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## Genetic diversity assessment of two *Medicago sativa* genes: CAD and WXP1

Camille Gréard, Philippe Barre, Sandrine S. Flajoulot, Sylvain Santoni,  
Bernadette Julier

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Camille Gréard, Philippe Barre, Sandrine S. Flajoulot, Sylvain Santoni, Bernadette Julier. Genetic diversity assessment of two *Medicago sativa* genes: CAD and WXP1. Joint meeting Breeding Grasses and Protein Crops in the Era of Genomics of Eucarpia Fodder Crops and Amenity Grasses Section and Protein Crops Working Group of Oil and Protein Crops Section, European Association for Research on Plant Breeding (EUCARPIA). FRA., Sep 2017, Vilnius, Lithuania. 267 p., 10.1007/978-3-319-89578-9\_41 . hal-02733670

**HAL Id: hal-02733670**

**<https://hal.inrae.fr/hal-02733670>**

Submitted on 2 Jun 2020

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# **BREEDING GRASSES AND PROTEIN CROPS IN THE ERA OF GENOMICS**

**BOOK OF ABSTRACTS**  
of the Joint Meeting of EUCARPIA Fodder Crops  
and Amenity Grasses Section and Protein Crops Working Group  
of Oil and Protein Crops Section



LITHUANIAN RESEARCH CENTRE  
FOR AGRICULTURE AND FORESTRY

Vilnius, Lithuania,  
September 11–14, 2017

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## Lithuanian Research Centre for Agriculture and Forestry

Lithuanian Research Centre for Agriculture and Forestry (LAMMC) is a public research institution with a mission to provide innovative solutions for agriculture and forestry through scientific research and experimental development. Institution focuses on five areas of interest: soil science, agroecology, food processing, phytopathology and plant breeding. Centre employs a total staff of 575 in three institutes - Institute of Agriculture, Institute of Horticulture and Institute of Forestry, and seven regional branches distributed across various regions of Lithuania. LAMMC carries long-term plant breeding programs in major field crops, vegetables, fruits and berries with more than 450 cultivars have been released since the establishment of plant breeding activities in 1922. Nowadays breeding of cereals, e.g. winter and spring wheat, spring barley, oats and field peas, and perennial grasses, e.g. perennial ryegrass, meadow fescue, *Festulolium*, Kentucky bluegrass, cocksfoot, timothy, red and white clover, is of major focus. Fruit trees, e.g. apple, pear and cherry, berries, e.g. black currant, strawberry, and vegetables, e.g. potato, carrot, onion, tomato and cucumber, are also being bred. Cultivars developed at the Lithuanian Research Centre of Agriculture and Forestry are being marketed in Lithuania, Latvia, Estonia and Belorussia.





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# Conference programme

A joint meeting of EUCARPIA Fodder Crops and Amenity Grasses Section and Protein Crops Working Group of Oil and Protein Crops Section

## Breeding Grasses and Protein Crops in the Era of Genomics

September 11–14, 2017 Crowne Plaza Hotel, Vilnius, Lithuania

Key-note presentations: 20 +10 min (presentation + questions)

Oral presentations: 15 + 5 min (presentation + questions)

Flash presentations: 5 min (presentation max 3 slides)

### Sunday, September 10, 2017

15:00–19:00	Registration Crowne Plaza Vilnius hotel, M. K. Čiurlionio str. 84, Vilnius
17:00–19:00	Welcome reception Crowne Plaza Vilnius hotel, M. K. Čiurlionio str. 84, Vilnius

### Monday, September 11, 2017

07:30–09:00	Registration Crowne Plaza Vilnius hotel, M. K. Čiurlionio str. 84, Vilnius
09:00–09:40	Opening ceremony. <b>Zenonas Dabkevičius</b> , <i>Lithuanian Research Centre for Agriculture and Forestry</i> <b>Eugenijus Butkus</b> , <i>Ministry of Education and Science</i> <b>Rimantas Krasuckis</b> , <i>Ministry of Agriculture</i> <b>Beat Boller</b> , <i>Eucarpia Past President</i> <b>Dirk Reheul</b> , <i>Eucarpia Fodder Crops and Amenity Grasses Section</i> <b>Antonio M. De Ron</b> , <i>Eucarpia Oil and Protein Crops Section</i>
09:40–10:00	Plant breeding and agriculture development in the Baltic region <b>Gintaras Brazauskas</b> , <i>LAMMC, Lithuania</i>

**SESSION 1: Utilisation of genetic resources and pre-breeding**

Chairmen: Roland Kölliker, Ulf Feuerstein

10:00–10:30	<b>Key-note 1</b> Utilization of genebank accessions to improve northern adaptation of perennial ryegrass ( <i>Lolium perenne</i> L.) <b>Odd Arne Rognli,</b> <i>University of Life Sciences, Norway</i>
10:30–10:50	Wild and primitive common bean populations in the germplasm collection at the MBG-CSIC <b>Antonio M. De Ron,</b> <i>Biology of Agrosystems, Misión Biológica de Galicia (MBG), Spanish National Research Council (CSIC), Pontevedra, Spain</i>
10:50–11:20	Coffee break
11:20–11:40	Patterns of spring growth and phenology in natural populations of <i>Lolium perenne</i> under contrasting field conditions <b>Anna Roschanski,</b> <i>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Germany</i>
11:40–12:00	Population structure of red clover ecotypes collected from Europe and Asia <b>Charlotte Jones,</b> <i>Aberystwyth University, UK</i>
12:00–12:20	Enhancing the sustainable use of <i>Lolium perenne</i> genetic resources from genebanks in plant breeding and research <b>Evelin Willner,</b> <i>Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Germany</i>
12:20–14:00	Lunch
14:00–14:20	Tetraploid <i>Festuca apennina</i> is prone to produce triploid hybrid progeny when crossed with diploid <i>Festuca pratensis</i> <b>Beat Boller,</b> <i>Agroscope, Switzerland</i>
14:20–14:40	Comparing mitotic and meiotic tetraploid red clover plants for seed yield <b>Gerda Cnops,</b> <i>ILVO, Belgium</i>
14:40–15:00	Domesticating shrubby native legumes for pastures and natural grasslands: a proposal <b>James P. Muir,</b> <i>Texas A&amp;M AgriLife Research, USA</i>



## Breeding Grasses and Protein Crops in the Era of Genomics

15:00–16:00	Flash presentations: <b>Tuna M.</b> [TR]: Interspecific hybridization in <i>Bromus</i> section <i>Pnigma</i> and nuclear DNA content of F1 progeny; <b>Ogrodowicz P.</b> [PL]: Effects of temperature on growth during <i>in vitro</i> embryo culture of field bean ( <i>Vicia faba</i> var. <i>minor</i> L.); <b>Norkevičienė E.</b> [LT]: Ecological and agronomic evaluation of <i>Dactylis glomerata</i> subsp. <i>lobata</i> ; <b>Ruņģis D.</b> [LV]: Yield and genetic composition of Latvian × <i>Festulium</i> cultivars and breeding material; <b>Liutukienė A.</b> [LT]: Genetic variability of the most important traits in alfalfa cultivars under Lithuanian conditions; <b>Akinroluyo O.</b> [LT]: Tetraploid induction in <i>Lolium multiflorum</i> ; <b>Cougnon M.</b> [BE]: The sense of testing sheep preference in tall fescue variety evaluation; <b>Norkevičienė E.</b> [LT]: Chemical composition of zigzag clover ( <i>Trifolium medium</i> L.); <b>Kemešytė V.</b> [LT]: Wild and semi natural ecotypes of perennial grasses and legumes for breeding purposes; <b>Petrauskas G.</b> [LT]: Breeding for improved seed yield of red clover.
16:00–16:45	Coffee + Discussion
16:45–18:15	Free time
18:15–20:00	Excursion to the Palace of the Grand Dukes <i>For more information and how to get there please see the page 73</i>

### Tuesday, September 12, 2017

#### SESSION 2: Genetic improvement of quality and agronomic traits

Chairmen: Piet Arts, James P. Muir

08:30–09:00	<b>Key-note 2</b> Breeding forage and grain legumes to increase EU's and China's protein self-sufficiency <b>Bernadette Julier,</b> <i>INRA, France</i>
09:00–09:20	Identification of loci controlling timing of stem elongation in red clover using GBS of pooled phenotypic extremes <b>Åshild Ergon,</b> <i>Norwegian University of Life Sciences, Norway</i>

09:20–09:40	<p><i>Lathyrus sativus</i> and <i>L. cicera</i> breeding: characterization of Iberian germplasm, QTL mapping, interspecific hybridization and cultivar development</p> <p><b>Diego Rubiales,</b> <i>Institute for Sustainable Agriculture, CSIC, Spain</i></p>
09:40–10:00	<p>Grain yield and morphology of dwarf vs tall white lupin in Mediterranean environments</p> <p><b>Luciano Pecetti,</b> <i>Research Centre for Fodder Crops and Dairy Productions (CREA-FLC), Italy</i></p>
10:00–10:20	<p>Deciphering grain legumes quality riddle: The genomics of bioactive compounds</p> <p><b>Maria Carlota Vaz Patto,</b> <i>Universidade Nova de Lisboa, Portugal</i></p>
10:20–10:40	<p>Manipulation of rumen nitrogen flows by use of <i>Festulolium</i> hybrids in rumen simulation fermenters</p> <p><b>Stephen Kamau Wanjiru,</b> <i>Aberystwyth University, UK</i></p>
10:40–11:10	Coffee break
<p><b>SESSION 3: Breeding for enhanced stress tolerance</b> Chairmen: Odd Arne Rognli, Diego Rubiales</p>	
11:10–11:40	<p><b>Key-note 3</b> Population genetics and GxE interaction analysis of the Eucarpia multi-site rust evaluation trial</p> <p><b>Torben Asp,</b> <i>Aarhus university, Denmark</i></p>
11:40–12:00	<p>Low crown rust resistance in Norwegian material of <i>Lolium perenne</i> and × <i>Festulolium</i></p> <p><b>Liv Østrem,</b> <i>NIBIO, Norway</i></p>
12:00–12:20	<p>Unravelling the genetic control of bacterial wilt resistance in ryegrass: achievements, prospects and challenges</p> <p><b>Roland Kölliker,</b> <i>ETH Zurich, Switzerland</i></p>
12:20–14:00	Lunch

