EUCARPIA

Fodder Crops and Amenity Grasses Section

The 1st Festulolium Working Group Workshop

7-8 October 2010, Poznań, Poland

FINAL REPORT

Editors

Arkadiusz Kosmala
Mike Humphreys
Marc Ghesquière
Zbigniew Zwierzykowski

Institute of Plant Genetics of the Polish Academy of Sciences
Poznań, Poland

The 1st EUCARPIA Festulolium Working Group Workshop
7-8 October 2010, Poznań, Poland

Introductory Welcome

Following invitation at the last EUCARPIA Fodder Crops and Amenity Grasses Section Meeting in La Rochelle in 2009, a new Working Group named the "*Festulolium Working Group*" was formed which was subsequently recognised officially by the Eucarpia Board.

We thank EUCARPIA sincerely for the opportunities this will provide to develop further our fodder grass crop research and its use by interested stakeholders within the EU agricultural industry. I would also personally like to thank the organisers of our very successful first Workshop led by Professor Zbigniew Zwierzykowski (Institute of Plant Genetics PAS, Poznań, Poland) in October 2010. A full Report of this meeting follows. I would also like to thank Dr Susanne Barth (TEAGASC, Carlow, Ireland) and colleagues for permitting us to organise our second Working Group Workshop Meeting in September 2011 commencing immediately before the main EUCARPIA Fodder Crops and Amenity Grasses Section Meeting taking place in Dublin.

We believe that *Festulolium* research and breeding is particularly important now given the need for food security in a world facing a changing and more extreme climate. We believe changes in EU Legislation which now recognise *Festulolium* as a single grass hybrid variety category, for the first time really enables us to exploit fully the outcomes of our research and provides us the ability to develop and commercialise any *Lolium* × *Festuca* species hybrid. The recent great advances in the genomics of *Festulolium* allows us like never before to design and develop new options for grassland agriculture in Europe. The integration of genomics with high throughput phenomics technologies currently under development will provide the opportunity for more precise and predictable designs for crop development in an outbreeding and heterogeneous grass crop.

To achieve our aims we must inform all interested parties the reasons why breeders/seed industry/farmers policy makers should support and use *Festulolium*. This is our first and I think main objective and therefore our meetings will attempt to bring these various bodies together. We believe that *Festulolium* cultivars can provide sustainable options for EU agriculture at a time of climate change. We stress the novelty and use of *Festulolium* compared to that of cultivars of the parent ryegrass and fescue species or currently marketed alternative grass crops. We will emphasise *Festulolium* multi-functionality with possibilities for improved resistance to abiotic stresses (drought, cold, flooding), for stabilising soils, for improved nutrient and water-use-efficiency, for improved crop persistency, and for maintenance and generation of biodiverse grassland communities, for improved soil structure and hydrology to reduce overland flow of water and nutrients and to protect scarce water and nutrient reserves, and protect water quality, and for C-sequestration.

I invite you to read the following detailed discussions and presentations of the first *Festulolium* Working Group Meeting in Poland and I invite you to join us and to participate at our second meeting on Sunday 4th September in Dublin this Year. For further information, do not hesitate to contact me.

Dr Mike Humphreys
Chair: Eucarpia *Festulolium* Working Group
IBERS, Aberystwyth University, Wales, SY23 3EB, UK
E-mail: mkh@aber.ac.uk
Tel. (direct) ++(44)(0)1970823098

Workshop 1 Introduction

The 1st EUCARPIA *Festulolium* Working Group Workshop was organized and hosted by the Institute of Plant Genetics of the Polish Academy of Sciences in Poznań, Poland from 7 to 8 October 2010. The Workshop had to be postponed due to the widespread flight cancellations following the large releases of volcanic dust from Iceland in April.

There were 34 participants from 10 countries (9 European and Japan) representing all sectors of *Festulolium* science, breeding, and the grass seed industry. Unfortunately, a few delegates who were to participate at the earlier arranged meeting that had to be postponed in April could no longer attend.

The Workshop was opened with an invited talk by Professor Neil Jones, Aberystwyth University, on the topic "*Two blades of grass where one grew before*". The 2 day Workshop was constructed around 4 half-day sessions and themes and was aimed at all sections of the *Festulolium* community. Each session was introduced by 3 to 4 short presentations each with a question to address by all workshop participants. The four themes were:

Session 1: *To describe what is special about Festulolium hybrids compared to current Lolium (ryegrass) and Festuca (fescue) cultivars?*
Session 2: *How best to develop Festulolium cultivars for EU grass seed markets – opportunities and obstacles?*
Session 3: *Descriptions of trait variability and analysis in Festulolium.*
Session 4: *Advances and state-of-the-art genomics studies in Festulolium.*

There was unanimous agreement by participants that the Workshop was a success and a desire to cooperate in field trials at different locations to compare performance of alternative *Festulolium* cultivars. The *Festulolium* Working Group Committee was extended from 3 to 5 members. It is now Dr Mike Humphreys (Chair) (UK); Prof. Zbigniew Zwierzykowski (PL); Dr Marc Ghesquière (F); Dr Liv Østrem (N), Dr Vladimir Černoch (CZ). The next *Festulolium* Working Group Workshop will be in Dublin, Ireland on Sunday 4th September 2011, directly preceding the 29th EUCARPIA Fodder Crops and Amenity Grasses Section Meeting.

In this Final Report of the Workshop proceedings including abstracts of all talks and posters presented and the subsequent joint discussions is completed.

The 1st *Festulolium* Working Group Workshop and publication of this Report have been supported by the Polish Ministry of Science and Higher Education and European Association for Research on Plant Breeding.

We thank Aleksandra Bocian, Arkadiusz Kosmala, Tomasz Książczyk (rapporteur), Izabela Pawłowicz (rapporteur), Magdalena Taciak, Włodzimierz Zwierzykowski, and Zbigniew Zwierzykowski, of the Local Organizing Committee, for their contributions to the success of the Workshop. Finally, we express our gratitude to the speakers and authors of posters.

Editors

Table of contents

Opening lecture

Two blades of grass where only one grew before

Neil Jones

IBERS Aberystwyth University, Edward Llwyd Building, Penglais Campus, Aberystwyth, Wales, UK, rnj@aber.ac.uk

Abstract. The pace of science is now moving so fast that a review paper, or even a manuscript, can be out of date before it is even submitted. We are now deeply immersed in the sequencing world, and already have the means to access syntenic genomes as well as the newly released sequence of *Brachypodium distachyon* (The International *Brachypodiun* Initiative. Nature 63: 763-768, 2010). These studies will no doubt enable rapid progress in the functional genomics of *Festulolium*, and greatly accelerate the progress in selection and utilisation of desirable traits, including those that are pertinent to breeding for rumen metabolism amongst many others. Whilst focussing on a number of new opportunities to increase the resolution of mapping and of genome organisation, we should also use these new molecular tools to advance or understanding of several key existing but as yet unsolved problems in the cytogenetics of *Festuloliums*.

How, for example, can we begin to manipulate recombination, and possibly release new forms of genetic variation that lie hidden within the chromosomes by the localisation of chiasmata. There is no doubt, based on knowledge from several plant species, that chiasma distribution has a genetics basis, but to uncover that and to utilise it is another sorry, although we are aware that inbred lines of *Lolium* have altered patterns of chiasma formation. In any event we still need some evidence that new variation is released when chiasmata are moved. It could be that optimised haplotypes have evolved over time. In any event studies are underway to explore this question.

How can we explain changes in genome balance, where *Lolium* dominates *Festuca* in allopolyploids, in a progressive and strident manner which totally alters the genome organisation in a way that makes the breeder is a victim of his/her objectives in maximising the benefits of combining complementary genomes into one functional alloploid. Is there a need here to study centromere organisation, and to develop probes that can distinguish and mark species-specific centromeres in the *Festuloliums*; bearing in mind that one species can impose it centromere sequences on another in certain situations (addition lines of maize chromosomes an oat background the maize Cen-H3 gene is silenced in the oat background, and the oat Cen-H3 functions to organize the kinetochore on the maize chromosomes). In making allopolyploids we clearly have to bear in mind the many known aspect of genome instability that often follows, and how this might even have influence in introgressed segments and chromosome substitutions. The history of *Festulolium* research has been rich and rewarding, especially with the advent of GISH and other new molecular tools, but the future promises much more, if the riches can be realised.

Session 1

To describe what is special about *Festulolium* hybrids compared to current *Lolium* (ryegrass) and *Festuca* (fescue) cultivars?

Novel uses of *Festulolium*

Mike Humphreys*, John Harper, Julie King, Sally O'Donovan and Lesley Turner

IBERS, Aberystwyth University, Gogerddan, Aberystwyth, Ceredigion, SY233EB; BARA: Bangor/Aberystwyth Research Alliance for Food Security through Plant Sciences, Wales, UK, *mkh@aber.ac.uk

Abstract. The introgression from wild crop relatives in plant breeding programmes is illustrated very well in natural or synthetic *Lolium* × *Festuca* hybrids where high frequencies of chromosome pairing and recombination allow a ready exchange of alleles and the development of novel plant genotypes that combine complementary agronomic and stress resistance traits. The extreme genetic and phenotypic variation found within genotypes of the *Festulolium* complex combined with the high frequencies of chromosome pairing and genome recombination encourage breakdown of linkage disequilibrium and facilitate widespread genome restructure and "dissection" of QTL controlling complex traits, thus providing an unique "window" into the evolution of adaptive traits within the Poaceae where species derive from a common ancestor. *Festulolium* genomics and phenomics will be integrated with that of the temperate fully-sequenced model monocot *Brachypodium* and with rice as the model tropical "grass"monocot. An introgression-mapping family at IBERS comprises monosomic chromosome addition lines of *F. pratensis* where each *Lolium* chromosome is replaced by an equivalent *Festuca* homoeologue. Introgression series developed from each *Festuca* chromosome enable the contributions of equivalent *Festuca* and *Lolium* alleles to a specific trait to be assessed, and genetic markers for their major determinants, developed. The outcome is a model for grass crop design where genes of *Lolium* and *Festuca* species are assessed for their efficacy. Whilst some traits are species specific, in other cases, heterosis effects between *Lolium* and *Festuca* genomes lead to an enhanced gene expression beyond the capabilities of either parental species. We have demonstrated in a BBSRC funded project how a *Festulolium* hybrid contributes through its extensive root turn-over to an improved soil hydrology to an extent that it reduces significantly surface run-off compared to either of its *Lolium* and *Festuca* parent controls thereby improving soil water retention, and reducing flooding and soil erosion.

More broadly this research is an exciting first step to realizing the potential to design grass genomes with precision to achieve both food production, improved crop resilience to increased abiotic stresses, and to deliver a key ecosystem service. Our objectives are to: (*i*) model and redesign forage grass morphology and ontogeny to optimise crop production under regimes of low water and nutrient availability; (*ii*) explore gene expression for adaptive traits within novel *Festulolium* genome combinations, and at different ploidy levels, to develop understanding of evolution of adaptive traits, the importance of heterosis, and to optimise gene expression in order to inform plant breeders of the most appropriate strategies to employ; (*iii*) undertake holistic approaches to multifunctional redesigns of forage grasses for improved water and nutrient-use, soil structure and hydrology, and animal nutrition by either increased forage production under stress conditions, or through co-adaptive traits

such as heat-shock protein expression, provide more efficient N-conversion in the rumen; (*iv*) explore with industrial partners multifunctional alternative uses for grass designs to mediate against flooding, to stabilise soils and to reduce erosion, for landscape design, for low-input green roofs, for landscape, for soil remediation, carbon sequestration, and for low input amenity and sports-turf designs.

