

In our project, we studied gene expression changes in reciprocal *Festuca pratensis* x *Lolium multiflorum* hybrids. We sequenced transcriptomes of both parents and using the Orthology Guided Assembly (*OGA*) strategy; we assembled and annotated 19,036 and 18,952 transcripts. Thereafter, we identified interspecific SNPs, which allowed discrimination of *L. multiflorum* and *F. pratensis* alleles in hybrids and were used for monitoring differential (DE) and allele specific (ASE) expression in parents and F1 and F2 hybrid generations.

The level of gene expression is highly similar in both parents for most of the genes and only several genes display different level of expression. Similarly, in most of the genes, there is an equal expression from both parental alleles in hybrids. Our data suggests that the ASE is heritable but is dependent upon the maternal plant. We have also discovered that *cis* elements are more prevalent then selection which manipulates *trans* elements. We speculate that the evolution favours small incremental changes per loci rather than manipulating *trans* elements whose effects would be broader and therefore less targeted.

Genotyping of Festulolium

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Interspecific hybrids originate from the merge of two genomes into one organism, frequently followed by polyploidization event. Pairing of homoeologous chromosomes is usually suppressed by chromosome pairing control system, such as Ph1 in durum and bread wheat. Ph1 is active also in interspecific hybrids of wheat with its relatives, such as rye or Agropyron. On the other hand, homoeologous chromosomes of fescues (*Festuca* L.) and ryegrasses (*Lolium* L.) pair promiscuously and recombine freely. This results in the large range of Festulolium genotypes with various genome compositions.

Recently, there are several methods used or having potential to be used for genome structure, evolution and genetic diversity analyses of Festulolium. Firstly, genomic in situ hybridization (GISH) provides an insight on the genomic constitution via labelling mitotic chromosomes by genomic DNA of parental species. Using this method, we analyzed all cultivars included in official EUCARPIA Festulolium trial and found large differences in the proportion of parental genomes (generally Festuca versus Lolium) within and among Festulolium cultivars. Despite the cost-efficiency and still unfulfilled potential (chromosome-specific probes are still not routinely used), this method is rather laborious and could hardly be used for large-scale screening. Secondly, DArT (diversity arrays technology) platform has been developed for Festuca-Lolium complex and has been revealed useful for Festulolium genotyping. DArT markers enable precise analysis on genetic diversity and the phylogenetic relationships of grass species as well as cultivars. It can also be used for the estimation of genomic constitution of interspecific hybrids such as Festulolium and hybrid ryegrasses. However, special attention should be paid to the interpretation of results. At the last, the most appropriate choice become SNPs (single nucleotide polymorphism) markers, which represent the incredible amount of markers, basically natural variations evenly distributed along the genome. During our last study, we identify several thousands of species-specific SNPs which could enable distinguishing parental genomes in hybrids. However, their conversion to cost-friendly platform has to be processed to employ them in the practical breeding.

From phenotype to genotype: the case-study of a common field trial initiated through Eucarpia for promoting Festulolium breeding

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Lolium x *Festuca* hybrids (so-called Festulolium) renews considerably grass breeding in conjunction to the development of new genomic technology, the context of the climate change and the stressing injunction towards the breeders to release better adapted cultivars. This presentation points out some of those issues from a field trial carried out across the Eucarpia network and underlines how molecular genotyping could be essential to tackle the challenge.

As hybrids between parental species quite differently adapted, Festulolium enlarge extensively genetic variability for many traits simultaneously. Two genome sources of genetic variability mix their effects within Festulolium hybrids. Thanks to preferential homologous chromosome pairing, strong linkage disequilibrium between parent species is mostly preserved within amphiploid hybrids. This contributes to maintain at intermediate level many traits simultaneously, of immediate overall advantage compared to parent species. Many experimental evidences then show that the gain of amphiploids tends to decrease as the number of generations following primary hybridization increases. When going through introgression stages into either parent species, the loss of effect on the phenotype is even more pronounced. Everything happens as if early large phenotype effects would result only from initial linkage disequilibrium, essentially at chromosome level. Thus, the exceptional high rate of homeologous recombination between *Lolium* and *Festuca* could make uneasy the retention of high linkage disequilibrium through long term selection and the recovery of substantial genetic progress.

On the other hand, high heterozygosity rate due to high level of interspecific recombination could be favourable per se to stabilize Festulolium performances across years and environments, in other words, for more resilience. Thus, a better evaluation of interspecific linkage disequilibrium appears to be of crucial importance both in terms of breeding methods and breeding objectives. The challenge of breeding Festulolium may return simply to how better monitor the interspecific recombination in accordance to the objectives, either promoting it for more resilience, or fixing it at a given stage of a breeding process to take advantage of optimum *Lolium-Festuca* linkage disequilibrium for a given forage usage or local climate conditions.

The new technology of genotyping by sequencing (GBS) has clearly this ability to assist Festulolium breeding. Genotype of individual plant as well as allele frequency in population can be assigned at thousands of single nucleotide polymorphic (SNP) loci throughout the genome, giving also access to allele dosage in polyploids which are frequent in Festulolium. In addition to the estimation of the average introgression rate among various Festulolium derivatives, GBS has also the stimulating perspective to estimate the true breeding value of traits of adaptation and how each *Lolium* vs *Festuca* genome counterpart may contribute for more precise breeding.

Results of multilocation × Festulolium trials

Vladimír Černoch

Members of Festulolium Eucarpia Working Group,

×Festulolium as a new genera in agriculture can potentially include positive traits from both parental genera, yield and feeding quality from *Lolium* and abiotic and biotic stress tolerance from *Festuca.* Participants of Eucarpia Festulolium Working Group decided to establish trial with existing loloid festulolim varieties (*Festulolium braunii, Festulolium loliaceum, Festulolium krasanii*) in different European climatic conditions in comparison with parental species.

15 festulolium varieties with 6 varieties of parental species were established in 3 reps trial on 7 locations (2UK, F, N, PL, SRB, CZ) in 2012 and later IRL 2013 and B 2014. DM yield potential, feeding quality and different agronomic traits were tested on all 21 entries in single trials and locations for 3 harvest years. Trials sown in 2012 were closed after harvest 2015, trials in IRL and B are still continuing.

According to present results *×Festulolium* varieties looks like good compromise in DM yield and feeding quality especially in more stressful conditions.

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