## Breeding Grasses and Protein Crops in the Era of Genomics

14:00–14:20	<p>Genotyping of Festulolium cultivars involved in EUCARPIA multi-site trial using DArT markers and GISH</p> <p><b>David Kopecký</b>, <i>Institute of Experimental Botany, Czech Republic</i></p>
14:20–14:40	<p>Transcriptomic analysis of drought-sensitive and tolerant genotypes of perennial ryegrass (<i>Lolium perenne</i> L.)</p> <p><b>Maria de la O Leyva Pérez</b>, <i>Aarhus University, Denmark</i></p>
14:40–15:40	<p>Flash presentations:</p> <p><b>Tanaka T.</b> [JP]: Possibility of paternal selection for forage yield improvement in timothy polycross breeding;</p> <p><b>Aper J.</b> [BE]: Selection of perennial ryegrass spaced plants in sward conditions;</p> <p><b>Westermeier P.</b> [DE]: Variation in intrinsic water use efficiency between perennial ryegrass genotypes differing for drought tolerance;</p> <p><b>Masajada K.</b> [PL]: Physiological indicators of tolerance to soil water deficit in <i>Lolium multiflorum</i>/<i>Festuca arundinacea</i> introgression forms;</p> <p><b>Trněný O.</b> [CZ]: Incidence of six grass species by <i>Fusarium</i> sp. as a cause of silvertop;</p> <p><b>Malenica M.</b> [DE]: Pyramiding of resistance genes in perennial ryegrass (<i>Lolium perenne</i>) using next-generation sequencing based Massive Analysis of cDNA Ends (MACE);</p> <p><b>Ruge-Wehling B.</b> [DE]: Anthracnose resistance in lupin – genetic analysis and molecular markers;</p> <p><b>Haase F.</b> [DE]: Localisation of stem rust resistance gene <i>LpPg1</i> in <i>Lolium perenne</i> genome based on transcriptomic data;</p> <p><b>Maršalkienė N.</b> [LT]: Variation of vegetation terms and productivity traits among spontaneous <i>Vicia villosa</i> Roth. Ecotypes</p> <p><b>Aavola R.</b> [EE]: Crown rust and brown blight infection of perennial ryegrass cultivars representing three maturity groups</p> <p><b>Górniewicz B.</b> [PL]: The dependence of seed yield and its components and chlorophyll fluorescence parameters in the narrow leafed lupin (<i>Lupinus angustifolius</i> L.)</p>
15:40–16:25	Coffee + Discussion
16:25–18:25	<p>Festulolium working group session:</p> <p><b>Zwierzykowski Z.</b> [PL]: A history of Festulolium in Poland <i>Institute of Plant Genetics, Polish Academy of Sciences, Poland</i></p> <p><b>Humphreys M.</b> [UK]: A sure-root to climate-smart grassland <i>IBERS, Aberystwyth University, UK</i></p> <p><b>Baert J.</b> [BE]: Current Festulolium breeding at ILVO <i>ILVO, Belgium</i></p>
18:25–19:25	Parallel board meetings

### Wednesday, September 13, 2017

Conference Tour: Lithuanian Research Centre for Agriculture and Forestry (LAMMC) Institute of Agriculture, Seed company *Dotnuva Baltic*, fertilizer factory *Lifosa*, livestock farm *Vaitelių ūkis*, conference Gala dinner at *Raudondvaris Manor*.

08:00	Departure from Crowne Plaza Hotel to: LAMMC – Farm – Dotnuva Baltic Farm – LAMMC – Lifosa Lifosa – Dotnuva Baltic – LAMMC <i>For more information about the tour objects see the pages 74–77</i>
19:00	Gala dinner at Raudondvaris Manor
22:30	Departure to Vilnius

### Thursday, September 14, 2017

<b>SESSION 4: Implementation of phenomic and genomic tools in breeding</b> Chairmen: Torben Asp, David Kopecký	
09:00–09:30	<b>Key note 4</b> Using LIDAR for forage yield measurement of perennial ryegrass ( <i>Lolium perenne</i> L.) field plots <b>Kioumars Ghamkhar,</b> <i>AgResearch, New Zealand</i>
09:30–09:50	An indoor screening method for reduced fall dormancy in alfalfa <b>Annick Bertrand,</b> <i>Quebec Research and Development Centre, Canada</i>
09:50–10:50	Flash presentations <b>Perić V. [RS]:</b> Application of multivariate analysis for genotype evaluation in soybean; <b>Zharikova D. [UA]:</b> Polymorphism of soybean cultivars and breeding lines by marker <i>Satt100</i> associated with <i>E7</i> locus; <b>Gréard C. [FR]:</b> Genetic diversity assessment of two <i>Medicago sativa</i> genes: <i>CAD</i> and <i>WXP1</i> ;

## Breeding Grasses and Protein Crops in the Era of Genomics

	<p><b>Aleliūnas A.</b> [LT]: Testing for freezing tolerance in perennial ryegrass;</p> <p><b>Barzyk P.</b> [PL]: Preliminary estimation of variation of alkaloids content in white lupin (<i>Lupinus albus</i> L.) collection;</p> <p><b>Vymyslický T.</b> [CZ]: The multifunctional role of legumes in vineyards and orchards;</p> <p><b>Knotová D.</b> [CZ]: Yields and quality of some perennial legumes in Czech Republic;</p> <p><b>Gasior D.</b> [UK]: Hydroponic evaluation of growth and nutrient uptake in a <i>Lolium/Festuca</i> introgression series;</p> <p><b>Julier B.</b> [FR]: Use of GBS for lucerne variety distinction.</p> <p><b>Jonavičienė K.</b> [LT]: Genome-wide and functional gene analysis of perennial ryegrass for improved growth under water limiting conditions (GrowGene)</p>
10:50–11:30	Coffee + Discussion
11:30–12:00	<p><b>Key note 5</b></p> <p>Genetics and Genomics to Advance Forage Grass Breeding</p> <p><b>Bruno Studer,</b> <i>ETH Zurich, Switzerland</i></p>
12:00–12:20	<p>Genomic selection for biomass yield of perennial and annual legumes</p> <p><b>Paolo Annicchiarico,</b> <i>Research Centre for Fodder Crops and Dairy Productions (CREA-FLC), Italy</i></p>
12:20–12:50	Business meeting
12:50–13:00	Closing of the meeting
13:00–14:30	Lunch
	Departure

## **Dear participants,**

This is the first joint meeting between the Fodder Crops and Amenity Grasses Section and the Protein Crops Working Group-Oil and Protein Crops Section of Eucarpia. Indeed both sections have growing common interests both regarding scientific developments as well as regarding the multiple uses of crops.

It was a real challenge for the organizers to compose an attractive programme appealing to participants of both sections and we are convinced they have utterly succeeded in this. The scientific contributions and the incomparable location of the meeting will facilitate the success of this event.

The presented topics allow to learn from each other and the joint meeting certainly will create opportunities to meet new colleagues, leading to innovative collaborations and developments, which exactly is the mission of Eucarpia.

We do convey our sincere thanks to the organizers of the Lithuanian Research Centre for Agriculture and Forestry and wish all of you a fruitful meeting.

Dirk Reheul

Antonio M. De Ron

Chair Fodder Crops and Amenity Grasses

Coordinator Protein Crops

## Plant breeding and agriculture development in the Baltic region

### Brazauskas G.

Lithuanian Research Centre for Agriculture and Forestry,  
Institute of Agriculture, Lithuania;  
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania  
E-mail: gintaras.brazauskas@lzi.lt

### Abstract

Baltic states, e.g. Lithuania (LT), Latvia (LV) and Estonia (EE), began their transition to a market economy after they regained their independence in 1990 since the collapse of Soviet Union. Economic and land reforms have dramatically affected agricultural performance in all three Baltic states. The restitution of the property rights as they were in 1940 was chosen as the main approach of land reform. Land reform resulted, as intended, in a complete breakup of the large scale collective and state farms. Restitution of land transferred land rights to the former owners, many of whom were often no longer active in agriculture. Furthermore, transition from a centrally planned economy to a market-oriented economy coincided with subsidy cuts and price liberalization, which in general caused input prices to increase and output prices to decrease. Purchased inputs were no longer affordable and the decrease in input use, e.g. fertilizer use declined in the early transition period by almost 80%, caused a decrease in agricultural output to about 50 to 60% of the pre-reform output. Output and productivity started to recover at the end of 1990s stimulated by consolidation of land in larger farming enterprises, substantial gains in labour productivity and investments through vertically integrated supply chains, especially after LT, LV and EE joined European Union (EU) in 2004. Today, farm structures in the Baltic countries are dominated by a mix of large corporate farms and medium-large sized family farms, with an average farm size of 17, 23 and 50 ha in LT, LV and EE, respectively. Agriculture accounts for approx. 3% of total gross value added (GVA) in the Baltic states in comparison with an EU average of 1.5% GVA. Animal output amounts to approx. 37 to 38% in LT and LV while it reaches 53% in EE. Subsequently forage plants reach 20% of total crop output in EE in terms of value in comparison with just 15 and 16% in LV and LT, respectively. Baltic states drastically increased cultivation of dry pulses in recent years with an area under dry pulses now reaching 3, 8 and 11% of total arable land in LV, EE and LT, respectively. Plant breeding activities in the Baltic states were established at the beginning of 1920s and are being carried out in all major agricultural crops, with extensive breeding programs in grasses, legumes and dry pulses.



## **Utilization of genebank accessions to improve northern adaptation of perennial ryegrass (*Lolium perenne* L.)**

**Rognli O. A.<sup>1</sup>, Aavola R.<sup>2</sup>, Aleliūnas A.<sup>3</sup>, Asp T.<sup>4</sup>, Brazauskas G.<sup>3</sup>,  
Gylstrøm K. H.<sup>5</sup>, Helgadóttir A.<sup>6</sup>, Isoahhti M.<sup>7</sup>, Kovi M. R.<sup>1</sup>,  
Kristjánadóttir T. A.<sup>6</sup>, Larsen A. S.<sup>8</sup>, Marum P.<sup>5</sup>, Paina C.<sup>4</sup>,  
Persson C.<sup>9</sup>, Rancāne S.<sup>10</sup>**

<sup>1</sup> Department of Plant Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, Ås, Norway

<sup>2</sup> Estonian Crop Research Institute, Jõgeva, Estonia

<sup>3</sup> Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto a. 1, Akademija, Kėdainiai distr. Lithuania

<sup>4</sup> Aarhus University, Department of Molecular Biology and Genetics, Slagelse, Denmark;

<sup>5</sup> Graminor AS, Ridabu, Norway

<sup>6</sup> Agricultural University of Iceland, Department of Land and Animal Resources, Reykjavík, Iceland

<sup>7</sup> Boreal Plant Breeding Ltd., Jokioinen, Finland

<sup>8</sup> DLF Research Center, Store Heddinge, Danmark

<sup>9</sup> Lantmännen ek. för., Svalöv, Sweden

<sup>10</sup> Latvia University of Agriculture, Jelgava, Latvia

E-mail: odd-arne.rognli@nmbu.no

### **Abstract**

Perennial ryegrass (*Lolium perenne* L.) is increasingly important as a forage grass in the Nordic and Baltic region, especially in the light of climate change. However, winter hardiness and persistence need to be improved. This article describes the long-term Nordic/Baltic public-private partnership for pre-breeding in perennial ryegrass, started in 2012. The basis for the project is detailed genotyping and phenotyping of nearly 400 genebank accessions, followed by generation of new broad-based diploid and tetraploid breeding populations, locally adapted and trait-based populations. Preliminary results confirm the need for more robust and stable cultivars with wider adaptations, and genebank accessions need to be introgressed and recombined with adapted materials followed by natural/artificial selection in order to create novel germplasm for these regions.