Festulolium hybrids compared to current *Lolium* (ryegrass) and *Festuca* (fescue) cultivars

Ulf Feuerstein

Euro Grass Breeding GmbH & Co. KG, Hof Steimke, 27330 Asendorf, Germany, ulf.feuerstein@eurograss.com

Abstract. Hybrids between the phylogenetically closely related *Lolium*- and *Festuca*-species are relative simple to generate and occur also naturally in permanent pastures. Since about 60 years these crosses are part of scientific work and forage breeding programmes. First varieties were listed in the USA followed by varieties from former Eastern Block countries. With the break down of the Iron Curtain also the interest of *Festulolium* in Western Europe increased. Many agricultural advisers and also seed companies propagated and recommended *Festulolium*. In 2004 the narrow and specific definition of *Festulolium* was broaden by the European Union. New National List tests were established in Germany and France and the first varieties were listed also in these countries. In these years' initially only little seed was available because of the collapse of parts of the seed production in Eastern Europe. Bit by bit the seed production was re-established in many European countries. For some years now plenty of seed appeared on the market – but there is only little demand.

The availability of seed of *Festulolium* coincides with a general decrease of the grass seed market. Therefore the small demand for *Festulolium* may have several reasons: (*i*) *Festulolium* has not found its place in grassland farming until now; (*ii*) In times of crisis farmers are looking for bargains and are not open for experiments; (*iii*) Due to mild winters in the last 20 years marginal locations which are actually most suitable for *Festulolium* cultivation have been sown more and more with *Lolium* dominated mixtures.

Dependent on their agricultural history there are countries in which farmers use mixtures for grassland sowing and other countries in which they use single varieties. In all cases their awareness of the individual merits of specific grass varieties is low and goes hand in hand with a low demand for *Festulolium*. To find its place in practical agriculture it is necessary to have a clear profile of a species – what are the strengths and weaknesses of the species. When a farmer orders perennial ryegrass seed he knows very well what to expect, the same is true for Italian ryegrass or meadow fescue. When he orders *Festulolium* he does not know what he will get because of many different types. It will be necessary to sharpen the profile of the species *Festulolium*.

Festulolium seed is more expensive than seed of Italian ryegrass or meadow fescue. It will be necessary to highlight the added value of *Festulolium* to increase the interest of farmers during a difficult time in the seed business. Looking at the immediate future it is likely that many perennial ryegrass varieties developed problems during the hard winter 2009/2010 so that in marginal locations *Festulolium* may be able to increase its market share.

Proteome analyses during cold acclimation of *Lolium-Festuca* species

Arkadiusz Kosmala[1*], Aleksandra Bocian[1], Marcin Rapacz[2], Barbara Jurczyk[2] and Zbigniew Zwierzykowski[1]

[1]Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland, [*]akos@igr.poznan.pl
[2]Agricultural University in Krakow, Department of Plant Physiology, Podłużna 3, 30-239 Kraków, Poland

Abstract. Winter-hardiness is a complex trait depending on the ability of plants to survive low temperatures, extreme temperature fluctuation, wind desiccation, ice nucleation, and snow moulds infection. Frost tolerance (FT) is thought to be the main component of winter-hardiness and one of the best indicators of plant ability to survive harsh winter conditions. The plant species growing in temperature regions can acquire FT through exposure to low, non-lethal temperatures, a phenomenon known as cold acclimation (CA). Perennial ryegrass (*Lolium perenne* L., *Lp*) is characterized by high nutritive values, rapid establishment rate and persistence. However, its poor ability to perform in harsh winter climates limits its distribution and cultivation. Inversely, meadow fescue (*Festuca pratensis* Huds., *Fp*), a species closely related to *Lp*, comprises lower nutritive values but is more frost tolerant and the most northerly distributed of all the forage grasses within the *Lolium-Festuca* complex. The molecular nature of frost tolerance, expressed differently in *Fp* and *Lp*, is not well recognized and the comprehensive molecular research performed on both species is required. Herein, we present the results of two proteomic projects, first one focused on *Fp* (finished; Kosmala et. al. 2009) and the second one on *Lp* (ongoing). The analyses involved the comparison of leaf protein accumulation profiles during CA between the plants with different levels of FT within each species by the use of two-dimensional electrophoresis and further identification of proteins which were accumulated differentially between the selected plants by the use of mass spectrometry. Possibilities of the application of similar proteomic approach into the analysis of *Festulolium* cultivars are discussed.

Kosmala A., Bocian A., Rapacz M., Jurczyk B., Zwierzykowski Z. (2009). Identification of leaf proteins differentially accumulated during cold acclimation between *Festuca pratensis* plants with distinct levels of frost tolerance. J. Exp. Bot. 60: 3595-3609.

The research was carried out in the frame of the projects of Polish Ministry of Science and Higher Education (nos. PBZ-MNiSW-2/3/2006/21 and 2 P06A 044 30).

Plant aquaporins and their role in maintenance of water balance during dehydrative stress

Izabela Pawłowicz* and Arkadiusz Kosmala

Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34,
60-479 Poznań, Poland, *ipaw@igr.poznan.pl

Abstract. Aquaporins constitute water channels that control the passive bidirectional flow of water and small polar molecules across cellular membranes. They are 26-30 kDa proteins belonging to the Major Intrinsic Proteins (MIP) superfamily of integral membrane proteins. MIPs show a tetrameric quaternary structure with each monomer consisting of six membrane spanning helices linked by three extra- and two intracellular loops. Two conserved amino acid motifs: Ar/R and NPA are responsible for specific substrate recognition. Plants aquaporins are divided into seven groups: PIPs, TIPs, NIPs, SIPs, GIPs, HIPs and XIPs. The plasma membrane integral proteins (PIPs) and tonoplast integral proteins (TIPs) have been studied extensively and more than thirty isoforms have been identified in *Arabidopsis thaliana*, *Oryza sativa* and *Zea mays*. Many studies revealed that genes belonging to these subgroups control many aspects of plant water relations like plant development and adaptations to abiotic stresses such as drought, cold and salinity. PIPs contribute to adaptation of plants to drought and also contribute to rehydration after water deficit. They are abundantly expressed in roots were they mediate most of soil water uptake. TIPs mediate water exchange between the cytosolic and vacuolar compartments and may play the central role in cell osmoregulation. Water stress-dependent expression of many *PIP* and *TIP* genes were described revealing their up- and down-regulation at the transcriptional level. Aquaporin genes expression analysis in transgenic plants shows that aquaporin overexpression has either beneficial or adverse effects on drought tolerance, depending on the gene or plant species. For example antisense inhibition of PIPs in *Nicotiana tabacum* and *A. thaliana* resulted in a marked defect in plants ability to recover from water stress. Although there is wide knowledge about transcriptional regulation of *MIP* genes, the role of particular aquaporins in water stress tolerance still needs to be investigated. Herein, we describe the proposal of molecular research to check the expression profiles of the selected aquaporin genes in *Lolium-Festuca* species and their intergeneric hybrids.

Cytogenetic stability and fertility in *Festulolium*

Zbigniew Zwierzykowski[1*], Arkadiusz Kosmala[1], Elżbieta Zwierzykowska[1], Magdalena Taciak[1], Włodzimierz Zwierzykowski[1], Tomasz Książczyk[1] and Wojciech Jokś[2]

[1]Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland, [*]zzwi@igr.poznan.pl
[2]Szelejewo Breeding Station Ltd., Szelejewo Drugie, 64-820 Piaski, Poland

Abstract. During last three decades more than twenty commercial *Festulolium* cultivars have been developed throughout the world using an amphiploidy approach. Most of them derive from reciprocal tetraploid hybrids of *L. multiflorum* (4x) × *F. pratensis* (4x) and *F. pratensis* (4x) × *L. multiflorum* (4x). All these amphiploid *Festulolium* cultivars are characterized by high yield and forage quality as well as good persistency, winter hardiness and drought tolerance. However, seed fertility and seed yield, which are crucial aspects in *Festulolium* breeding, in amphiploid cultivars still express unsufficient levels, much lower comparing to values of these traits in cultivars developed *via* an introgression approach.

Cytogenetic studies of genome structure of *Festulolium* cultivars carried out using genomic *in situ* hybridization (GISH) demonstrated that homoeologous chromosome pairing resulted in extensive recombination between chromosomes of the parental genomes leading to aneuploidy and genetic instability in following generations of breeding populations. Recently, genomic constitution of *Festulolium* cultivars, including cultivars derived from reciprocal hybrids of tetraploid *F. pratensis* and *L. multiflorum*, was described and a large range of variation in the proportions of parental genomes and in the extent of intergeneric recombination was revealed (Kopecký et al. 2006).

In contrast to *F. pratensis* × *L. multiflorum* hybrids, tetraploid *F. pratensis* (4x) × *L. perenne* (4x) hybrids are used in breeding relatively rarely, generally due to unsufficient level of seed set, even in case, when selection for improving fertility was applied during several generations. In this lecture we present results of molecular cytogenetic investigations on genome structure and dynamics of homoeologous recombination in selected breeding populations of eight successive generations developed from *F. pratensis* (4x) × *L. perenne* (4x) hybrids (Zwierzykowski et al. 2006). The breeding programme was developed at the Szelejewo Plant Breeding Station by W. Jokś. We also demonstrate a new project focused on studies of cytogenetic stability and fertility in following generations of unselected materials derived from the allotetraploid *F. pratensis* × *L. perenne*.

Kopecký D., Loureiro J., Zwierzykowski Z., Ghesquière M., Doležel J. (2006). Genome constitution and evolution in *Lolium* × *Festuca* hybrid cultivars (Festulolium). Theor. Appl. Genet. 113: 731-742.

Zwierzykowski Z., Kosmala A., Zwierzykowska E., Jones N., Jokś W., Bocianowski J. (2006). Genome balance in six successive generations of the tetraploid *Festuca pratensis* × *Lolium perenne*. Theor. Appl. Genet. 113: 539-547.

This work was supported in part by the Polish Ministry of Science and Higher Education (grant No. N N310 090736).

General discussion

Is it heterosis that makes Festulolium special?

Voices in the discussion: Liv Østrem, Mike Humphreys, Arkadiusz Kosmala, Marc Ghesquière, Beat Boller, Neil Jones, David Kopecký, Vladimir Černoch

Advantages and disadvantages of amphiploid and introgression forms of *Festulolium* were discussed. In the conclusion it was said that heterosis is not the most beneficial trait of *Festulolium* hybrids. Biodiversity, complementary traits between species and genera, multifunctional capabilities and genome variations for stress adaptations make *Festulolium* hybrids useful for breeders and scientists.
Subsequent Meeting Note: Complex grass trait genetics is not sufficiently advanced to categorically answer this question. What is now apparent is that there are now examples where *Lolium* and *Festuca* genomes may interact to induce an enhanced plant phenotype superior to that of either the parent species. Also note next discussion:

Are the genes transferred from Festuca to Lolium expressed in the same way in Lolium genomic background as in Festuca background?