## **Wild and primitive common bean populations in the germplasm collection at the MBG-CSIC**

**De Ron A. M.<sup>1</sup>, Pose J. D.<sup>1</sup>, Menéndez-Sevillano M. C.<sup>2</sup>, Ferreyra M. J.<sup>2</sup>**

<sup>1</sup> Biology of Agrosystems, Misión Biológica de Galicia (MBG), Spanish National Research Council (CSIC), Pontevedra, Spain

<sup>2</sup> Active Bank of Northwestern Argentina (BANOA), EEA Salta, National Agricultural Technology Institute (INTA), Salta, Argentina  
E-mail: amderon@mbg.csic.es

### **Abstract**

In the Misión Biológica de Galicia, Spanish National Research Council (MBG-CSIC) there is a germplasm collection of common bean (*Phaseolus vulgaris* L.) that includes wild and cultivated accessions. In the case of the cultivated populations from the Andean Region, some of them are considered “primitive” since they are unimproved landraces. The aim of this study was to describe some characteristics of the wild and primitive populations and their area of origin.



## Patterns of spring growth and phenology in natural populations of *Lolium perenne* under contrasting field conditions

Roschanski A. M.<sup>1</sup>, Barre P.<sup>2</sup>, Escobar- Gutiérrez A.<sup>2</sup>, Sampoux, J. P.<sup>2</sup>  
Muylle H.<sup>3</sup>, Thomas I.<sup>4</sup>, Dehmer K. J.<sup>1</sup>, Willner E.<sup>1</sup>

<sup>1</sup> Leibniz Institute of Plant Genetics and Crop Plant Research (IPK),  
Inselstr. 9, 23999 Malchow/Poel, Germany

<sup>2</sup> INRA, Centre Nouvelle-Aquitaine-Poitiers, UR4 (UR P3F), F-86600 Lusignan, France

<sup>3</sup> ILVO, Plant Genetics and Breeding Section, ILVO, Caritasstraat 21 B,  
9090 Melle, Belgium

<sup>4</sup> IBERS-Aberystwyth University, Plas Gogerdan, Aberystwyth, Ceredigion,  
United Kingdom

E-mail: roschanski@ipk-gatersleben.de

### Abstract

The ecotypic diversity of perennial ryegrass (*Lolium perenne* L.) is a major genetic resource for breeding programs. In three replicated micro-sward trials in France, Belgium and Germany, we measured spring growth and recorded heading date of round 400 genebank accessions from the natural diversity of *L. perenne* that were selected as to represent the wide range of variability in this species. We observed marked differences between trial locations as well as interaction between accessions and locations in the timing of spring growth rates along growing-degree-days (GDDs). These preliminary results are part of a wider project aiming to investigate the natural adaptation of perennial ryegrass to various regional climates across its spontaneous area of presence in Europe.

## Population structure of red clover ecotypes collected from Europe and Asia

Jones C.<sup>1</sup>, De Vega J.<sup>2</sup>, Lloyd D.<sup>1</sup>, Hegarty M.<sup>1</sup>, Ayling S.<sup>2</sup>, Powell W.<sup>3</sup>, Skøt L.<sup>1</sup>

<sup>1</sup> Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Gogerddan, Aberystwyth, SY23 3EE, UK

<sup>2</sup> The Earlham Institute, Norwich Research Park, Norwich, NR4 7UH, UK

<sup>3</sup> Scotlands Rural College (SRUC), Peter Wilson Building, Kings Buildings, West Mains Road, Edinburgh, EH9 3JG  
E-mail: cgj@aber.ac.uk, lfs@aber.ac.uk

### Abstract

Red clover (*Trifolium pratense* L.) is a highly adaptable species, with a wide geographic distribution. At the genetic level, wild ecotypes possess high variability, which enables them to adapt rapidly to diverse environments. This variation can be identified, via molecular techniques, and used to determine the differences between populations that may otherwise be indistinguishable. The results presented here are part of a wider study undertaken with a view to assess the available genetic diversity and use it to facilitate future introgression of useful traits into red clover breeding populations.



## **Enhancing the sustainable use of *Lolium perenne* genetic resources from genebanks in plant breeding and research**

**Bachmann-Pfabe S.<sup>1</sup>, Willner E.<sup>2</sup>, Oppermann M.<sup>3</sup>,  
Weise S.<sup>3</sup>, Dehmer K. J.<sup>1</sup>**

<sup>1</sup> Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben,  
Genebank, Satellite Collections North, Parkweg 3a, 180190 Groß Lüsewitz, Germany

<sup>2</sup> Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben,  
Genebank, Satellite Collections North, Inselstraße 9, 23999 Malchow (Poel), Germany

<sup>3</sup> Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben,  
Genebank Documentation, Corrensstraße 3, 06466 Stadt Seeland, Germany  
E-mail: willner@ipk-gatersleben.de

### **Abstract**

*Lolium perenne* is one of the most important grass species in Europe and subject of intensive breeding and research. Hence, there is a substantial need for well described, diverse and easily available genetic resources. The Satellite Collections North of the IPK Genebank continuously characterize and evaluate genebank accessions of oil and fodder crops. As an example, results from characterization and evaluation trials of 336 *L. perenne* accessions collected in Bulgaria, Croatia, Ireland and Spain were presented. Traits considered were, amongst others, phenological development, ear emergence, biomass production, disease susceptibility and winter hardiness. Results from the three-year evaluation trials showed a high variability regarding these traits. Generally, the Spanish ecotypes revealed a high biomass production level, while the Bulgarian ecotypes were characterized by a low rust susceptibility. The Irish ecotypes showed a better growth after winter than the ecotypes from the other countries in the third scoring year. These results have been transferred to the in-house developed Genebank Information System (GBIS) and to the international EURISCO information system. Their access via EURISCO is demonstrated.

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## **Tetraploid *Festuca apennina* is prone to produce triploid hybrid progeny when crossed with diploid *Festuca pratensis***

**Boller B.<sup>1</sup>, Felder T.<sup>1</sup>, Kopecký D.<sup>2</sup>**

<sup>1</sup> Agroscope, Reckenholzstrasse 191, 8046 Zürich, Switzerland

<sup>2</sup> Institute of Experimental Botany, Centre of Plant Structural and Functional Genomics, Šlechtitelů 31, 783 71 Olomouc-Holice, Czech Republic

E-mail: beat.boller@agroscope.admin.ch

### **Abstract**

*Festuca apennina* De Not. is a species growing at altitudes of 1100 to 2000 m a.s.l. in different regions of Europe. To test cross fertility of different cytotypes of *F. apennina*, pairwise crosses were made with tetraploid and triploid *F. apennina*, as well as diploid and colchicine-induced tetraploid *Festuca pratensis* Huds. Tetraploid *F. apennina* appeared to be quite cross-fertile with diploid *F. pratensis* and produced triploid progeny in both cross directions. When *F. apennina* plants were pollinated with diploid *F. pratensis*, they yielded even more seed per inflorescence than those pollinated with another *F. apennina* plant. About 25 % of the progeny of this cross were triploids; the remaining 75 % were tetraploid, presumably resulting from self-pollination. Much less seeds were obtained on diploid *F. pratensis* pollinated with *F. apennina*, but the progeny consisted of as much (52 %) triploid hybrids as of diploid selfs (48 %). Seeds harvested on *F. apennina* giving rise to triploid progeny appeared to have no cold requirement for germination, as opposed to tetraploid progeny from self-pollination or crosses among *F. apennina*. Colchicine-induced tetraploids and diploids of *F. pratensis* were much less cross-fertile. Few viable seeds were obtained, with a great majority (91 %) resulting from self-pollination, with only 9 % triploid hybrids. The surprisingly high cross fertility of tetraploid *F. apennina* with diploid *F. pratensis*, the apparent absence of a triploid block in their progeny, and the fact that seeds of *F. apennina* giving rise to triploid progeny have no cold requirement, probably contribute to the frequent occurrence of triploid forms at the low end of *F. apennina* altitudinal distribution.

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## **Comparing mitotic and meiotic tetraploid red clover plants for seed yield**

**Vleugels T., Roldán-Ruiz I., Cnops G.**

ILVO (Flanders research institute for Agriculture, Fisheries and Food), Plant Sciences  
Unit, Caritasstraat 39, 9090 Melle, Belgium  
E-mail: tim.vleugels@ilvo.vlaanderen.be

### **Abstract**

New tetraploids can be created by chromosome doubling (mitotic tetraploids), or through unreduced gametes (meiotic tetraploids). In this study, meiotic tetraploids were generated in interploidy crosses, their seed yield was compared against mitotic tetraploids with similar genetic background, and the relation between genetic diversity and seed yield was explored. A total of 15 meiotic tetraploids were obtained in 16 interploidy crosses between diploid Lemmon or Merian genotypes and tetraploid Elanus genotypes. Mitotic tetraploids were created by crossing plants from tetraploidized Lemmon or Merian populations with Elanus genotypes. Tetraploid progeny from cross-pollinations were identified with flow cytometry and SSR marker analysis. The efficiency of interploidy crosses to generate meiotic tetraploids was low (0.5% in diploid and 0.7% in tetraploid mother plants) and the degree of self-pollination was high (95.0% in diploid and 94.1% in tetraploid mother plants). Using seeds from the diploid parent was more time and cost efficient because most undesired progeny was identified by flow cytometry, leaving fewer plants to be screened with SSR markers. Meiotic tetraploids yielded significantly more seeds than mitotic tetraploids with similar genetic background (15.9g vs. 10.4 g), but their genetic diversity was not higher (2.6 vs. 2.9 alleles per SSR locus). Seed yield and the average allele number per locus were not correlated among all progeny plants.

## **Domesticating shrubby native legumes for pastures and natural grasslands**

**Muir J. P.<sup>1</sup>, Forrest F. S.<sup>2</sup>, Brady J.<sup>1</sup>, Dubeux Jr. J. C.<sup>3</sup>, Pitman W. D.<sup>4</sup>**

<sup>1</sup> Texas A&M AgriLife Research, USA\*

<sup>2</sup> South Texas Natives, Texas A&M Kingsville, USA

<sup>3</sup> Institute for Food and Agricultural Science, University of Florida, USA

<sup>4</sup> Louisiana State University Agricultural Center, USA

\*E-mail: j-muir@tamu.edu

### **Abstract**

As ruminant species diversify in natural and managed ecosystems in an effort to increase production, so does the need for greater plant diversity. Legume shrub propagation is rarely targeted for this purpose. As an example, the wide range of native perennial, shrub (0.5-3 m) legumes in Texas indicates untapped potential for multiple uses such as forage, ecosystems services, wildlife habitat, fuel, and possibly pulse crops. We propose that concerted programs to identify, collect, characterize, domesticate and commercialize these legumes could lead to multiple benefits in native and cultivated ecosystems characterized by herbaceous, shrubby and arboreal canopies, each contributing to greater herbage and animal productivity than mono-canopy systems. This approach could be applied in Europe and throughout the world.



## Interspecific hybridization in *Bromus* section *Pnigma* and nuclear DNA content of F1 progeny

Şahin B., Yılmaz E. C., Erenler S., Savas Tuna G., Nizam İ., Tuna M.