Voices in the discussion: Arkadiusz Kosmala, Mike Humphreys, David Kopecký

The expression of particular genes is influenced by other genes (the genetic background). Thus, it may matter whether the genes are expressed in their "natural" or in an alien genetic environment (alien chromatin from the other species). It is possible to analyze the expression of genes transferred from *Festuca* species to *Lolium* species both at transcript and protein level. Then, it is possible to compare the expression profiles obtained for *Festulolium* hybrids to the expression profiles obtained for parental forms. This comparison is especially important in analyzing gene expression during stress conditions, as stresses such as drought and frost are polygenic traits. The first (unpublished) results suggest that the protein accumulation profiles could be quite different in hybrids compared to their parental species and an induced protein network obtained during a stress treatment may be a characteristic only found in hybrids. As a consequence, it would be difficult or even impossible to draw conclusions solely on the basis of the parental profiles or predict from the hybrid genomic structure. The knowledge of basic *Festulolium* genomic structure with respect to presence or absence of particular chromosomes does not necessarily predict the level of abiotic stress resistance achieved by a plant. Moreover, the same genes can show different expression level in different hybrids depending on the host species. Furthermore, in amphiploids, not only are the genes of special interest transferred but may gene sets segregate throughout the genome, including possibly the regulatory genes derived from the donor species. In the case of the introgression lines the genetic machinery of the recipient is used to express the transferred genes. Furthermore, it was possible to observe interactions between *Lolium* and *Festuca* genes. Chromosome 3 is a good example of those interactions.

The role of HSP70 protein in drought and cold stress was also discussed. *F. glaucescens* plants accumulated high level of HSP70 protein in response to water deficit. In the case of cold-acclimated *L. perenne*, HSP70 accumulated at different amounts in a low and a high frost tolerant genotype. A greater level of accumulation of HSP70 was observed for low frost tolerant plants during the whole process of cold acclimation (21 days).

Is it possible: (i) to optimise the genetics of Festulolium towards significant improvements of the main target traits; (ii) to sharpen the product profile of Festulolium towards competitive usages in agriculture?

Voices in the discussion: Zbigniew Zwierzykowski, Ulf Feuerstein,
Mike Humphreys, Marc Ghesquière, Liv Østrem, Vladimir Černoch, Beat Boller

First, the definition of *Festulolium* was discussed, which is very broad and includes both amphiploid and introgression forms. These may differ in ploidy number, fertility and in seed yield. In the amphiploid cultivars, a greater difference between fertility and seed yield is observed than is found in introgression forms. As a consequence farmers do not accurately know what kind of material they have. In many cases we can only describe cultivars as fescue-type or ryegrass-type. There is a need to create effective registration rules (Index System) that will better describe new cultivars to solve this problem. The name of hybrid *Festulolium* cultivars should include its genetic and its species background.

Another problem discussed was the testing method employed in cultivar assessments. These days every country has its own testing system which make cultivar comparisons difficult and their respective stress resistance unknown. Standardization is required in plant material-selections during transfers of target traits.

Session 2

How best to develop *Festulolium* cultivars for EU grass seed markets – opportunities and obstacles?

Insights on crucial aspects for *Festulolium* development: official testing, seed productivity and genetic progress

Marc Ghesquière*, Thierry Bourgoin and Jean-Louis Durand

French National Institute for Agricultural Research (INRA), BP6 – 86600 Lusignan, France, *Marc.Ghesquiere@lusignan.inra.fr

Abstract. *Festulolium* deals in general with many attractive concepts and technologies in the field of sustainable agriculture, genetics and breeding. However, variety development and acknowledgement of genetic progress enhanced from *Lolium* × *Festuca* hybridization is still poor. So far, only 42 varieties were registered in a national list of *Festulolium*, if not in a tall fescue list in some instances. Although a large portfolio of well-referenced varieties does not necessarily evidence good genetics and breeding, this can greatly help to lead future genetics/genomics in interspecific hybrids. In this context, the feed-back of large-scaled assessment of diversified and contrasted varieties of *Festulolium* over locations and years may represent an essential "phenotyping" for more genomics.

Three crucial aspects are identified for enhancing variety development in *Festulolium*: (*i*) Better regulation for registration in national lists. The new definition of *Festulolium* in 2004 has neither make the officials standardize their DUS and VCU procedures over countries nor the process of decision making. A list of recognized *Festulolium* varieties to be used as controls is not available; there is no guarantee that the seed batches used in trials are of the same generation; the crossing diagram of controls is often unknown which does not facilitate fair VCU evaluation; the weighing of traits to estimate overall value is still based on general means and do not emphasize the benefit of improved persistency in particular stressing locations. While in most countries, the candidates are tested against *Festulolium* controls more or less of a same nature, in France, they are expected to perform better than the mid value of pure species controls, *i.e.* to exhibit heterosis for all traits. However, it's true that the officials would not promote any better assessment of *Festulolium* as long as the annual number of candidates for registration will not increase significantly; (*ii*) Better mastered seed production, especially in amphiploid types. At the scale of the field for seed multiplication, it seems more and more obvious than residual male sterility may limit seed potential yet assessed to be of correct level in plot trials in experimental station. More investigations are essential to elucidate whether full restoration of fertility could come down to a few key-genes or it is controlled by many genes. In this latter case, the question clearly becomes how large can be overall "hybridity" in *Festulolium* to not preclude seed production, with important consequences to define the right interspecific genome balance we could breed within; (*iii*) Better understanding of functional mechanisms of stress tolerance and persistency in the grasses. Recent results from sampling plants in swards of *Festulolium* show that better persistency towards abiotic stresses may be not so univocal in terms of genome contribution, possibly including positive effect from *Lolium*. To specify genome contribution on the direction of phenotypic variation and/or of response to selection will contribute to more clear-cut genetic progress and facilitate registration of varieties.

All these aspects have obviously close interrelationships which encourages to coordinate efforts for better defining what could/should be a realistic variety of *Festulolium* in the future and to better defend *Festulolium* as a model perennial grass.

Utilization of present *Festulolium* varieties and future breeding goals

Vladimir Černoch[1*], David Kopecký[2], Michal Klíma[1] and Jaroslav Doležel[2]

[1]Šlechtitelská stanice Hladké Životice, s.r.o., Fulnecká 95, 742 47 Hladké Životice, Czech Republic, [*]vc@pbhz.cz
[2]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany of the AS CR, Sokolovská 6, CZ-77200 Olomouc, Czech Republic

Abstract. *Festulolium* is a "new" grass species with a great potential for agricultural and non-agricultural using. It originated from crosses of different *Festuca* and *Lolium* species and in spite of some *Festulolium* hybrid types are known from the nature, registered varieties are new and come from a long-term breeding work. To date over thirty varieties of different *Festulolium* types have been registered and more are in the process of breeding and registration all over the world.

The reason for forming of ×*Festulolium* varieties is opportunity to combine positive traits from species representing both genera. Persistency and stress tolerance from *Festuca* and quality and quick development from *Lolium* species. Combination of desirable traits in one variety is a big advantage of the existing *Festulolium* varieties, which started to replace pure species of both parental genera especially in the Central Europe and Scandinavia.

Breeding of new *Festulolium* varieties is hampered by lack of methods suitable for selection of superior genotypes and until now, only morphological selection has been applied. It is expected development of biochemical and DNA markers which will help breeders in the near future and make a breeding process efficient and faster. Recently a DArT chip with 3884 polymorphic markers for single *Festuca* and *Lolium* species has been developed and was shown suitable for verifying the presence of parental genomes and/or their parts in *Festulolium* hybrids.

It is safe to predict, that the role of *Festulolium* varieties will continue increasing along with the global climate changes (no matter if the changes are natural or due to human activity). Breeding of the first generation of *Festulolium* varieties took a long time. Now, more than fifty years after the humble beginnings the production of new hybrid varieties will speed up due to the experiences from breeding and registration process of the first *Festulolium* generation and availability of high-throughput screening systems. In the light of this, it will become important for breeders to define new breeders goals on *Festulolium* for particular climatic conditions, what would be difficult for pure parental species. The *Festulolium* make it possible to combine acceptable feeding quality with drought or frost tolerance and forming of new varieties with different growth characteristics such as winter growing, winter dormancy, summer growing, fast spring development, etc.

This work was supported by the Ministry of Agriculture of the Czech Republic (grant award NAZV QH71267).

Festulolium for northern growing conditions

Arild Larsen[1*], Liv Østrem[2] and Odd Arne Rognli[3]

[1]Graminor Ltd, N-8049 Bodø, Norway, [*]arild.larsen@graminor.no
[2]Norwegian Institute for Grassland and Environmental Research, Fureneset, N-6967 Hellevik i Fjaler, Norway
[3]Norwegian University of Life Sciences, N-1432 Ås, Norway

Abstract. European cvs. of *Festulolium* showed in early Norwegian tests high yielding capacity and good forage quality, but low winter persistence. However, the positive DMY potentials evoke interest for these types of hybrid. The first amphitetraploid materials of *Lolium perenne* and *L. multiflorum* crossed with *Festuca pratensis* and *F. arundinacea* were received from IGER, Aberystwyth, Wales, to Norway. The hybrids were subjected to climatic selection for several cycles, and produced promising candidate cvs. National breeding was started within The Norwegian Crop Research Institute in 1995. The goal was to combine yielding capacity and high forage quality from *Lolium* with winter hardiness, persistence and disease resistance from *Festuca*, using the introgression method. Winter hardy cvs. of tetraploid *L. perenne* were crossed with adapted Nordic cultivars of *F. pratensis* and the primary hybrids were backcrossed to *L. perenne* (2n). A high number of BC_1 /BC_2-families have been produced and tested at different locations. Later, amphitetraploids of *L. perenne / L. multiflorum* and *F. pratensis* were made, as well as androgenetic plants. Following cooperation with DLF Trifolium, Denmark, and Hladke Zivotice, Czech Republic, plant materials of *F. arundinacea* × *L. multiflorum* origin*,* were introduced to the breeding programme.

From mass selections and progeny testing, a number of candidate cvs. have been produced and tested with market cvs. In Norway yield and persistence are highly dependent on winter survival and a vigorous stand in spring. Yield ranking of cultivars may therefore be different in South East, West and North Norway. Attack of snow mould (*Fusarium nivale*), late growth cessation in fall causing low hardening, and low tolerance to frost and ice cover, are important factors leading to winter damage in *Festulolium*. Amphitetraploids of *L. perenne* × *F. pratensis* origin, and hybrids containing *F. Arundinacea,* have shown best wintering ability. Winter damage will delay growth start in spring, causing low yield at early cuts, even though the stand may recover late in the season. Cultivars with vigorous stand in spring compete well with cultivar of timothy (*Phleum pratense*), meadow fescue and perennial ryegrass. The forage quality is mainly comparable to perennial ryegrass.

Genomic resources for molecular markers for *Festulolium* breeding are being developed at the Norwegian University of Life Sciences (UMB). These comprise DaRT markers mapped in *F. pratensis*, SNP markers in candidate genes for frost tolerance, EST library in *F. pratensis* which is being analyzed for SNP markers and blasted against a *Lolium* EST library to identify species-specific SNPs. Several of the key genes involved in lignin biosynthesis in *F. pratensis* are cloned and sequenced and will develop species-specific markers by comparison with *L. perenne* also for these genes affecting nutritive quality. Today *Festulolium* breeding in Norway is conducted by Graminor Ltd focusing on comprehensive progeny testing of BC-families and composition of synthetic cultivars. Internal tests have brought forward

some candidates to official variety testing (VCU) of which one is recommended for listing. Important problems are controlled transfer of resistance genes, good markers for use in plant breeding programs and better understanding of gene expression from the two species in the hybrid materials.