Namik Kemal University, Faculty of Agriculture, Department of Field Crops,  
Tekirdag, Turkey  
E-mail: mtuna@nku.edu.tr

### Abstract

There are about 150 species with a wide geographic distribution in the genus *Bromus* L. The genus contains annuals, biennials and perennials ranging from self to cross pollinating. Smooth brome grass, *Bromus inermis* Leyss., is the most important and widely grown species of the genus. It has high yield of highly digestible forage and highly tolerant to cold and specially drought. However, it is strongly rhizomatous and it has slow regrowth after defoliation. The objective of this study was to cross smooth brome with some of its close relatives, included in the section *Pnigma* to improve agronomic usefulness of smooth brome as forage crop. Crosses among brome grasses (*B. inermis* ( $2n=8x=56$ ), *B. riparius* ( $2n=10x=70$ ), *B. pumpellianus* ( $2n=8x=56$ ) and *B. biebersteini* ( $2n=10x=70$ )) were easily made. All the crosses set seeds but, the percentage of seed set varied approximately between 5% and 80% based on species, ploidy and genotype. 2C nuclear DNA content of the hybrids varied between nuclear DNA content of parent plants.



## Effects of temperature on growth during in vitro embryo culture of field bean (*Vicia faba* var. *minor* L.)

Ogrodowicz P.<sup>1</sup>, Surma M.<sup>1</sup>, Adamski T.<sup>1</sup>, Kaczmarek Z.<sup>1</sup>,  
Święcicki W. K.<sup>1,2</sup>, Stopyra P.<sup>3</sup>, Kuczyńska A.<sup>1</sup>,  
Krystkowiak K.<sup>1</sup>, Mikołajczak K.<sup>1</sup>

<sup>1</sup> Institute of Plant Genetics, Polish Academy of Sciences, Poznań, Poland

<sup>2</sup> Poznań Plant Breeders Ltd., Tulce, Poland

<sup>3</sup> Plant Breeding Strzelce Ltd., Co. – IHAR-PIB Group, Poland

E-mail: [pogr@igr.poznan.pl](mailto:pogr@igr.poznan.pl)

### Abstract

Attainment of homozygous lines in grain legumes is multi-year procedure. It may be shortened by applying the single seed descent technique under greenhouse conditions. Additionally, this technique can be combined with in vitro culture of embryos dissected from immature seeds. The aim of this study was to establish in vitro conditions for the culture of field bean embryos as the first step in the development of homozygous lines. Four temperature regimes were applied and their influence on embryo development was evaluated. Under *ex vitro* conditions plant survival was observed. Our results indicate a good potential of embryo culture to shorten the time required for obtaining homozygous lines of field bean.

## Ecological and agronomic evaluation of *Dactylis glomerata* subsp. *lobata*

Norkevičienė E.<sup>1</sup>, Balsevičius A.<sup>2</sup>, Kemešytė V.<sup>1</sup>, Butkutė B.<sup>1</sup>,  
Petrauskas G.<sup>1</sup>

<sup>1</sup> Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry,  
Instituto a. 1, Akademija, Kėdainiai distr. Lithuania

<sup>2</sup> Botanical Garden of Vytautas Magnus University, Kaunas, Lithuania  
E-mail: egle.norkeviciene@lzi.lt

### Abstract

Widening of ecological adaptation of forage grasses (including tolerance of climatic stresses, enhancement of genetic resistance to pests and diseases, expansion of geographical adaptability) warrant exploration and utilization of wild relatives of a wide range of species. This study set out to investigate the ecological characteristics of *Dactylis glomerata* subsp. *lobata* and to compare its agronomic traits and yield with those of the most common and economically valuable species of the genus, *D. glomerata* subsp. *glomerata*. The current study revealed that *D. glomerata* subsp. *lobata* is less productive than *D. glomerata* subsp. *glomerata*. Though, *D. glomerata* subsp. *lobata* agrobiological characteristics suggest that it might be a valuable genetic resource for forage production.

## Yield and genetic composition of Latvian × *Festulolium* cultivars and breeding material

Bērziņš P.<sup>1</sup>, Ruņģis D.<sup>2</sup>, Rancāne S.<sup>1</sup>, Gailīte A.<sup>2</sup>, Beļeviča V.<sup>2</sup>, Stesele V.<sup>1</sup>, Vēzis I.<sup>1</sup>, Jansons A.<sup>1</sup>

<sup>1</sup> Latvia University of Agriculture, Institute of Agriculture, LV-5101, Skrīveri, Latvia

<sup>2</sup> Genetic Resource Centre, Latvian State Forest Research Institute “Silava”,

Salaspils, LV-2169, Latvia

E-mail: dainis.rungis@silava.lv

### Abstract

Interspecific hybridization of forage grasses has been undertaken at the Institute of Agriculture since the end of the 1970's. *Lolium perenne* (Lp) and *Festuca pratensis* (Fp) aneuploid hybrids and a tetraploid Fp cultivar 'Patra' have been utilized for crossing. Two cultivars – 'Saikava' and 'Vizule', which contain Lp, *L. multiflorum* (Lm) and Fp germplasm have been registered. These x*Festulolium* cultivars were assessed in field conditions, and the genetic composition was determined by morphological parameters as well as by utilization of species-specific molecular markers. In total, a higher proportion of *Lolium* (O/L) genotypes were identified in the analysed loci (62%), compared to hybrid genotypes (F/L – 34%). *Festuca* genotypes (F/O) were found in 3% of all loci. A higher proportion of *Lolium* alleles were found within all the x*Festulolium* cultivars: Ape – 74.9%, Lorry – 81.8%, Vizule – 72.9%, Saikava – 75.8%, Lofa – 74.3%, Punia – 58.9%.

## **Genetic variability of the most important traits in alfalfa cultivars under Lithuanian conditions**

**Liatukienė A.**

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry,  
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania  
E-mail: aurelija@lzi.lt

### **Abstract**

Alfalfa (*Medicago sativa* L.) is an important perennial legume for animal feeding. A basic requirement for the successful breeding of this species is variability of the initial material. The collection under study consisted of 37 cultivars of different origin. The current study aimed to determine the genetic variability of the most important traits, including disease resistance, seed yield, fresh and dry matter yield, plant height during spring regrowth and at flowering stage and stem density. The statistical analysis of the data (ANOVA) revealed significant variability among the cultivars for all traits investigated. The highest variability was found for the seed yield (46.5 %), while the lowest one (5.9 %) was estimated for resistance to spring black stem and leaf spot. A cluster analysis was used to graphically illustrate the genetic diversity of alfalfa cultivars. The high level of genetic variability among the alfalfa cultivars in this collection highlights their potential for being used in the future breeding schemes.

## **Tetraploid induction in *Lolium multiflorum***

**Akinroluyo O., Kemešytė V., Statkevičiūtė G.**

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry,  
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania  
Email: kelvin.akinroluyo@lzi.lt

### **Abstract**

The induction of tetraploids from diploid cultivars of *L. multiflorum* is important in improving the germplasm. The aim of the study is to investigate the optimal concentration and time duration to induce tetraploids from diploid cultivars of *L. multiflorum*. Seedlings developing from excised embryos were subjected to different concentration of mitosis inhibitors and for different time intervals. The survival and induction percentages were determined for each induction approach.

### **Acknowledgements**

The research project was funded by the Research Council of Lithuania, grant No. MIP-064/2015 (ADAPTGENAS)



## The sense of testing sheep preference in tall fescue variety evaluation

Cougnon M.<sup>1</sup>, Baert J.<sup>2</sup>, Reheul D.<sup>1</sup>

<sup>1</sup> Department of Plant Production, Ghent University, Gent, Belgium

<sup>2</sup> ILVO Plant, Melle, Belgium

E-mail: mathias.cougnon@ugent.be

### Abstract

Tall fescue (*Festuca arundinacea* Schreb.) has a low digestibility and a low intake when grazed compared to ryegrasses. To make progress in these traits, we introduced a new way of testing candidate varieties (candivars) in our breeding program. We ran simultaneously a yield trial, in which yield and digestibility were measured, and a preference trial where sheep preference was evaluated using two methods: a qualitative (visual) and a quantitative measurement. In this paper we question whether such a preference test has an added value. Based on results of 18 candivars, we conclude that measuring sheep preference makes sense, because not all varieties with a high digestibility have good preference.

## Chemical composition of zigzag clover (*Trifolium medium* L.)

Butkutė B.<sup>1</sup>, Lemežienė N.<sup>1</sup>, Padaruskas A.<sup>2</sup>, Norkevičienė E.<sup>1</sup>, Taujenis L.<sup>2</sup>

<sup>1</sup> Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry, Instituto al. 1, Akademija, Kėdainiai distr., Lithuania

<sup>2</sup> Department of Analytical and Environmental Chemistry, Vilnius University, Vilnius, Lithuania

E-mail: brone@lzi.lt

### Abstract

The study deals with the assessment of zigzag clover (*Trifolium medium* L.) for nutritional and mineral composition as well as for the distribution of isoflavones in whole aerial plant part at vegetative and full flowering stages, and morphological parts of flowering plants. Zigzag clover proved to be valuable protein- mineral- and isoflavone-rich legume crop with low NDF content. Plants at the vegetative stage exhibited higher concentrations of most components tested. According to the total concentration of isoflavones, the plant parts ranked as follows: leaves >> stems >> flowers. The peculiarities of distribution of nutritive, mineral components and isoflavones, revealed in the study, could be useful for exploiting species both in animal nutrition and in the development of health-benefiting food ingredients.

## Wild and semi natural ecotypes of perennial grasses and legumes – for breeding purposes (a review)

Lemežienė N., Stukonis V., Kemešytė V., Norkevičienė E.

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry,  
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania  
E-mail: nijole@lzi.lt

### Abstract

Between the years 1994–2014, perennial grass and legumes wild and semi-natural ecotypes were collected in different geographic locations and regions of Lithuania, Baltic littoral (Latvia), Kaliningrad region (Russia), Precarpathian, Carpathian, Zacarpathian and Poese regions (Ukraine). Altogether about 2500 seed accessions of 32 species of grasses and 18 species of legumes were collected.

On the basis of most promising wild ecotypes, the following forage type varieties of grasses and legumes have been developed: smooth-stalked meadow grass (*Poa pratensis* L.) 'Rusnė', black medic (*Medicago lupulina* L.) 'Arka', meadow foxtail (*Alopecurus pratensis* L.) 'Valentas', reedtop (*Agrostis gigantea* Roth.) 'Violeta', reed canary grass (*Phalaris arundinacea* L.) 'Pievys DS'. For the development of forage-type varieties of some species, such as timothy, ryegrass and meadow fescue, wild ecotypes were in most cases insufficiently luxuriant. About 3 % of the forage-type plants of perennial grasses exhibited resistance to foliar diseases.

According to a complex of valuable agro morphological characteristics, small part (about 1.5 %) of the wild ecotypes of various species were found to be promising for the application in turf grass breeding. Some turf grass varieties have been developed on the basis of the most valuable wild ecotypes: Canada bluegrass (*Poa compressa* L.) 'Odrė DS', wood bluegrass (*Poa nemoralis* L.) 'Luka DS', red fescue (*Festuca rubra* L.) 'Gludas', 'Varius' and 'Alkas', hard fescue (*Festuca trachyphylla* (Hack.) Krajina) 'Astravas'.