General discussion

Do we need Festulolium varieties?

Voices in the discussion: Marc Ghesquière, Neil Jones, Arild Larsen,
Mike Humphreys, Ian Armstead, Simen Sandve

Marc Ghesquière mentioned a need for better assessment of *Festulolium* varieties and of a better description of present and especially new *Festulolium* varieties. In his opinion, special attention should be placed on the plant material used in *Festulolium* development and its reproducibility so that comparable experiments may be conducted. Emphasis should be placed on achieving the availability of complete plant genealogy and complete genetic descriptions of current *Festulolium* varieties to maintain standardised seed batches in public institutions and research centres. It is difficult to obtain standard seed batches in the absence of any knowledge of their pedigree. Criteria are necessary to evaluate the homogeneity of *Festulolium* varieties. The discussion was broadened by Neil Jones who questioned what was it that was interesting for breeders from current and planned research being undertaken on chromosome genetics. *Festulolium* research needs breeders and geneticists to come together, and making *Festulolium* varieties is the best way to achieve this. A need to extend the *Lolium* area grown in Europe was proposed by Arild Larsen. Other participants suggested we should categorize *Festulolium* species hybrids based on their traits to better direct material to the suitable market.

It is accepted that *Festulolium* hybrids are difficult to stabilize as amphiploids. Plant hybridisation, speciation and developments of polyploids give us a “window” into how to stabilise plant genomes. To understand how chromosomes pair preferentially and segregate at meisosis.

The participants also discussed several questions of importance: (i) a case for physical mapping and how to manipulate the knowledge, (ii) why genotypes having specific alien introgressions have drought resistance or better persistency than others. Considering the main question of the discussion: “Do we need *Festulolium* cultivars?” It was concluded that this would depend on their economic value. In practice, some people only require a variety to function, and they are not so interested in its sale to the markets. Another conclusion was that science is a business, and must have an impact. Science should give something back to nature, and we have a role to play – to create a variety of a commercial value which will be used in a widespread manner to deliver environmental service.

During discussion, it was also said there is a need to develop markers which could be used easily to compare and identify genetic maps. Marker development was essential for use in introgression study. This point of view was heightened with the desired idea to design better plants with valuable genes, what is far from being achieved. The participants agreed that it would be better to look for candidate genes and to localize the genes of special interest with their help. The genetic aspect of the discussion was reinforced by developments in introgression-mapping. The practical use of introgression mapping was emphasized in discussion, and of single *Festuca* introgression lines, which have already been identified and currently being employed in crop improvement programmes. It was noted that the *Festulolium* system is of great value to trait genetics research due to the vast availability of genetic and phenotypic variation and the high frequencies of chromosome recombination, which together make them attractive to basic research. It is often the case that researchers do not focus their science on areas of interest and concern to breeders.

Results from the introgression breeding programme used in Norway show that resistance to snow mould (*Microdochium nivale*) does not seem to have been transferred from *F. pratensis* to new *Festulolium* cultivars.

How can the transfer of a resistance gene be better controlled in a breeding programme and are we sure that resistance genes from Festuca are fully expressed in a Lolium genomic background?

Voices in the discussion: Neil Jones, Mike Humphreys, Simen Sandve,
Arkadiusz Kosmala

At the beginning of the discussion, Neil Jones asked for an explanation, what the breeders understand under "transfer" conception. The expression of resistance genes in a *Lolium* genomic background is not well recognized and still remains as an open problem. During discussion, a very important trait, crown-rust resistance was mentioned, which has QTL associated with *Festuca* chromosome 4. It was also mentioned that is better to transfer *Festuca* chromosomes into a *Lolium* genomic background. Considering an answer to the question about conviction if the resistance genes are fully expressed in *Lolium* genomic background, Arkadiusz Kosmala explained precisely how to check the level of expression both in the hybrids and their parental species. He mentioned that it is possible to monitor it for a single gene trait, when the gene controlling the trait is known, both on the transcriptome (*real time* PCR) and proteome (Western blot) level.

Is it possible to increase seed yield in amphiploid Festulolium breeding materials?

Voices in the discussion: Mike Humphreys, Zbigniew Zwierzykowski,
Marc Ghesquière, Vladimir Černoch

The participants mentioned several aspects which would be helpful to increasing seed yield including discovery of suitable genetic markers. If there is possibility to find gene(s) to regulate chromosome pairing, improvement of seed set will then be possible. Zbigniew Zwierzykowski brought up the fact concerning pollen fertility and seed set in successive generations of *Festulolium* hybrids. He spoke about the comparison of pollen fertility and seed set and patterns which can be typical for particular varieties. He gave some examples of comparison in successive generations of *F. pratensis* × *L. multiflorum* and *F. pratensis* × *L. perenne* hybrids. He also stated that between F_1 and F_6 or F_8 generation, a significant increase of pollen fertility has been observed in both kinds of *Festulolium* hybrids. In parallel, the increase of seed set was not comparable, and its value was very often close to 50%. For comparison, the mean seed set in diploid cultivars of ryegrass and meadow fescue reaches 55-65%. It was also mentioned that such findings were found in materials under selection and they were plants created by an amphiploidy approach. Marc Ghesquière and Zbigniew Zwierzykowski referred to research showing correlations between seed fertility and seed set, but that no correlation was found necessarily between seed set and chromosome pairing behavior. No correlations between the pollen fertility and the seed set was also mentioned. Selection on a seed set level should be considered.

Some discussion was had on differences in germination of *Festulolium* achieved by an amphiploidy or an introgression approach, which gave some values where germination was lower in amphiploid forms of *Festulolium*.

Vladimir Černoch mentioned that we have practical experience derived from seed yield trials and seed production for different amphiploid varieties of *F. pratensis* × *L. multiflorum*. Seed yield is between both parental species with stable differences among varieties (more than 20%) and the best one is close to *L. multiflorum*. We should discuss how to improve seed yield of amphiploid forms of *Festulolium* either by scientific or traditional breeding methods. To conclude the discussion, Marc Ghesquière commented that if the seed set value was low, and costs too high a *Festulolium* variety would never sold in the market-place.

Session 3

Descriptions of trait variability and analysis in *Festulolium*

Characterisation and interpretation of phenotypic diversity in *Festulolium* using the example of rooting traits

Lesley B. Turner[1], M. Farrell[1], Sarah Hawkins[1], Sally A. O'Donovan[1], C. Watts[2], W.R. Whalley [2], Ian King[1], John A. Harper[1] and Mike W. Humphreys[1*]

[1]Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University, Gogerddan Campus, Aberystwyth, SY23 3EB, Wales, UK, [*]mkh@aber.ac.uk; BARA:Bangor/Aberystwyth Research Alliance for Food Security through Plant Sciences
[2]Rothamsted Research (RRES), Harpenden Research Station, Hertfordshire, AL52JQ, UK

Abstract. Many of the temperate grass species used in agriculture can survive periods of mild to moderate drought. However, breeding for traits that favour continued growth and yield both during and after conditions of stress would be of great economic value. Ryegrass and fescue species plus several *Festulolium* hybrid accessions were grown in 1m deep pipes of compost. Measurements of both above and below ground growth were carried out over two years. The effect of drought, imposed by withholding water on some of these traits was assessed in the first year. These plant materials showed considerable variation for above and below ground growth and for response to drought. In particular, there were significant effects on the growth of new roots during and after a period of drought and in the impact of this on drought tolerance.

The data provide some information on the genetic control of these traits in forage grasses. Furthermore it is concluded that *Lolium* and *Festuca* can be considered as a single complex of grasses, and that these are a useful source of genetic variation for breeding drought resistant grasses for sustainable agriculture. *Festulolium* systems provide opportunities to dissect the genetic control of useful traits and this study has suggested that at least some of the genetic control of relevant rooting and drought tolerance traits is located on chromosome 3. Ryegrass/fescue chromosome 3 is syntenic with rice chromosome 1 on which root and drought trait QTL have frequently been declared. This study has confirmed the extent of the considerable genetic variation for root traits available in ryegrasses and fescues, and shown considerable evidence of close associations between below and above ground growth in the group of plant accessions studied.

The roots of the long term drought resistance of forage production with *Festuloliums*

Jean-Louis Durand* and Marc Ghesquière

French National Institute for Agricultural Research (INRA) BP6 – 86600 Lusignan, France, *jean-louis.durand@lusignan.inra.fr

Abstract. Drought resistance consists in: a minimum production in summer, for pasture and forage production, high density survival, fast recovery of the canopy in autumn and winter. Many plant properties are associated to that complex target, involving many traits more or less intermingled. Economic drought is not restricted to plant survival, which mainly is associated to a very low production in summer. The maintenance of an efficient canopy in summer depends on: leaf growth, leaf senescence, crop water status (soil water absorption – stomatal regulated transpiration), net photosynthesis.

Leaf production depends on tillering, leaf apparition and elongation rates, while the crop water status depends on the balance between water abortion from the soil by roots and the canopy transpiration. On the other side, the root water absorption first depends on the depth of soil explored. It means that the capacity y of the crop to produce deep roots before and maintain them during summer is essential. This is possible at the expense of energy, which cannot be further used for leaf production or tillering. *Lolium* and *Festuca* species exhibit much contrasted traits with respect to tillering, leaf area production and root growth and maintenance. The hybridization is hence a unique manner of combining these characters. Roots are more difficult to study and only recently was it proved that intra-specific variation in depth of water extraction could be investigated within populations of grasses. Recent findings will be described showing the use of natural abundance in oxygen isotopes in ranking the depth of water extraction in *Festuloliums* in various conditions of swards and nurseries.

Photoinhibition avoidance in cold and freezing tolerance in the *Lolium-Festuca* complex

Marcin Rapacz

University of Agriculture in Kraków, Faculty of Agriculture and Economics, Department of Plant Physiology, Podłużna 3, 30-239 Kraków, Poland, rrrapacz@cyf-kr.edu.pl

Abstract. During winter plants cope not only freezing but also other environmental stresses. Among them tolerance to cold-induced photoinhibition seems to be closely related to freezing tolerance. This relationship comes from partially common mechanisms of acclimation to both stresses. During cold acclimation increasing reduction of PSII observed with decreasing temperature may act as one of the signals triggering gene expression. On the other hand exposition to excess light compared to

the energy demand in dark photosynthetic reactions means photoinhibitory conditions, which must be avoided to protect destructive processes not only to photosystems but also to the entire cells due to the overproduction of reactive oxygen species. As a consequence during cold acclimation mechanisms of photosynthetic acclimation to photoinhibitory conditions are also induced. It was demonstrated that the capabilities for photosynthetic acclimation to the cold by *Lolium* × *Festuca* hybrids were correlated with genotypic differences in winter survival. An evidence that plants impaired in photosynthetic acclimation to cold were also not capable to increase freezing tolerance during cold acclimation was also presented in BC_3 progeny derived from a triploid hybrid of *F. pratensis* (2x) × *L. multiflorum* (4x) backcrossed three times onto diploid *L. multiflorum.* However, the approximate correlation between winter hardiness (or freezing tolerance) and the ability of photosynthetic acclimation to cold was about 0.7, which means that, freezing tolerance and the tolerance to cold-induced photoinhibition are, at least partially, independent.

Two main strategies of photosynthetic apparatus acclimation to relative excess of light can be observed in higher plants during cold hardening. The first strategy consists of increasing energy demand by increasing carbon assimilation and carbon metabolism which is called photochemical mechanism. The second, more efficient strategy relies on an intensification of protective non-photochemical mechanisms that harmlessly dissipate excess excitation energy as heat. The studies performed on androgenic plants of allotetraploid *Festulolium* cultivars (*F. pratensis* × *L. multiflorum*) confirmed that plants with higher winter hardiness were characterized with efficient non-photochemical dissipation of excess energy. Further studies showed that this mechanism is characteristic to *F. pratensis*, whereas in *L. multiflorum* only increasing photochemical energy quenching is observed. It was shown that the transfer of the non-photochemical mechanism from *Festuca* to *Lolium* results in increasing freezing tolerance of hybrids. The identification of non-photochemical mechanism of energy dissipation in cold in *Lolium* × *Festuca* hybrids may be considered an easy and relatively inexpensive (chlorophyll fluorescence measurements) physiological marker of freezing tolerance and winter hardiness. The preliminary evidence of a role for genes found on chromosome 4 of *F. pratensis* for increased non-photochemical energy dissipation was also shown.

Fungi of *Fusarium* spp. decrease yield quantity and quality of forage grasses – how we can improve new cultivars of *Festulolium*?

Agnieszka Płażek* and Ewa Pociecha

University of Agriculture in Kraków, Faculty of Agriculture and Economics, Department of Plant Physiology, Podłużna 3, 30-239 Kraków, Poland, *rrplazek@cyf-kr.edu.pl

Abstract. Winter hardiness of grasses and cereals is closely associated with frost and snow mould resistance. Winter pathogens are a specific group of fungi which infect overwintering plants under snow cover at low temperature, high humidity and limited light intensity. In Central Europe *Microdochium nivale* is a main agent

causing leaf injuries, and in the case of crown infection death of the whole plants. Fungi of *Fusarium* spp. are characterized by secretion of many mycotoxins from trichotecens group, dangerous for animals and human. *M. nivale* was earlier included to *Fusarium* genus and the main trichotecens produced by fungi of this species were named from *M. nivale*, for example nivalenol, deoxynivalenol (DON) etc. Further studies proved that *M. nivale* does not produce any harmful toxins, but some investigations demonstrated opposite data. In summer leaves and heads of forage grasses and cereals are infected by *F. graminearum* and *F. oxysporum*, producing such trichotecene toxins as T-2 toxin, zearalenone, nivalenol, DON or HT2-toxin. These compounds demonstrate nephrotoxic, hepatoxic influences, cause injury of reproductive and digestive systems of animals.

In the study relating to cultivation of *Festulolium* as forage grass its susceptibility to *M. nivale* and mentioned *Fusarium* species as well as content of trichotecens in leaves should be investigated. Moreover, the study should involve the regrow after snow mould infection and its influence for further growth rate, fresh and dry matter yield as well as seed yield. Some literature data indicate that sometimes *Fusarium* infection process proceeds without visual symptoms, but in leaves or seeds many toxins might be found. It means that study relating to indicate of biochemical (stress symptoms) and molecular (gene expression) differences between leaves of healthy plants and that from plants which had been infected and then regrown should be performed.

General discussion

What traits do we prioritize to assemble in Festulolium to produce a sustainable forage grass suitable for the 21st century and how do we best accomplish this?

Voices in the discussion: Marc Ghesquière, Beat Boller, Mike Humphreys, David Kopecký, Simen Sandve, Tomasz Książczyk

Mike Humphreys reminded about EU FPV project SAGES. During its realization the major traits important for drought resistance like root growth, and for winter hardiness were identified. Characterization of gene for root growth is important for *Festulolium*, as a relationship between root activity and drought resistance is observed. Drought resistance in perennial grasses is based on:

- persistency of the sward rather than summer productivity although for non-extreme climates such as the UK some continued plant growth and crop production is also necessary;
- priority was to capture water rather than to use it efficiently for growing. Again this would depend on the level of the stress. Water-use-efficiency (growth/unit water consumed) is an important UK target trait;
- priority of Depth of Water Extraction (DWE).

Characterization of the gene for root growth is also important. Drought resistance genes are mapped on *F. glaucescens* chromosome 3 and it was possible to transfer these genes into ryegrass. Five generations were produced, which are going to be tested in the field and selected for the market.

How are tillering, leaf morphogenesis and depth of water extraction genetically associated?

Voices in the discussion: Marc Ghesquière, Mike Humphreys

It is important to have the balance between physiology and morphology of the plant. If we have too much deep-rooting plant its drought resistance can be reduced.

It also depends on plant growing area (terrain topography). The main agronomic problem connected with improvement of morphologic traits in hybrids is that highly drought-resistant plants are characterized with loss of the yield, compared to more drought-sensitive genotypes.

Are chlorophyll fluorescence measurements a good tool for the discrimination of various stress resistance in Lolium-Festuca hybrids? Better than elsewhere?

Voices in the discussion: Marcin Rapacz, Beat Boller, Marc Ghesquière

The discussion was started by Marcin Rapacz who asked if chlorophyll fluorescence could be a marker for stress-resistance. Chlorophyll fluorescence measurement is effective and not time-consuming tool, quite often used by plant physiologists and breeders. Breeders are generally satisfied with the results, however sometimes interpretation can cause trouble. Chlorophyll fluorescence measurement can be done in the field (*in vivo* conditions), but it is more convenient to collect leaves from the plant growing in the field and take the measurements in the laboratory. The effectiveness of this method strongly depends on the plant species. It was successfully used for water deficit resistance differentiation of tall fescue population and in banana as well as freezing tolerance discrimination in wheat and triticale. On the other hand, it does not have its application in drought-treated barley. In tall fescue even the correlation between some chlorophyll fluorescence parameters and soil water potential can be assigned.

ABA-system in Festulolium

Voices in the discussion: Marc Ghesquière, Marcin Rapacz, Agnieszka Płażek

The problem of plant resistance to mould pathogens was discussed. Moulds are caused by various *Fusarium* species. They are very dangerous for animals and humans due to secretion of many toxins. Defence response to *Fusarium* diseases involves synthesis of such compounds as pathogenesis-related proteins, phenolic compounds, carbohydrates, and generation of reactive oxygen species. During influence of abiotic and biotic stresses various signal compounds are synthesized in plants. Abscisic acid (ABA) is often engaged in signal transduction and gene expression. ABA influences frost resistance and resistance to snow mould pathogens. However, in some plant species resistance to various pathogens can be decreased or increased by this hormone. The creation of new improved plant cultivars more resistant to diseases is complicated. Resistance to many pathogens has wide physiological and genetic background. Resistance to snow moulds and many *Fusarium* species is controlled by many genes. According to present knowledge not all of such genes were recognized. The problem of toxin production by *Microdochium nivale* var. *nivale* and *M. nivale* var. *majus* was discussed. It was suggested that an influence of snow mould infection on development of winter grasses and their yield (green mass and number of seeds) should be examined in further studies.

Session 4

Advances and state-of-the-art genomics studies in *Festulolium*

Introgression genomics in *Lolium/Festuca*

Julie King*, Ian Armstead, John Harper, Caron James, Dagmara Gasior, Luned Roberts, Rhys Kelly, Ann Thomas and Ian King

Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, UK, *juk@aber.ac.uk

Abstract. Introgression maps have been developed for each of the seven linkage groups of *Lolium/Festuca*. These introgression maps have been used to determine the physical and genetic location of SNPs derived from the coding sequence from approximately 1000 BACs from the rice genome. Many of the SNPs developed are from rice sequences previously used to generate primers that have been genetically mapped in barley. Cross species markers developed at the John Innes centre, Norwich, UK, have also been introgression mapped. The sequences used to develop the SNPs have also been aligned with the newly developed *Brachypodium* physical map.

Thus this work is providing a genome wide comparative analysis of gene order and distribution in *Lolium*, rice, *Brachypodium*, wheat and barley. It will also allow grass to "talk to" the other monocot species providing the mechanism for the amalgamation of data on the genetic control of target traits across the monocots. The introgression maps and primers developed during this project also provide the basis for the initial alignment of the *Lolium perenne* physical map to established chromosome-based introgression and genetic maps. Contigs developed during the physical mapping will be aligned to the bins on the introgression maps using common markers.

We have also demonstrated that a substantial component of the coding sequences in monocots is localised proximally in regions of very low recombination frequencies. The implication of these findings is that during domestication of monocot plants, selection has concentrated on genes located in the terminal regions of chromosomes within areas of high recombination frequency. Thus a large proportion of the genetic variation available for selection of superior plant genotypes has not been exploited.

Developing a physical map of the *Lolium perenne* genome based on High-Information Content BAC Fingerprinting and BAC-end sequencing

Ian Armstead[1*], Julie King[1], Lin Huang[1], Helen Ougham[1], David Kudrna[2] and Ian King[1]

[1]IBERS, Aberystwyth University, Aberystwyth, Ceredigion, SY23 2EL, UK,
[*]ipa@aber.ac.uk
[2]Arizona Genomics Institute, University of Arizona, Tucson, AZ8572, USA

Abstract. The development of next generation sequencing technologies has meant that sequencing throughput *per se* is no longer a significant technological limiting factor, even for larger genome plant species. The major challenge to targeted and complete sequencing of novel plant genomes becomes the downstream analysis, *i.e.,* the physical assembly, genic annotation and validation. One of the necessary steps in this process is the establishment of a comprehensive physical map of the target genome, based upon the fingerprinting and sequential compilation of overlapping bacterial artificial chromosome (BAC) sequences (contigs). Such a project is just being initiated at IBERS and the major aims can be summarised as:

a) creation of c. 10x genome coverage BAC libraries of an inbred *L. perenne* genotype;
b) construction of BAC DNA pools for efficient PCR screening of the BAC libraries for assigning markers to BACs;
c) creation of a BAC-end-sequencing (BES) database of the BAC library;
d) generation of High Information Content Fingerprints (HICF) of the BAC libraries with Fingerprint Contig (FPC) software alignments;
e) production of a manually-edited HICF-based physical map incorporating BES and marker data;
f) alignment of this *L. perenne* physical map with existing introgression, genetic and comparative maps;
g) creation of a user-friendly web interface for integrated data and map visualisation, exploration and distribution.

The target genotype (designated p226/135/16) has undergone 8 rounds of selfing and marker and sequence information indicate considerably reduced heterozygosity. BAC library construction, HICF and end sequencing for this project are being undertaken at the Arizona Genomics Institute. To date, genomic DNA from this genotype has been used to construct a HindIII BAC library consisting of 120960 clones with an average insert size of 134 kb. This should represent c. 5-6x genome coverage. BAC PCR screening pools have been developed, by Amplicon Express, which cover the entire library and which should allow mapping of PCR-based markers to individual BACs. A separate set of pools has also been developed to explore the feasibility of similarly assigning DArT markers. A second BAC library of similar size is in the process of construction using the restriction enzyme BstY1 and PCR screening pools will also be constructed from this library. Both libraries will move forward to HICF and BAC-end sequencing within the next few months.