## Breeding for Improved Seed Yield of Red Clover

**Petrauskas G., Mikaliūnienė J., Norkevičienė E.,  
Statkevičiūtė G., Kemešytė V.**

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry,  
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania  
E-mail: giedrius.petrauskas@lzi.lt

### **Abstract**

Red clover (*Trifolium pratense* L.) is valued for crude protein and water soluble carbohydrates content, dry matter digestibility and other properties. On the contrary poor seed yield is often disappointing. Seed yield is a very important criterion in red clover breeding programs, since it is crucial for the commercialization of cultivars on the market. Therefore this study was aimed to compare the seed yield of different red clover cultivars and the most promising breeding lines. The findings revealed that seed yield decreased more than half in the second year of harvest. Only a few diploid Lithuania-bred cultivars tended to produce seed yield significantly higher than average (0.35 t ha<sup>-1</sup>). The seed yield of breeding line No. 2114 was found to be lower than that of the other breeding lines; however, it was the only one stable in both years of harvest. Therefore breeding line No. 2114 is valuable as breeding material and can be useful in subsequent stages of selection.



## **Breeding forage and grain legumes to increase EU's and China's protein self-sufficiency**

**Julier B.<sup>1</sup>, Skøt L.<sup>2</sup>, Weise S.<sup>3</sup>, Karagić Đ.<sup>4</sup>, Roldán-Ruiz I.<sup>5</sup>,  
Barre P.<sup>1</sup>, Lloyd D.<sup>2</sup>**

<sup>1</sup> P3F, INRA, 86600 Lusignan, France

<sup>2</sup> Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, Aberystwyth, UK

<sup>3</sup> Leibniz Inst of Plant Genet & Crop Plant Res (IPK) Gatersleben  
06466 Stadt Seeland, Germany

<sup>4</sup> Inst Field & Vegetable Crops IFVCNS, Forage Crops Dept, Novi Sad, Serbia

<sup>5</sup> Inst Agr & Fisheries Res ILVO, Plant Sci Unit, Melle, Belgium  
E-mail: bernadette.julier@inra.fr

### **Abstract**

Both Europe and China have a low level of protein autonomy. They import 30% and 60%, respectively, of the world plant protein production for both human and animal nutrition. However, in both continents, regions with appropriate agro-ecological conditions for the production of plant proteins exist. The project EUCLEG, recently granted by the H2020 programme of European Union, has the ambition to give a new momentum to forage legume (alfalfa and red clover) and grain legume (pea, faba bean and soybean) breeding. The scientific objectives of this project are i) to broaden the genetic base of legume crops, ii) to analyse the genetic diversity of European and Chinese legume accessions using phenotypic traits (yield, quality in a context of climate change) and molecular markers, iii) to analyse the genetic architecture of key breeding traits using association genetics (GWAS) and iv) to evaluate the benefits brought by genomic selection (GS) to create new legume varieties. Comprehensive searchable databases containing passport data, as well as agronomic and genetic features will be developed. The breeders that are members of EUCLEG will exploit the results to create the new varieties needed to improve protein production.

## Identification of loci controlling timing of stem elongation in red clover using GBS of pooled phenotypic extremes

Ergon Å.<sup>1</sup>, Milvang Ø.W.<sup>1</sup>, Milvang O.<sup>2</sup>, Kovi M. R.<sup>1</sup>

<sup>1</sup> Faculty of Biosciences, Norwegian University of life Sciences, P.O. Box 5003, N-1432 Ås, Norway

<sup>2</sup> Brennaveien 143, 1279 Oslo, Norway  
E-mail: ashild.ergon@nmbu.no

### Abstract

A population of red clover (*Trifolium pratense* L.), was characterized for timing of stem elongation. Out of the 672 plants tested, 52 of the earliest and 52 of the latest were chosen for genetic characterization using genotyping by sequencing (GBS). The 52 individuals from each group were randomly divided into three subgroups and equal amounts of DNA from each individual in each subgroup was combined in a pool, creating a total of six pools, that were subject to GBS. Single nucleotide polymorphisms (SNPs) with allele frequencies that differed significantly between the early and the late group were identified by the use of pairwise  $F_{ST}$ -values for each SNP. At the highest stringency, we found 57 biallelic SNPs with significantly different allele frequencies in the early and the late groups, of which 37 could be mapped to a chromosome and the rest to unplaced scaffolds ( $P < 0.01$ , false discovery rate  $< 0.001$ ). The 37 SNPs corresponded to 22 loci when defining a locus as a  $\pm 500$  Kb region surrounding a SNP, an interval known to be associated with an almost complete decay of linkage disequilibrium in the studied population. Significant SNPs were distributed among all chromosomes, with the highest number on chromosome 6 and 7.



## ***Lathyrus sativus* and *L. cicera* breeding: characterization of Iberian germplasm, QTL mapping, interspecific hybridization and cultivar development**

**Rubiales D.<sup>1</sup>, González-Bernal M. J.<sup>1</sup>, Carrillo-Perdomo E.<sup>1</sup>, Almeida N. F.<sup>2</sup>, Gonçalves L.<sup>2</sup>, Vaz Patto M. C.<sup>2</sup>**

<sup>1</sup> Institute for Sustainable Agriculture, CSIC, Córdoba, Spain

<sup>2</sup> ITQB NOVA, Universidade Nova de Lisboa, Oeiras, Portugal

E-mail: diego.rubiales@ias.csic.es

### **Abstract**

Grass pea (*Lathyrus sativus*) and chickling pea (*L. cicera*) are two legume crops used for food or feed since ancient times and are ancient crops that are largely neglected today. We have characterized Iberian landraces for resistance against a number of diseases in both species, what is complemented now with aphid and weevil screenings, starting with *L. sativus*. We are also generating mapping populations in both species that are being studied.

In an attempt to increase the variability, interspecific crosses were made between *L. sativus* and *L. cicera* generating valuable pre-breeding material. This research activity was complemented with breeding and cultivar development.

## Grain yield and morphology of dwarf vs tall white lupin in Mediterranean environments

**Annicchiarico P.<sup>1</sup>, Carroni A.M.<sup>2</sup>, Manunza P.<sup>2</sup>, Huyghe C.<sup>3</sup>, Pecetti L.<sup>1</sup>**

<sup>1</sup> Research Centre for Fodder Crops and Dairy Productions (CREA-FLC), Lodi, Italy

<sup>2</sup> Research Unit for Mediterranean Agro-Pastoral Systems (CREA-AAM), Sanluri, Italy

<sup>3</sup> Institut National de Recherche Agronomique (INRA), Lusignan, France

E-mail: luciano.pecetti@crea.gov.it

### Abstract

There is lack of information on the preferable plant architecture of white lupin (*Lupinus albus* L.) for Mediterranean environments. The objective of this study was to compare tall vs dwarf near-isogenic lines from two gene pools for grain yield and plant morphology. The four lines were assessed in a Mediterranean-climate site of Sardinia for two seasons under two sowing densities (25.4 and 35.5 seeds/m<sup>2</sup>). On average, one gene pool (#15) exhibited a semi-determinate growth habit on the basis of its high seed proportion on the main stem relative to the other, indeterminate gene pool #16 (54.9 % vs 45.5;  $P < 0.05$ ). As expected, dwarfism reduced plant stature (106 vs 124 cm) and increased the harvest index (0.293 vs 0.250). Compared with the tall variant, dwarfism led to significantly ( $P < 0.05$ ) higher grain yield in the gene pool #16, and lower yield in the gene pool #15, giving rise to significant plant stature  $\times$  gene pool interaction ( $P < 0.05$ ). Sowing densities and seasons did not differ for grain yield, nor interacted significantly with plant stature or gene pool for this variable ( $P > 0.05$ ). Although preliminary, our results for Mediterranean environments highlight the genotype-specific usefulness of dwarfism and suggest that this trait might be beneficial in germplasm with indeterminate growth habit.



## **Deciphering grain legumes quality riddle: the genomics of bioactive compounds**

**Vaz Patto M. C.<sup>1</sup>, Mecha E.<sup>1</sup>, Pereira A. B.<sup>2</sup>, Leitão S. T.<sup>1</sup>,  
Alves M. L.<sup>1</sup>, Bronze M. R.<sup>1,2,3</sup>**

<sup>1</sup> ITQB NOVA, Instituto de Tecnologia Química e Biológica António Xavier,  
Universidade Nova de Lisboa, Oeiras, Portugal

<sup>2</sup> iBET, Instituto de Biologia Experimental e Tecnológica, Oeiras, Portugal

<sup>3</sup> iMED, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal  
E-mail: cpatto@itqb.unl.pt

### **Abstract**

Grain legumes are a vital source of inexpensive plant-based proteins and amino acids and can be eaten as part of a healthy diet; they help to prevent chronic diseases. Potential health benefits of grain legumes have been mainly attributed to the presence of secondary metabolites with antioxidant activity. Breeding for improved end-user's quality is a complex task due to trait interaction. Some of these metabolites, such as phenolic compounds, that act as health-promoting agents are also considered anti-nutrients, influencing both taste and consumers' acceptability. Under the scope of LEGATO and BEGEQA projects, we studied the genetic architecture of the total phenolic compounds content in grass pea and common bean seeds. Association mapping approaches were applied to both common bean and grass pea germplasm collections and the genomic regions controlling these bioactive compounds concentration were identified. This work will contribute to the development of grain legume varieties with better nutritional quality, meeting consumers' expectations at the same time that support the improvement and production of a plant resource with a vital role in sustainable agriculture.

## **Manipulation of rumen nitrogen flows by use of *Festulolium* hybrids in rumen simulation fermenters**

**Kamau S., Belanche A., Humphreys M., Kingston-Smith A.\***

Institute of Biological Environmental and Rural Sciences, Aberystwyth University, UK

\*E-mail: ahk@aber.ac.uk

### **Abstract**

*Festulolium* is an interspecific hybrid between *Lolium* and *Festuca* species which combines the superior forage quality characteristics of ryegrass with fescue's ability to grow in harsh environments. The aim of this study was to perform an integrated study of rumen nitrogen metabolism by using rumen simulation to explore the extent to which swards containing *Festulolium* populations previously shown to have decreased rates of endogenous protein degradation can confer advantageous protein utilization in comparison with a currently market leading diploid ryegrass. An *in vitro* experiment was therefore conducted using three *Festulolium* hybrids: *L. perenne* × *F. glaucescens* (LpFg); *L. perenne* × *F. mairei* (LpFm); and *L. multiflorum* × *F. glaucescens* (LmFg) and *Lolium perenne* (Lp) as a control. *Festulolium* hybrids had higher conversion ratios of nitrogen into microbial protein than *Lolium perenne* thus suggesting greater potential to improve ruminal nitrogen use efficiency in a fresh feeding system. This would help to increase production efficiency as well as decreasing the environmental impact of livestock agriculture.