The BAC libraries, PCR screening pools and associated datasets will, wherever possible, be made available to the *Lolium/Festuca* research community. It is hoped that these resources will form the initial focus of an international effort towards the comprehensive molecular characterisation of the *L. perenne* genome.

Chromosome research in the *Festuca-Lolium* complex

David Kopecký

Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany of the AS CR, Sokolovská 6, CZ-77200 Olomouc, Czech Republic, kopecky@ueb.cas.cz

Abstract. The presentation will provide an overview of the grass research program in the Laboratory of Molecular Cytogenetics and Cytometry during the past five years. The program uses *Festuca* and *Lolium* species to study chromosomes and their behavior in poor species and intergeneric hybrids. This is facilitated by several attributes, which make *Festuca* and *Lolium* excellent cytogenetic models. (a) Homoeologous parental chromosomes pair and recombine readily in hybrids. (b) Chromosomes of parental species in hybrids can be easily distinguished by genomic *in situ* hybridization (GISH). (c) Chromosomes are identifiable based on morphology and using cytogenetic markers. (d) Chromosomes are large enough to identify introgressed segments and to study the distribution and frequency of homoeologous recombination events. As part of our research program, we have been developing novel materials and tools for the analysis of genomes of *Festuca* and *Lolium* species and their hybrids. These include unique germplasm and cytogenetic stocks, cytogenetic mapping, flow cytometry for ploidy screening and estimation of genome size, and a DArTFest array for characterization of genome constitution in ×*Festulolium* hybrids and construction of genetic maps in *Festuca* and *Lolium*.

This research has been supported by the Ministry of Agriculture of the Czech Republic (grant award NAZV QH71267).

DArT marker association with frost tolerance in a *Lolium perenne* × *Festuca pratensis* hybrid population

Simen R. Sandve[1*], David Kopecký[2], Jan Bartoš[2], Arild Larsen[3], Liv Østrem[4] and Odd Arne Rognli[1]

[1]Norwegian University of Life Sciences, Department of Plant and Environmental Sciences, Norway, [*]simen.sandve@umb.no
[2]Laboratory of Molecular Cytogenetics and Cytometry, Institute of Experimental Botany, Olomouc, Czech Republic; [3]Graminor, Bodø, Norway; [4]Bioforsk Vest Furuneset, Norway

Abstract. A *Festulolium* line originating from a *Lolium perenne* × *Festuca pratensis* cross was screened for frost tolerance using the re-growth method. Eleven plants with high frost tolerance (HFT) and ten with low frost tolerance (LFT) were genotyped with the DArT-FEST chip. HFT group had an average frost tolerance score of 7.06 (±0.22) and the LFT group had an average frost tolerance of 2.59 (±0.61). DArT markers that differed in their distribution between HFT and LFT groups were identified and 72 markers with significantly different distribution ($q<0.05$) were subsequently sequenced. Of these 72 markers, 46% had blast hits with an E-value<1e-10, and 21 genes could be classified with a putative functional class according to GO terms and manual classification. The largest group was transcription associated genes. Using existing genetic map positions of DArT markers (unpublished results) we were able to place 58 of the DArT markers from this study onto *Lolium* and *Festuca* linkage groups. One of the mapped loci, marker D562536 situated on the top half of LG4, was significantly associated with frost tolerance phenotype ($q=0.041$). However, most markers that associate with the frost tolerance phenotypes have unknown genomic position. Preliminary analysis with GISH on a subset of the HFT and LFT plants indicate that selection for LFT favored tetraploid genotypes, while selection for HFT increased the frequency of diploid plants and the occurrence of large introgressions of *F. pratensis* into the *L. perenne* background.

General discussion

Is cytogenetics already dead discipline? Can it survive in "Next Generation and High-Throughput Age"?

Voices in the discussion: David Kopecký, Mike Humphreys, Simen Sandve, Tomasz Książczyk, Ian Armstead

Starting the discussion, David Kopecký related to this question said that in era of new generation sequencing and SNPs, analyzing a single plant by cytogenetic approach will be of low-throughput and time consuming, in other words highly in-effective. The use of more advanced techniques, such as DArT arrays, should ensure us fast characterization of genome constitution in *Festulolium* hybrids and in construction of genetic maps in *Festuca* and *Lolium*. Characterization of differences in sequence between parental cultivars using DArT arrays, should be of much importance. On the other side, the FISH technique was recognized as a valuable technique and also it was pointed out that there is still a lot of work using FISH with different probes to attend. Tomasz Książczyk disagreed that cytogenetics was a discipline without a future. He disagreed with the opinion that cytogenetics is a dead discipline and stated it could survive in "Next Generation and High-Throughput Age". For basic studies Tomasz stated, use of cytogenetics has much importance, reminding us of its usefulness in many plants as a basic tool of genome characterization and chromosome identification. There are scientists who are not interested in genetic mapping or High-Throughput sequencing as well as combining physical and genetic maps`. On the other side, physical mapping of genes responsible for a quality trait requires definitely well establish cytogenetic maps and chromosome identification of each grass species. Localization of different DNA sequences using FISH (e.g. rDNA-FISH, BAC-FISH) allows determination of markers for particular chromosomes, which is critical for future gene localization leading to grass quality improvement. This is especially important for grasses such as *Lolium* or *Festuca* species, which have numerous chromosomes with a reduced number of chromosome-specific physical markers. The usefulness of the BAC clones coming from *L. perenne* BAC library was considered as a potential source of chromosome-specific markers. Advantages and disadvantages of this study were discussed, indicating that the BAC clones might be used in those experiments, but we should be careful with employing BAC-FISH to find chromosome-specific markers.

Can Festuca- and Lolium-specific markers be identified, and what would be the best strategy for the development of such markers?

Voices in the discussion: Marc Ghesquière, Beat Boller, Vladimir Černoch, Simen Sandve

The main problem of the strategy is connected with a choice of the species-specific markers. Some markers, which are specific for one population, can be non-specific for another population and, a population-specific marker after strong selection could become non-specific at the end of selection process. On the other hand, the participants were able to obtain some real species-specific markers. It was also taken into consideration, that different ecotypes and populations must be compared with each other to test and choose suitable markers. One possible strategy is the Fisher Exact Test to identify markers with different allele distribution, as an example of marker assisted phenotype.

How to relate bioinformatic and sequence information to the phenotype?

Voices in the discussion: Ian Armstead, Neil Jones, David Kopecký, Vladimir Černoch, Piet Arts, Mike Humphreys

Concerning the question, it was pointed out that such relations are difficult to establish due to epigenetic effects. There is a mass of information within one genome with in these grass species; every genotype being different, which makes understanding the genetic controls of complex traits very difficult. Breeders must work, select and monitor many traits. There is no simple relationship between genotype and phenotype, but moves in some crop plants, like in maize were underway to employ whole genome associations with crop phenotype. Following a question, how can breeders and researchers come together to undertake research? The participants agreed that there was a great advantage in collaboration, but they also pointed out that it could be a competing or a real collaboration. Selling varieties to the markets will provoke competition for both breeders and scientists. An important point is that breeders will always require new plant materials, new *Festulolium* cultivars to sell, so it would be very helpful if research developers would be interested in breeding new cultivars.

It also should be stated, and what this forum encompasses, that there are new techniques to design new varieties, and grasses could have better adaptation to the climate which is itself changing. There will be new and improved opportunities for *Festulolium.* A few words concerned the economy which is very important in breeding new varieties; many varieties to maintain require a large financial outlay. Some participants were rather sceptical whether it will be possible to relate bioinformatics with the phenotype, giving an example of *Zea mays*, in which a relationship of the genome and phenotype is poor at this moment.

What would you like Lolium/Festuca genomics to be able to do?

Voices in the discussion: Neil Jones, Mike Humphreys, Ian Armstead

A question regarding which modern methods can be used to study genomics begun the discussion on what will we be able to do next. The participants described a range of many techniques that could be useful in *Festulolium* genomics and they were positively composed to further research, suggesting an open "genomic platform". In relation to this, some participants believed that there are great possibilities to use the outcomes from rice genomics. It was worth mentioning that the *L. perenne* physical map development as well as alignment of physical contigs to genetic/introgression maps will provide a modern genomic tool in *Festulolium* research for the new era.

Posters

Chromosome pairing in introgression lines of *Lolium perenne*/ *Festuca pratensis*

Magdalena Taciak[*], Elżbieta Zwierzykowska and Zbigniew Zwierzykowski

Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland; [*]mtac@igr.poznan.pl

Abstract. *Festuca pratensis* is an important source of genes for abiotic stress resistance for *Lolium multiflorum* and *L. perenne* cultivars. For several years at IPG PAS in Poznań a backcross breeding programme for the transfer of frost tolerance genes from winter hardy *F. pratensis* into high quality *L. perenne* cultivars has been performed. Partially fertile, triploid hybrids of *F. pratensis* × *L. perenne* (2n = 3x = 21; FpLpLp) were produced by intercrossing the diploid *F. pratensis* (2n = 2x = 14), used as the female parent, with the autotetraploid *L. perenne* (2n = 4x = 28), used as the male parent. The F1 hybrids were backcrossed twice onto diploid *L. perenne* (2n = 2x = 14) cv. Arka to produce BC1 and BC2 generations. Both backcross progenies were studied in respect of their chromosome number, genomic structure and fertility. In many investigations genomic *in situ* hybridization (GISH) has been used successfully to differentiate genomes in *Festuca* × *Lolium* hybrids and to identify alien chromosomes and chromosome segments in allopolyploid and introgression derivatives of *Festuca-Lolium*. Until now only a small number of papers have been published on *Festuca-Lolium* hybrids where GISH was used to study meiosis.

In this work we present results of chromosome pairing at metaphase I of pollen mother cells (PMCs) in different *L. perenne* introgression derivatives: (*i*) substitution lines, having 13 *Lolium* chromosomes and 1 complete *Festuca* chromosome, (*ii*) addition lines, with 14 *Lolium* chromosomes and 1(2) complete *Festuca* chromosome(s), and (*iii*) introgression lines with 13 complete *Lolium* chromosomes and 1 recombinant *Lolium* chromosome with a single terminally located *Festuca* chromosome segment. In substitution lines a complete *Festuca* chromosome pair mostly in a LpFp bivalent, similarly as a *Lolium* chromosome with a single *Festuca* segment. In addition lines *Festuca* chromosomes remained as univalents.

rDNA loci patterns in triploid and tetraploid hybrids of *Festuca pratensis* × *Lolium perenne*

Tomasz Książczyk[*], Magdalena Taciak and Zbigniew Zwierzykowski

Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland, [*]tksi@igr.poznan.pl

Abstract. The *Festuca-Lolium* complex contains several species of high agronomic importance, which are also good models for studying the evolution of genomes through the use of chromosome markers. Physical mapping of 5S and 18S–5.8S–25S (45S) rRNA genes by fluorescence *in situ* hybridization (FISH) provides valuable chromosomal landmarks, and their characteristic positions enable chromosome identification. The aim of our research was (i) an analysis of ribosomal DNA (rDNA)

loci number and localization in *F. pratensis* and *L. perenne* cultivars, and in two intergeneric hybrids – triploid (2n = 3x = 21) *F. pratensis* (2x) × *L. perenne* (4x) and tetraploid (2n = 4x = 28) *F. pratensis* (4x) × *L. perenne* (4x), (ii) a determining the parental genomes using genomic *in situ* hybridization (GISH), (iii) an assignment of known chromosomal markers to corresponding genomes in these hybrids (Książczyk et al. 2010). Among triploid and tetraploid F_1 hybrids, FISH with 5S and 25S rDNA probes showed various numbers of rDNA loci, indicating the genome reorganization with a tendency to reduce the number of 45S rDNA loci. This tendency is accompanied by the amplification of 5S rRNA gene loci in *Festuca* genome-like chromosomes in the F_1 plants studied. Nevertheless, this assay did not allow indicating particular rDNA-bearing *Lolium* chromosome pairs in triploid and tetraploid hybrids, because of undifferentiated 45S rDNA loci pattern. On the other hand, FISH/GISH analyses in triploid and tetraploid hybrids proved that *Festuca* chromosome pairs 2 and 3, and only the *Lolium* chromosome pair 3 can be easily tracked and should be used to discern specific rDNA loci patterns in the reorganization of genomes in other *Festuca* × *Lolium* hybrids and their derivatives. Whilst rDNA sequences seem not to be an effective marker of *Lolium* chromosomes, in other case, an application of new landmarks for the remaining chromosomes is still needed. Therefore, it will be our intention in the collaboration with Dr Glyn Jenkins (IBERS, Aberystwyth University, UK) to use a set of *Lolium* BAC clones for physically mapping and to identify *Lolium* chromosomes in *Festuca* × *Lolium* hybrids. BAC-FISH experiments will be now being carried out, using chromosome-specific BAC clones, which should further facilitate parental chromosome identification in intergeneric *Festuca* × *Lolium* hybrids as well as to analyze the restructuring of genomes in their successive generations.

Książczyk T., Taciak M., Zwierzykowski Z. (2011). Variability of ribosomal DNA sites in *Festuca pratensis*, *Lolium perenne* and their intergeneric hybrids revealed by FISH and GISH. J. Appl. Genet. (submitted).

This work has been supported in part by the Polish Ministry of Science and Higher Education (grant No. N N310 090736).

A study of the naturally occurring ×*Festulolium* hybrid – ×*Festulolium loliaceum* and its progeny

John Harper*, Dagmara Gasior, Julie King, Ian King, Ianto Thomas and Mike Humphreys

IBERS, Aberystwyth University, Gogerddan, Aberystwyth, Ceredigion, SY233EB, Wales, UK, *oah@aber.ac.uk

Abstract. ×*Festulolium loliaceum* is a natural hybrid formed between the widespread and common forage grass species *Lolium perenne* and *Festuca pratensis*. *L. perenne* is the most widely sown grass species in UK agriculture and is therefore considered of great economic importance. *F. pratensis,* although not used widely in agriculture in the UK due to its poor agronomic performance is a close relative of *L. perenne* and is a potential source of novel and complementary gene variation. For example cold

tolerance and disease resistance genes have been transferred from *Festuca* into the *Lolium* species. The greater resilience to abiotic stresses found commonly in *Festuca* species compared to those of *Lolium* has led to much effort both in the UK and across Europe to create synthetic intergeneric hybrids (as alternatives to the natural hybrid ×*Festulolium loliaceum*) that combine the attributes of good growth and forage quality found in *Lolium* cultivars with the greater resilence to stresses found in *Festuca,* and this has taken on priority as a durable and sustainable strategy to combat future climate change.

The hybrid ×*Festulolium loliaceum* is found across the UK and Europe especially in mature meadows on land prone to flooding. They are found in three forms as diploids and as triploids where they may have either two genomes of *L. perenne* and one of *F. pratensis* or alternatively two genomes of *Festuca* and one of *Lolium*. The triploids arise probably due to unreduced female gametes. The naturally occurring hybrids and their location in more waterlogged soils than their parent species can provide an insight into the selection pressures that have favoured the hybrid generation and in genetic studies their relationship with neighbouring *Lolium* and *Festuca* parent species' populations. The opportunities for gene flow and introgression between the hybrids and the parent species can also be studied.

In this study we have examined the genomic constitution and fertility of hybrids collected in Oxfordshire. We have been able to produce a number of progeny from these hybrids which have given insights into how polyploids might be naturally produced and how 'semi sterile' hybrid plants can gain a degree of fertility which could allow exchange of genetic material between the two species. If these natural hybrids have evolved to survive in niche environments such as waterlooged meadows then they could be a source of important variant alleles that could be transferred into the agricultural species. Alternatively heterosis associations between *Lolium* and *Festuca* genes may confer ×*Festulolium loliaceum* with a selective advantage in flooded conditions compared to *L. perenne* and *F. pratensis*.

The production of a wide hybrid between *Lolium multiflorum* and the North African *Festuca* species *Festuca arundinacea* subsp. *atlantigena*

John Harper*, Dagmara Gasior, Mike Humphreys, Markku Farrell, Julie King and Ian King

IBERS, Aberystwyth University, Gogerddan, Aberystwyth, Ceredigion, SY233EB,Wales, UK; *oah@aber.ac.uk

Abstract. The grass species *Festuca arundinacea* subsp. *atlantigena* is found in the Atlas Mountains in North Africa. We know it is an octoploid with 2n=56 chromosomes and is a deep rooted perennial fescue. The hybrids were produced by crossing a tetraploid *Lolium multiflorum* cultivar with *F. atlantigena* creating a F1 hexaploid 42 chromosome hybrid having 28 chromosomes from *F. atlantigena* and 14 chromosomes from *L. multiflorum*. We then used a combination of pair crossing and polycrossing between the F1 plants and were successful in getting viable seed.

The progeny have been grown on and we have used a combination of cytological techniques to establish the chromosome number of the progeny and their genomic constitution. In the progeny we are observing a loss of chromosomes and in the plants studied to date this has involved loss of *F. atlantigena* chromosomes. The preferential reduction of the *Festuca* compared to the *Lolium* chromosome complement that develops over generations resembles conclusions drawn from alternative studies that used *L. perenne* or *L. multiflorum* × *F. pratensis* amphiploids where it is hypothesised that meiotic drive favours preferential transmission of *Lolium* chromosomes.

The progeny are being phenotyped for '*Festuca* like' and '*Lolium* like' phenotypes. We have already observed interesting traits such as rhizome and deep rooting in these hybrids:-they will be the subject of further study to look at potentially important traits. The ability to redesign *Festulolium* genotypes and phenotypes provides novel and multifunctional uses for *Festulolium* grasses.

The study will also allow us to gain insights into the possible progenitors of the North African fescues, both the octoploid *F. atlantigena* and the decaploid *F. letourneuxiana* have been relatively little studied. It is thought that *F. glaucescens* and *F. mairei* may be possible progenitors. We are using ribosomal DNA to establish the number and position of ribosomal sites in these species. In past studies ribosomal probes have been invaluable in providing 'chromosome landmarks' both in *Festuca* and *Lolium* species. We are also using genomic DNA from the possible progenitors as genomic probes singularly and in combination to confirm whether or not they constitute the ancestral genomes of *F. atlantigena*. This combination of approaches should give valuable information not only on the origins of the North African fescues but also add to our understanding of how polyploids are created and how polyploidy gives them an advantage over their progenitors by enabling them to survive or colonise areas or habitats unavailable to the progenitor species.

Accumulation of the selected amino acids and sugars during cold acclimation of perennial ryegrass genotypes distinct in the level of frost tolerance

Aleksandra Bocian[1*], Arkadiusz Kosmala[1], Marcin Rapacz[2] and Zbigniew Zwierzykowski[1]

[1]Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland; [*]boc@igr.poznan.pl
[2]Agricultural University in Kraków, Faculty of Agriculture and Economics, Department of Plant Physiology, Podłużna 3, 30-239 Kraków, Poland

Abstract. *Lolium perenne* (perennial ryegrass) is one of the most important forage grass species. Similarly to all winter-hardy plant species, *L. perenne* has the ability to increase its frost tolerance after exposition to low sublethal temperatures (this adaptive process is known as cold acclimation, CA). Cold acclimation influences numerous physiological and biochemical pathways in plants, reprogramming cell metabolism and gene expression. The most important plant reactions to temperature decrease are changes in proportion and composition of membrane lipids, and

accumulation of compatible solutes (mostly sugars and amino acids known as osmoprotectants) in the cell's cytoplasm. To recognize the metabolomic response to CA in *L. perenne*, the comprehensive research was initiated, including: (i) the selection of two genotypes with distinct levels of frost tolerance – high frost tolerant (HFT) genotype and low frost tolerant (LFT) genotype, (ii) the analysis of primary leaf metabolites (sugars and amino acids) accumulation at eight different time points of CA (2, 8, 26 hours, and 3, 5, 7, 14 and 21 days), as well as one day before CA, using gas chromatography combined with mass spectrometry, (iii) the comparison of the obtained accumulation profiles between HFT and LFT genotypes. Two control samples were used – the first collected before cold acclimation after two hours of light and second one after 8 hours of light to eliminate potential influence of illumination on metabolite levels. No differences between this two control samples were observed within a group of sugars but higher level of accumulation was estimated in "8h control" in the case of aspartic acid (both genotypes) and proline (only HFT genotype). The most intensive accumulation during CA was observed in the case of proline and raffinose. These compounds are thought to be the most important and universal compatible solutes identified in plants. The abundance of the other analyzed sugars remained unchanged during CA with the exception of fructose (its abundance increased slightly in both genotypes but was always higher in HFT genotype and it could be connected with hydrolysis of the fructans). On the other hand, the abundance of the most analyzed amino acids increased during CA, however, glutamic and aspartic acids showed similar levels of accumulation in both genotypes, whereas the levels of lysine and asparagine were higher in LFT genotype. The accumulation process of presented compatible solutes may be connected with development of frost tolerance during cold acclimation in *L. perenne*. However, according to these results the importance of given osmoprotectants is inconclusive.

This work was supported in part by the Polish Ministry of Science and Higher Education (grant no. PBZ-MNiSW-2/3/2006/21).

Physiological backgrounds of *Festulolium* snow mould resistance – current state of knowledge and perspectives

Ewa Pociecha[1*], Agnieszka Płażek[1] and Zbigniew Zwierzykowski[2]

[1]University of Agriculture in Kraków, Faculty of Agriculture and Economics, Department of Plant Physiology, Podłużna 3, 30-239 Kraków Poland, [*]rrchilmo@cyf-kr.edu.pl
[2]Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland

Abstract. The resistance of *Festulolium* to environmental factors, especially to frost and snow mould pathogens, usually are not positively correlated; however, hardening to frost causes positive physiological and structural changes which are important for both mentioned factors. The physiological backgrounds of differences in resistance to frost and fungus *Microdochium nivale* were studied on six androgenic genotypes generated from the amphidiploid *Festuca pratensis* × *Lolium multiflorum* (2n = 4x = 28) hybrid (*Festulolium*). Metabolic changes during prehardening (2 weeks at 12ºC),

during hardening (3 weeks at 2ºC) and during first days of pathogenesis caused by *M. nivale* were analyzed.