## Low crown rust resistance in Norwegian material of *Lolium perenne* and $\times$ *Festulolium*

Østrem L.<sup>1</sup>, Asp T.<sup>2</sup>, Ghesquière M.<sup>3</sup>, Sanada Y.<sup>4</sup>, Rognli O. A.<sup>5</sup>

<sup>1</sup> The Norwegian Institute of Bioeconomy Research (NIBIO), Fureneset,  
6967 Hellevik i Fjaler, Norway

<sup>2</sup> Department of Molecular Biology and Genetics, Aarhus University,  
4200 Slagelse, Denmark

<sup>3</sup> INRA/URP3F, Le Chêne, RD 150, CS 80006, Lusignan 86600, France

<sup>4</sup> Hokkaido Agricultural Research Center, NARO Hitsujigaoka, Toyohira,  
Sapporo, 062-8555, Japan

<sup>5</sup> Department of Plant Sciences, Faculty of Biosciences, Norwegian University  
of Life Sciences, 1432 Ås, Norway

E-mail: liv.ostrem@nibio.no

### Abstract

Norwegian cultivars and breeding materials of perennial ryegrass and *Festulolium* were planted at three locations in Denmark, France and Japan for testing of resistance against leaf diseases. In general, all plant materials were susceptible to crown rust. The highest incidence of rust attack occurred at the French site, which due to its climatic conditions might be the most suitable testing site for future scoring of similar plant material. Entries based on introgressed genetic materials from UK were most resistant towards crown rust. Crown rust resistance needs increased focus as a breeding objective in the Nordic region due to climate changes, which will most likely lead to increased infection of leaf diseases.



## Unravelling the genetic control of bacterial wilt resistance in ryegrass: achievements, prospects and challenges

Kölliker R.<sup>1,2</sup>, Knorst V.<sup>1,2</sup>, Hersemann L.<sup>2</sup>, Widmer F.<sup>2</sup>, Studer B.<sup>1</sup>

<sup>1</sup> Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich, Zurich, Switzerland

<sup>2</sup> Molecular Ecology, Agroscope, Zürich, Switzerland  
E-mail: roland.koelliker@usys.ethz.ch

### Abstract

*Xanthomonas translucens* pv. *graminis* (Xtg) causes bacterial wilt, one of the most important forage grass diseases in temperate grasslands. Molecular genetics and genomic tools have the potential to significantly benefit resistance breeding and to enable targeted resistance management. In the past, a major QTL for bacterial wilt resistance was identified in *Lolium multiflorum* and Xtg was shown to rely on a non-canonical type III secretion system for plant infection. Recently, a number of candidate genes for bacterial wilt resistance were identified by comparing genomic sequences of resistant and susceptible parental plants and their progeny. Comparative genomics of different *X. translucens* pathovars allowed identifying virulence traits characteristic for Xtg. These candidate plant resistance genes together with the bacterial virulence factors provide an invaluable resource for the development of genomics assisted selection strategies. In addition, the well characterised plant genotypes and bacterial strains serve as an ideal model system to fully understand the complex *L. multiflorum*-Xtg interaction.



## Genotyping of *Festulolium* cultivars involved in EUCARPIA multi-site trial using DArT markers and GISH

Kopecký D.<sup>1</sup>, Baert J.<sup>2</sup>, Barth S.<sup>3</sup>, Bartoš J.<sup>1</sup>, Černoč V.<sup>4</sup>, Doležel J.<sup>1</sup>,  
Grogan D.<sup>5</sup>, Harper J.<sup>6</sup>, Humphreys M.<sup>6</sup>, Książczyk T.<sup>7</sup>, Østrem L.<sup>8</sup>,  
Paszkowski E.<sup>9</sup>, Sokolović D.<sup>10</sup>, Zwierzykowski Z.<sup>7</sup>, Ghesquière M.<sup>11</sup>

<sup>1</sup> Institute of Experimental Botany, Centre of the Region Hana for Biotechnological and Agricultural Research, Šlechtitelů 31, 78371 Olomouc, Czech Republic

<sup>2</sup> ILVO, Caritasstraat 21, 9090 Melle, Belgium

<sup>3</sup> Teagasc, Oak Park Crops Research Centre, Carlow, Ireland

<sup>4</sup> DLF Seeds, s.r.o., Fulnecká Fulnecká 95, 742 47 Hladké Životice, Czech Republic

<sup>5</sup> Dept. of Agriculture, Food & Marine, Davis St., Tipperary, Ireland

<sup>6</sup> IBERS, Aberystwyth University, SY23 3EE, Aberystwyth, United Kingdom

<sup>7</sup> Institute of Plant Genetics, Strzeszyńska 34, 60-479 Poznań, Poland

<sup>8</sup> NIBIO, Fureneset, 6967 Hellevik i Fjaler, Norway

<sup>9</sup> DANKO Plant Breeding Ltd., Szelejewo Drugie, 64-820 Piaski, Poland

<sup>10</sup> Institute of Forage Crops, 37251 Globoder, Kruševac, Serbia

<sup>11</sup> INRA, 86600 Lusignan, France

E-mail: kopecky@ueb.cas.cz

### Abstract

A comprehensive set of *Festulolium* cultivars from on-going field trials in the Eucarpia network was characterised at the chromosome level using genomic in situ hybridization (GISH) and by Diversity Array Technology (DArT) markers. Both technologies were found to be complementary in describing the breeding history of the plant material. The genomic composition of the *Lolium* × *Festuca* cultivars varied from those that comprised equivalent proportions of their parental genomes to introgression lines where small chromosome segments of *Festuca* had been translocated onto *Lolium* chromosomes. The breadth of genotype combinations found within the grass cultivars described represents an important resource of genetic variations necessary to combat the diverse abiotic stresses encountered within Europe, including safeguards against prolonged exposure to harsh weather conditions. It is likely that in future plant breeding, genotyping will contribute to precision-transfers of targeted *Festuca* genes into *Lolium* germplasm in order to enhance resilience to climate change.

## **Transcriptomic analysis of drought-sensitive and tolerant genotypes of perennial ryegrass (*Lolium perenne* L.)**

**Leyva M. O.<sup>1</sup>, Nagy I.<sup>1</sup>, Hentrup S.<sup>1</sup>, Byrne S.<sup>2</sup>, Asp T.<sup>1</sup>**

<sup>1</sup> Molecular Biology and Genetics, Aarhus University, Denmark

<sup>2</sup> Teagasc, Crop Science Department, Oak Park, Carlow, Ireland

E-mail: torben.asp@mbg.au.dk

### **Abstract**

Ten perennial ryegrass genotypes were tested for response to water deficiency in greenhouse under controlled conditions. Four genotypes with contrasting response to drought were selected for RNAseq transcriptomic analysis. In total, we found 5321 differentially expressed genes (DEGs) during drought but only 232 DEGs were shared across all genotypes. We could identify 7 genes with contrasting expression profiles in two tolerant genotypes compared to the sensitive genotypes. Most of these genes code for regulatory proteins that can be utilized for future selection strategies in breeding material for enhanced drought tolerance.



## Paternity test for forage yield improvement in timothy polycross breeding

Tanaka T.<sup>1</sup>, Tamura K.<sup>2</sup>, Ashikaga K.<sup>1</sup>, Fujii H.<sup>1</sup>, Yamada T.<sup>3</sup>

<sup>1</sup> Hokkaido Research Organization Kitami Agricultural Experiment Station,  
Kunneppu, Hokkaido, Japan

<sup>2</sup> Hokkaido Agricultural Research Center, NARO, Sapporo, Hokkaido, Japan

<sup>3</sup> Field Science Center for Northern Biosphere, Hokkaido University, Sapporo,  
Hokkaido, Japan

E-mail: tanaka-tsuneki@hro.or.jp

### Abstract

Paternal identity knowledge can enhance selection gains in polycross breeding of forage grasses. This study assessed paternal parents selected through timothy (*Phleum pratense* L.) breeding without paternal information in Japan. Paternal assignment was conducted based on a simple exclusion way using 27 genomic-SSR markers. General combining ability (GCA) for forage yield was examined based on polycross progeny tests. Results showed an unbalanced number of indirectly selected paternal parent and inferior paternal parent in GCA for forage yield. These results indicate that paternal selection can give continuous improvement avoiding inbreeding depression and that it can enhance selection gains for forage yields in timothy polycross breeding.

## **Selection of perennial ryegrass spaced plants in sward conditions**

**Aper J., Ghesquiere A., Baert J.**

Plant Sciences Unit, Flanders research institute for Agriculture,  
Fisheries and Food, Melle, Belgium  
E-mail: [jonas.aper@ilvo.vlaanderen.be](mailto:jonas.aper@ilvo.vlaanderen.be)

### **Abstract**

We evaluated growth, winter damage and rust susceptibility of diploid and tetraploid perennial ryegrass genotypes that were cloned and planted as spaced plants in three 'environments': on bare soil, in a red fescue sward and in a white clover sward. Scores were most repeatable in bare soil. Over all, correlation was higher between the scores from plants on bare soil and in red fescue than between the scores on bare soil and in white clover. Some accessions showed different growth reactions in one of the three swards.



## Variation in intrinsic water use efficiency between perennial ryegrass genotypes differing for drought tolerance

Westermeier P.<sup>1</sup>, Schäufele R.<sup>2</sup>, Hartmann S.<sup>1</sup>

<sup>1</sup> Institute for Crop Science and Plant Breeding,  
Bavarian State Research Center for Agriculture, Freising, Germany

<sup>2</sup> Grassland Group, Technische Universität München, Freising, Germany  
E-mail: peter.westermeier@lfl.bayern.de

### Abstract

Drought tolerance of perennial ryegrass (*Lolium perenne* L.) is one of the constraints for the use of this forage species in drought prone areas. Global climate change with increasing fluctuation in rainfall patterns will further limit its use in larger areas of Europe. The aim of this study was to investigate whether there is genotypic variation for carbon isotope discrimination in drought tolerance of selected perennial ryegrass as a measure for water use efficiency. The phenotypic selection for drought tolerance is not generally correlated with carbon isotope composition within the material investigated. For one genotype with good phenotypic drought tolerance a significant difference in intrinsic water use efficiency was found under stress conditions. It is also shown, that water use efficiency is not the only drought tolerance mechanism within perennial ryegrass, as other drought tolerant genotypes show no significant differences in intrinsic water use efficiency but a good recovery after drought stress at the same time.

## **Physiological indicators of tolerance to soil water deficit in *Lolium multiflorum*/*Festuca arundinacea* introgression forms**

**Masajada K., Augustyniak A., Perlikowski D., Ratajczak D., Zwierzykowski W., Pawłowicz I., Kosmala A.**

Institute of Plant Genetics, Polish Academy of Sciences,  
Poznan, Poland  
E-mail: kmas@igr.poznan.pl

### **Abstract**

*Festuca arundinacea* is a model plant species for a group of forage grasses, comprising the *Lolium-Festuca* complex to recognize the crucial mechanisms of tolerance to water deficit and recovery after stress cessation. The *L. multiflorum*/*F. arundinacea* introgression forms are excellent plant materials for dissecting these important traits into several components. Here, four genotypes of the introgression forms with a different capacity in response to abiotic and biotic stresses, were analyzed with respect to their physiological performance under water deficit conditions and further recovery. It was revealed that the Relative Water Content and Electrolyte Leakage parameters could be good indicators of tolerance to soil water deficit and ability to recover after stress cessation in the *L. multiflorum*/*F. arundinacea* introgression forms.



## **Incidence of six grass species by *Fusarium* sp. as a cause of silvertop**

**Trněný O., Nedělník J.**

Agricultural Research, Ltd., The Czech Republic

E-mail: trneny@vupt.cz

### **Abstract**

Silvertop is the big threat to grass seed production because of its devastating effect on yield. Screening of three *Fusarium* species in silvertop stalks confirmed interdependence between symptoms of diseases and *Fusarium poae* infection. Pooled samples of six grass species were tested for the presence of infection by PCR detection of *F. poae*, *F. graminearum* and *F. culmorum*. *F. poae* were detected in 67 % of silvertop pooled samples and only in 8 % in asymptomatic pooled samples. *F. culmorum* were not detected in any case and *F. graminearum* were not detected at a significant level in comparisons silvertop and asymptomatic samples.



## Pyramiding of resistance genes in perennial ryegrass (*Lolium perenne*) using next-generation sequencing based Massive Analysis of cDNA Ends (MACE)

Malenica M.<sup>1</sup>, Haase F.<sup>2</sup>, Bojahr J.<sup>1</sup>, Rotter B.<sup>3</sup>, Böhm C.<sup>1</sup>,  
Ruge-Wehling B.<sup>2</sup>, Winter P.<sup>3</sup>

<sup>1</sup> Saatzucht Steinach GmbH & Co KG, Germany

<sup>2</sup> Federal Research Centre for Cultivated Plants (JKI), Institute for Breeding Research on Agricultural Crops, Germany

<sup>3</sup> GenXPro GmbH, Germany

E-mail: milka.malenica@saatzucht-steinach.de

### Abstract

Due to its high nutritive values, rapid establishment and persistence perennial ryegrass (*Lolium perenne* L.) is the most important temperate forage grass species. Its productivity and nutritive content are impaired by various fungal and bacterial diseases including crown rust (*Puccinia coronata* f. sp. *lolii*), stem rust (*Puccinia graminis* f. sp. *graminicola*) and bacterial wilt (*Xanthomonas translucens* pv. *graminis*). As the use of antibiotics and fungicides is economically and environmentally inefficient, the most powerful option to control these diseases is breeding for resistant varieties. Up to now breeding for combined resistance is based on artificial seedling inoculation and multi-year field observations. This selection procedure, however, is costly and time-consuming. Gene pyramiding using molecular markers may speed up this process and eventually generates perennial ryegrass varieties with multiple resistances to crown rust, stem rust and bacterial wilt that exceed the standards of the National Listing.

Resistance genes from *L. perenne* and *L. multiflorum* were introduced into our breeding material. Donors of resistance to the three diseases were backcrossed several times with elite material, and the offspring (n = 907) rated for resistance after artificial infection. To facilitate the pyramiding of resistance genes, we first develop candidate molecular markers for each resistance gene by combining bulked segregant analysis with genome-wide, NGS-based massive analysis of cDNA ends (MACE) transcriptome profiling. To this end, various full-sib mapping populations (n ≥ 200) were generated from crosses between two genotypes with contrasting resistance levels and phenotyped with the respective pathogen. Then single locus marker assays for each resistance gene are developed and validated. For the stem rust resistance locus *LpPg1* this approach yielded 27 closely linked “Exclusive Transcripts in Resistant (ETR)” and 3 SNP markers (Bojahr et al. 2016). From this study three ETR markers internally referred to as *LpETR\_SZS1*, *LpETR\_SZS2* and *LpETR\_SZS3* were validated on phenotyped offspring (BC1 x L. p.) chosen for gene pyramiding. Therefore, 116 resistant (disease severity scores 1 to 2) and 74 susceptible (disease severity score ≥ 5) genotypes were selected and screened for resistance to stem rust. In more than 93 % of the cases results from markers *LpETR\_SZS1* and *LpETR\_SZS2* confirmed the results of the artificial seedling test whereas marker *LpETR\_SZS3* was validated in 83 % of the cases.

In conclusion, the validation of the three published ETR markers for determining *LpPg1*-carrying genotypes demonstrates the great potential of these markers for gene pyramiding by marker-assisted backcrossing. The development of similar markers for crown rust and bacterial wilt is on its way using the same approach. In future these generated diagnostic markers could be used for high-throughput genotyping in early generations to implement smart resistance breeding.

## Anthracnose resistance in lupin – genetic analysis and molecular markers

Ruge-Wehling B.<sup>1</sup>, Fischer K.<sup>1</sup>, Dieterich R.<sup>2</sup>, Rotter B.<sup>3</sup>,  
Winter P.<sup>3</sup>, Wehling P.<sup>1</sup>

<sup>1</sup> Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Agricultural Crops, Groß Lüsewitz, Germany,

<sup>2</sup> Saatzucht Steinach GmbH & Co. KG, Bocksee, Germany,

<sup>3</sup> GenXPro GmbH, Frankfurt/Main, Germany  
E-mail: brigitte.ruge-wehling@julius-kuehn.de

### Abstract

Anthracnose, caused by the fungus *Colletotrichum lupini*, represents the most important disease in lupin cultivation worldwide. To obtain high and stable yields it is necessary to grow resistant cultivars as there are no possibilities to overcome the fungus via pesticides. Sets of European cultivars and plant genetic resources of sweet narrow-leaved and yellow lupin were assessed in their susceptibility to anthracnose in an artificial greenhouse resistance test (Yang et al. 2004). In both lupin species breeding lines were identified which displayed a qualitative resistance. Genetic analysis of F2 populations of narrow-leaved lupin (N = 368) and yellow lupin (N = 206) revealed a monogenic dominant inheritance of resistance in each crop species. F3 progeny tests confirmed the identification of novel dominant resistance genes. Multi-annual field tests with the novel resistant resources of narrow-leaved and yellow lupins validated a high and stable level of anthracnose resistance. The underlying genes were designated *LanrBo* (*L. angustifolius*) and *Llur* (*L. luteus*). Marker development was based on anchor markers (Nelson et al. 2010), AFLPs, as well as transcriptome data from MACE and RNAseq experiments. Linkage analysis allowed us to locate *LanrBo* on linkage group NLL-11 in *L. angustifolius*. Two flanking markers resulted in a marker bracket with a joint recombination of less than 0.2 %. The two markers are already integrated in breeding programs and open up the possibility to combine different resistance genes in one breeding line as well as introduce resistance into high-yielding lines. Regarding *Llur*, highly polymorphic SNPs are available and currently used for the genetic mapping of this gene.

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## Localisation of stem rust resistance gene *LpPg1* in *Lolium perenne* genome based on transcriptomic data

Haase F.<sup>1</sup>, Bojahr J.<sup>2</sup>, Rotter B.<sup>3</sup>, Winter P.<sup>3</sup>, Ruge-Wehling B.<sup>1</sup>

<sup>1</sup> Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Agricultural Crops, Rudolf-Schick-Platz 3a, OT Groß Lüsewitz, 18190 Sanitz, Germany

<sup>2</sup> Saatzucht Steinach GmbH & Co KG, Wittelsbacherstrasse 15, 94377 Steinach, Germany

<sup>3</sup> GenXPro GmbH, Altenhöferallee 3, 60438 Frankfurt am Main, Germany  
E-mail of corresponding author: florian.haase@julius-kuehn.de

### Abstract

Ryegrass (*Lolium* sp.) is the most important cool-season forage crop in temperate regions. Severe fungal diseases like stem rust [caused by *Puccinia graminis* subsp. *graminicola*] can considerably affect the seed production of ryegrasses. Thus, the development of molecular markers for stem rust could improve a marker-assisted selection for crop breeding and an accelerated development of ryegrass cultivars with pathogen resistance and agronomic adaptation to Germany's agricultural conditions. The previously identified qualitative stem rust resistance locus *LpPg1* affords efficient resistance against the pathogen (Beckmann et al., 2010). Development of molecular markers was accomplished by a combination of bulked segregant analysis (BSA) and massive analysis of cDNA ends (MACE) transcriptome profiling based on a next generation sequencing approach. In full-sib mapping populations (n = 276), leaf segments of individual plants were inoculated with the pathogen and RNA was isolated from bulks of susceptible and resistant segregants, respectively. Genetic polymorphisms and altered gene expression levels serve a basis for bioinformatical processing of the data. Thus, 30 molecular markers were genetically mapped to a 50.8 cM region surrounding the stem-rust resistance locus *LpPg1*. MACE sequences of resistant-specific transcripts and exclusively SNPs were annotated to reference genome of *Brachypodium distachyon* and peaked on the region between 26.0 and 34.5 Mbp on *Bd1*. The syntenic relationship between perennial ryegrass and *Brachypodium* was used for the assumed assignment of *Lolium* LG 2, 4 and 7. Transcript data were compared to [1] perennial ryegrass GenomeZipper (Pfeifer et al., 2013) and [2] whole genome shotgun scaffolds by Byrne et al. (2015) to predict the unambiguous genetic location of stem rust resistance locus *LpPg1*. The *in silico* prediction of gene position was used to single out anchor-markers with potential association to the resistance locus. Therefore 96 anchor-markers were used to localize *LpPg1* via genetic linkage mapping. Two markers indicating genetic linkage to the resistance locus are located on LG7 in *Lolium perenne* and were successfully integrated into abovementioned genetic map.

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## Variation of vegetation terms and productivity traits among spontaneous *Vicia villosa* Roth. ecotypes

Maršalkienė N., Sliesaravičius A., Dastikaitė A.

Institute of Biology and Plant Biotechnology, Aleksandras Stulginskis University,  
Kaunas distr., Lithuania  
E-mail: nijolemars@gmail.com

### Abstract

Winter vetch (*Vicia villosa*) is among the best of the legumes in their ability to be productive in low fertility or acid soils and used for forage, hay, silage and green manure. The experimental objects were spontaneous *V. villosa* ecotypes from different places of Lithuania. Regarding to the vegetation cycle terms, four of *V. villosa* groups were distinguished. According to green mass, mature seed number per plant and herbage protein content, the group of early ecotypes was distinguished as the most productive. Owing to the protein content in air-dried herbage mass, the most valuable part of the total plant mass were leaves and flowers. The end of the growing season was influenced by the spread of diseases and in some year by drought.