The obtained results demonstrated that in the laboratory conditions resistance to frost of androgenic *Festulolium* forms was positively correlated with resistance to *M. nivale*. During prehardening and hardening the significant relationship between increased abscisic acid concentration and decreased salicylic acid concentration and resistance to *M. nivale* of studied genotypes was found. It was observed that cold acclimation induces changes of the total phenolic pool, and that their patterns are different for resistant and susceptible plants. It confirms the positive role of phenolic compounds in plant protection against stress conditions. Decrease of osmotic potential, usually accompanying cold acclimation, was also found as a form of plant adaptation to biotic stress. Decreasing of water potential was partly a result of soluble carbohydrates accumulation which is considered to be a possible link between the resistance to frost and fungi. The relationship between photosynthetic efficiency with a low level of molecular weight carbohydrates and pathogen resistance might render the measurements of chlorophyll fluorescence useful in the evaluation of the degree of plant resistance to snow mould. During first few days of pathogenesis, based on the results of the study, potential indicators of resistance to *M. nivale* in *Festulolium* include the following factors: increased soluble carbohydrate content and phenolics content, higher hydrogen peroxide accumulation, decreased catalase activity, increased abscisic acid content and reduced heat emission. In general, the results obtained confirm the existence of an overlap between signaling networks controlling responses to abiotic and biotic stress. The relationship between the resistance to frost and snow mould pathogens and physiological parameters analyzed herein, as well as the others, such as the impact of different hormones, will be discussed in detail.

Chemotaxonomic relationships within the *Festuca-Lolium* complex on the basis of phenolic secondary metabolites

Anna Piasecka*, Aneta Sawikowska, Zbigniew Zwierzykowski and Piotr Kachlicki

Institute of Plant Genetics of the Polish Academy of Sciences, Strzeszyńska 34, 60-479 Poznań, Poland, *akar@igr.poznan.pl

Abstract. Most knowledge on the taxonomy and phylogenetic relationships in grasses within the *Festuca-Lolium* complex came from morphological, cytogenetic as well as seed-protein and DNA markers studies. The aim of the present work was to determine whether secondary metabolites can be used to characterize *Festuca* and *Lolium* species, their cultivars or intergeneric *Festuca* × *Lolium* (*Festulolium*) hybrids. Chlorogenic acids and flavonoids play a crucial role in plant response to unfriendly environmental conditions and their presence influences the feeding quality of plant material. However, information regarding composition of these phenolic secondary metabolites in grasses is limited. We report results of studies on the variation in phenolic compounds that are present in leaves of chosen cultivars of *F. pratensis*, *F. arundinacea*, *L. multiflorum* and *L. perenne,* as well as *Festulolium*

hybrids. Additionally, two cultivars representing other grass species, *Agrostis gigantea* and *Phleum pretense,* have been studied. The analyses have been performed using two liquid chromatography systems: HPLC with ultraviolet UV diode array detector (DAD) and electrospray ionization (ESI) ion trap mass spectrometer as well as UPLC/UV instrument. The applied systems provide opportunities to identification of 120 different compounds. Among them tri-, di- and monoglucosides of flavons: apigenin, chrysoeriol and tricin as well as flavonols: quercetin, kaempferol and isorhamnetin have been found. The identified sugar derivatives include mainly glucosides, gentiobiosides, sophorosides, rutinosides and in a lesser proportion glucuronates. The identified compounds are mainly *O*-glycosides, but some *C*-glycosides have also been detected. Some glucosides are esterified with malonic, *p*-coumaric, caffeic, or ferulic acid. In addition isomeric chlorogenic acids occur in the studied plants in relatively high concentrations. UPGMA (Unweighted Pair Group Method with Arithmetic) trees have been created on the basis of the secondary metabolites profiles data. The classical UPGMA method has been computed using GenStat software and dendrograms have been drawn from the phenolic-derived distance matrix of the grass accessions studied. The dendrograms show a very high conformity of phenolic compounds composition and taxonomic classification of the studied grass cultivars.

Developing tetraploid *Festulolium* strains with greater summer survival in warm region of Japan

Kazuhiro Uchiyama[1*], Toshinori Komatsu[2], Akira Arakawa[1] and Kazuhiko Mizuno[1]

[1]National Institute of Lovestock and Grassland Science, Senbonmatsu 768, Nasushiobara, Tochigi 329-2747, Japan; [*]kazuuchi@affrc.go.jp;
[2]National Agricultural Research Centre for Hokkaido Region, Hitsujigaoka 1, Toyohira-ku, Sapporo 062-8555, Japan

Abstract. Summer stress tolerance, which influences persistency and productivity, is the most important characteristics for forage grass breeding in warm region of Japan. Introduced cultivars of *Festulolium* from foreign countries were not adapted to the warmer regions of Japan because of the lack of summer stress tolerance (Uchiyama et al., 2004). On the other hand, Japanese cultivars of Italian, perennial and hybrid ryegrass and meadow fescue showed better performance than the foreign *Festulolium* cultivars. Hence we decided to improve summer stress tolerance of *Festulolium* by two approaches. One is to produce intergeneric hybrids between Japanese meadow fescue and ryegrass cultivars, and the other is to improve summer stress tolerance of foreign *Festulolium* cultivars by phenotypic recurrent selection. We developed two strains of tetraploid *Festulolium*, 'Nakei 1' and 'Nakei 2' in 2008.

'Nakei 1' was developed from intergeneric hybridization between Japanese meadow fescue and ryegrass cultivars. As female parents, we selected 2 genotypes each from 4x 'Tomosakae' and 4x 'First' (meadow fescue). As male parents, we selected 3 genotypes from 'Akiaoba 3' (Italian ryegrass), 2 genotypes from 'High flora' (hybrid ryegrass) and 1 genotype each from 'Yatsugatake H-2' (hybrid

ryegrass) and 'Yatsukaze 2' (perennial ryegrass). We crossed the genotypes between the meadow fescue and ryegrass, and obtained 29 intergeneric hybrids through embryo rescue method in 2003. The F_1 hybrids were intercrossed for producing F_2 seeds in the isolated greenhouse in 2004. 1120 plants of 29 half-sib families were planted in 2004, and then 66 plants with vigorous regrowth after summer were selected and intercrossed in 2007. This generation was designated as 'Nakei 1'.

'Nakei 2' was developed from foreign cultivars of *Festulolium* by 3 cycles of phenotypic selection for summer survival. In the first cycle of selection (2001-2003), we planted 1120 plants of 7 foreign cultivars (Barfest, Becva, Evergreen, Paulita, Perun, Prior and Tandem) as initial breeding materials. We selected 42 genotypes and intercrossed among them. In the second cycle (2003-2005), we planted 1260 plants of 33 half-sib families and selected 41 plants of 18 half-sib families for intercrossing among them. In the third cycle (2006-2008), we planted 1570 plants of 41 half-sib families and selected 65 plants of 17 half-sib families for intercrossing among them. This generation was designated as 'Nakei 2'.

Now the local adaptability test of these 2 strains is being done in 9 locations all over Japan from 2008 to 2012. We hope they will be registered in the national cultivar list of Japan in 2013.

Effect of water stress on leaf greenness (SPAD index) and dry matter yield of *Festulolium braunii* (K. Richt.) A. Camus

Mariola Staniak

Department of Forage Crop Production, Institute of Soil Science and Plant Cultivation – State Research Institute, Czartoryskich 8, 24-100 Puławy, Poland, staniakm@iung.pulawy.pl

Abstract. Environmental stress, especially drought stress, can play an important role in the reduction of crop yield. Drought in Poland are hardly predictable. It is difficult to forecast the term of their occurrence, duration, territorial range and intensity. The driest region of Poland is almost the entire central region, as well as northwestern and mid-eastern parts. In that region summer water deficits strongly limit forage production and this could be improved by new grass cultivars.

Chlorophyll can be considered an indicator of the annual yield of grasses and grass regrowth, since there exists a positive correlation between its content in the leaf blades of grasses and dry matter yield. Chlorophyll concentration is a reliable indicator of plant vigor and resistance to environmental stressors.

The aim of the study was to determine the effects of water stress on leaf greenness (SPAD index) and dry matter yield of three *Festulolium* varieties. A pot experiment was performed in 2008, in a greenhouse of the Institute of Soil Science and Plant Cultivation–State Research Institute in Puławy (51°21'N; 21°40'E), at two soil moisture levels: 70% field water capacity (optimum moisture content) and 40% field water capacity (water stress). It was study the responses of three ×*Festulolium braunii* varieties: Felopa, Sulino and Agula to water stress. In order to maintain the appropriate soil moisture, water losses were made up on a daily basis, to achieve a specified weight of the pot with soil. The experiment was performed in four replications.

Over the vegetation season leaf greenness (SPAD index) with a SPAD 502 optical chlorophyll meter (Minolta) was measured. This device measures the difference between light absorption by a leaf at a wavelength of 650 and 940 nm, and the quotient of these values represents indexed leaf greenness correlated with chlorophyll content. The chlorophyll concentration was measured on the youngest, fully developed leaf of shoots selected randomly of each pot. The plants were defoliated three times over the grooving season. The yield of dry matter was determined.

It was found that the water deficit in soil had the significant effect on the chlorophyll content of leaves. All tested varieties showed higher values of the SPAD index under conditions of soil moisture deficiency. Chlorophyll concentration in leaves increased on average by 15%, compared with the control treatments. The differences between varieties were not significant. The highest SPAD index value were found in the second regrowth, and the lowest in the first regrowth. The dry matter yield of all tested varieties examined was at a comparable level. Water stress considerably affected dry matter yield. The mean decrease in dry matter yield was 37%, in comparison with control treatments. The strongest response was observed in var. Agula, where yield decrease was approx. 38%, and the slightest in var. Sulino, where yield was lower by 35%.

Understanding the genetic basis for slow plant-mediated proteolysis in *Festulolium* hybrids

Sally O'Donovan*, Alison Kingston-Smith and Mike Humphreys

IBERS, Aberystwyth University, Gogerddan, Aberystwyth, Ceredigion, SY23 3EB, Wales, UK, *sso@aber.ac.uk

Abstract. Ruminant farming uses approximately half of the UK land area and has benefitted from continual developments in forage species for use in improved pastures. The use of improved pasture land for ruminant production has had a major impact on productivity but inefficiencies in the rumen (associated with excessive rates of degradation of forage protein) mean that capture of resource is still poor compared with potential gains. Increased rumen efficiency through improved utilisation of feed protein will also decrease emissions of environmentally damaging wastes and greenhouse gas emissions. Previous studies have identified that part of the inefficiency in feed use by ruminants is due to plant-mediated proteolysis, induced by the adverse conditions in the rumen (temperature of 39°C and anaerobic). Furthermore, species-specific differences in rates of induced proteolysis in *Festuca* and *Lolium* species indicate that induced proteolysis is under genetic control. The aim of this project is to exploit the ability to make intraspecific *Festulolium* hybrids to produce a genotype with the palatability of *Lolium* plus the enhanced preservation of plant protein in the rumen of *Festuca*. Specifically we are testing the effect of genome dosage on the ability to capitalise on protein protection in relation to differences in endogenous mechanisms for survival under heat and drought stress.

This project is funded as a studentship through EBLEX (English Beef and Lamb Executive) and HCC (Meat Promotion Wales) levy boards for England and Wales.

Author index