## **Crown rust and brown blight infection of perennial ryegrass cultivars representing three maturity groups**

**Aavola R., Sooväli P., Tamm S., Bender A.**

Estonian Crop Research Institute, Jõgeva, Estonia  
E-mail: rene.aavola@etki.ee

### **Abstract**

Infection of perennial ryegrass with fungal pathogens was assessed during three years on a basis of a variety type. The scoring served a goal of searching for donor varieties for resistance breeding and distinguish likely differences characterising the variety types. Six types representing three maturity classes and two ploidy levels were compared. From amongst 33 cultivars, lates Barnhem (2x), Elgon (4x) and Herbie (2x) turned out to be most resistant against crown rust (1.8–1.9 pts). Brown blight proved to be the prevailing disease, which infected the least foliage (3.0–3.5 pts) in cv. Arvicola, Maurice (both 4x) and Aberdart (2x). Eight tetraploid cultivars preceded the most resistant diploid Herbie on the basis of combined resistance against the two fungi. Three healthiest tetraploids were late-maturing. Among the most resistant diploids four were late-maturing and 1 was intermediate. Direct effects of cultivars and years to the resistance against both diseases were highly significant ( $p < 0,001$ ) in both ryegrass ploidy groups. Cultivar x year co-effects did not impact ( $p > 0,05$ ) the brown blight resistance in tetraploid cultivars.



## **Correlation of seed yield and its components and chlorophyll fluorescence parameters in the narrow leafed lupin (*Lupinus angustifolius* L.)**

**Górynowicz B.<sup>1</sup>, Święcicki W.<sup>1</sup>, Pilarczyk W.<sup>2</sup>, Mikulski W.<sup>1</sup>**

<sup>1</sup> Institute of Plant Genetics of the Polish Academy of Sciences, Genomics Department, Legumes Comparative Genomics Team, Poznań, Poland

<sup>2</sup> Poznań University of Life Sciences Department of Mathematical and Statistical Methods, Poznań, Poland  
E-mail: bgom@igr.poznan.pl

### **Abstract**

Narrow leafed lupin (*Lupinus angustifolius* L.) is one of the most important legumes species and it is a fundamental element for plant and animal production due to high protein content. The aim of this study was to assess the correlation of seed yield and its components on selected chlorophyll fluorescence parameters in narrow leafed lupin cultivars at the flowering and maturation phases. In 2011-2014 field experiments with 10 narrow leafed lupin cultivars were conducted at 4 locations and in 2012-2014 chlorophyll fluorescence measurements in vivo of plants in field conditions were made. The results were analysed statistically. The significant correlation of seed yield on ETO/CS and ETO/RC at the end of the flowering phase was found. These parameters were positively correlated with seed yield, number of pods and number of seeds in the pod, and negatively correlated with thousand seed weight. The results of this study suggests the direction of further research.

## A history of *Festulolium* in Poland

Zwierzykowski Z.<sup>1</sup>, Kosmala A.<sup>1</sup>

<sup>1</sup> Institute of Plant Genetics, Polish Academy of Sciences,  
Strzeszyńska 34, 60-479 Poznań, Poland  
E-mail: zzwi@igr.poznan.pl

### Abstract

Intergeneric hybridization within the *Lolium-Festuca* complex in Poland (at the Institute of Plant Genetics, Polish Academy of Sciences in Poznań) began in the middle of 1960s. From this moment numerous intergeneric hybrid combinations mostly between agriculturally important *Lolium* species (*L. multiflorum* Lam. and *L. perenne* L.) and *Festuca* species (*F. arundinacea* Schreb. and *F. pratensis* Huds.) have been created. These hybrids have been used to develop various amphiploid and/or introgression derivatives and then some of them were used in breeding programmes as well as genetic, cytogenetic and molecular studies.

Due to close cooperation with Szelejewo Breeding Station Ltd., first Polish *Festulolium* cultivars (Felopa, Sulino, Agula and Rakopan) developed from *F. pratensis* × *L. multiflorum* hybrids have been bred.

The introgression derivatives of *Lolium* × *F. pratensis*/*F. arundinacea* intergeneric hybrids are unique research materials for the studies to recognize molecular mechanisms of tolerance to a wide range of abiotic stresses in the *Lolium-Festuca* forage grasses. *Lolium multiflorum*/*F. arundinacea* introgression forms are used in the research on tolerance to water deficit, salinity and low temperature, while *L. multiflorum*/*F. pratensis* forms are excellent models to study molecular basis of cold acclimation and frost tolerance. In the comprehensive research to decipher a complex response of *Lolium-Festuca* grasses to environmental stresses, modern tools of physiology and molecular biology, including genomics, proteomics and metabolomics, are applied.

Since in different intergeneric *Lolium* × *Festuca* hybrids interspecies chromosomal rearrangements have been observed, these plant materials are good models to analyze the processes involved in genomes interactions and chromosomal aberrations, which lead to karyotype reshuffling in hybrids. In this research, tools of molecular cytogenetics, genomic (GISH) and fluorescent (FISH) *in situ* hybridization, are successfully used. In the last few years, in our team, these studies were focused mainly on *L. perenne* × *F. pratensis* hybrids and their introgression forms.



## **A sure-root to climate-smart grassland**

**Humphreys M.<sup>1</sup>, Marley C.<sup>1</sup>, Loka D.<sup>1</sup>, Harper J.<sup>1</sup>, Gasior D.<sup>1</sup>, Collins R.<sup>1</sup>,  
Marshall A.<sup>1</sup>, Kingston-Smith A.<sup>1</sup>, Dodd R.<sup>2</sup>, Jones D.<sup>2</sup>, Dungait J.<sup>3</sup>**

<sup>1</sup> Institute of Biological Environmental and Rural Sciences, Gogerddan,  
Aberystwyth University, Aberystwyth, Ceredigion, Wales, UK, SY23 5EE.

<sup>2</sup> Bangor University, Environmental Centre Wales, Deiniol Road, Bangor,  
Gwynedd, Wales, UK, LL57 2UW.

<sup>3</sup> North Wyke Research Station, Rothamsted Research, North Wyke,  
Okehampton, Devon, UK, EX20 2SB.  
E-mail: mkh@aber.ac.uk

### **Abstract**

We highlight progress from two major UK research programmes: SureRoot (<http://www.sureroor.uk/>) and Climate-Smart Grassland (<http://www.nrn-lcee.ac.uk/climate-smart-grass/>).

The SureRoot programme uses two BBSRC-funded National Capability facilities to select novel *Festulolium* and white clover populations with enhanced root systems that improve soil hydrology to mitigate flooding following episodes of intense rainfall, and/or maintain sustainable forage production at times of drought. The grasses and clovers are assessed under eight alternative livestock management systems at different UK locations to assess their potential widespread use.

Four hypotheses are being assessed: H1 Variations in individual plant root phenotype in grasses and legumes may be replicated in crops grown under field conditions; H2 Variants for grass or legume root growth and turnover can benefit soil structure and hydrology; H3 Interspecific grass and clover hybrids grown as mixtures can further enhance environmental service. H4 Modified root phenotype for environmental service is achievable without compromise to agronomic performance.

Climate-Smart Grassland measures the impacts of multiple stresses (drought, flooding, ozone) on grasslands and identifies “tipping-points” when a sequence of these may lead to crop failure.

Five hypotheses are being assessed: H1. Multiple extreme weather events within a year make the ecosystem more pre-disposed to a non-reversible change in ecosystem functioning than single extreme events. H2 Grassland recovery periods are much longer after multiple interacting extreme weather events than from single events. H3 The duration of stress needed to induce a complete loss of vegetation function is much shorter than the time required to induce a loss in below-ground soil functioning. H4 Soil mesofauna rather than soil microorganisms are most sensitive to extreme weather events and this contributes to a long-term decline in soil quality. H5 Multiple extreme events result in more severe soil water repellence negatively affecting the infiltration characteristics and soil moisture storage in soils.



## Current Festulolium breeding at ILVO

**Baert J., Aper J., Van Laere K.**

Flanders Research Institute for Agriculture, Fisheries and Food (ILVO),  
Plant applied genetics and breeding, Caritasstraat 39, 9090 Melle, Belgium  
E-mail: joost.baert@ilvo.vlaanderen.be

### Abstract

In order to introduce more abiotic stress tolerance in *Lolium* fodder grasses, we carried out at ILVO in the years 2011 and 2012 more than 12000 crosses between the *Lolium* species *perenne* (Lp) or *multiflorum* (Lm) and the *Festuca* species *pratensis* (Fp), *glaucescens* (Fg) or *arundinacea* (Fa). The *Lolium* genotypes were tetraploid, the *Festuca* genotypes were tetraploid or hexaploid. Well over 4000 embryos were put in vitro for rescue. Finally we obtained 30 F1-plants that were scored in the field for spring growth, crown rust resistance, drought tolerance, leaf softness and winter hardiness. After selection three polycrosses were made with 3 plants of the Lp x Fa cross, 3 plants of the Lm x Fa cross and 5 plants of the Lp x Fp cross. The first polycross produced almost no seeds. The F2-seeds of the latter polycrosses were tested in a plot trial.

The two F2 *Festulolium*-populations had a higher annual dry matter yield, a better rust resistance and digestibility than standard varieties of a similar parental composition and than their *Lolium* parental species. Their original F1 components were backcrossed to the *Lolium* parent. The F2 populations are now multiplied to F3 populations. Chromosome countings and karyotyping by GISH of the parental, F1, F2 and BC1 genotypes show the segregation and recombination of the *Lolium* and *Festuca* genomes. The expected genomic instability and shift towards the *Lolium* genome in the progeny seem to be confirmed.



## Using LIDAR for forage yield measurement of perennial ryegrass (*Lolium perenne* L.) field plots

Ghamkhar K.<sup>1</sup>, Irie K.<sup>2</sup>, Hagedorn M.<sup>2</sup>, Hsiao J.<sup>2</sup>, Fourie J.<sup>1</sup>, Gebbie S.<sup>3</sup>, Flay C.<sup>1</sup>, Barrett B.<sup>1</sup>, Stewart A.<sup>4</sup>, Werner A.<sup>2</sup>

<sup>1</sup> Forage Science, Grasslands Research Centre, AgResearch, Palmerston North, New Zealand

<sup>2</sup> Lincoln Agritech Ltd, Lincoln, New Zealand

<sup>3</sup> Development Engineering, Lincoln Research Centre, AgResearch, Lincoln, New Zealand

<sup>4</sup> PGG Wrightson Seeds, Christchurch, New Zealand  
E-mail: kioumars.ghamkhar@agresearch.co.nz

### Abstract

Perennial ryegrass (*Lolium perenne* L.) is the most important forage species in New Zealand and one of the most important species in Europe and Australia. A LIDAR-based tool for non-invasive estimation of plant biomass in perennial ryegrass field plots was developed with the aim to measure the variation for plant foliage yield among 12 cultivars. Phenotypic data were collected both with a mechanical harvester and using the LIDAR system over the growing season. Significant ( $P < 0.05$ ) variation was observed among cultivars. Significant ( $P < 0.0005$ ) correlations were observed between processed LIDAR data and fresh and dry weights of plant foliage biomass with  $R^2$  values of 0.78 and 0.76, respectively.

## **An indoor screening method for reduced fall dormancy in alfalfa**

**Bertrand A., Claessens A., Rocher S.**

Quebec Research and Development Centre, Agriculture and Agri-Food Canada,  
Québec, QC, Canada, G1V 2J3  
E-mail: Annick.bertrand@agr.gc.ca

### **Abstract**

Fall dormancy (FD) is a determinant component of alfalfa yield in northern climate, but selection for that trait is often done at the expense of freezing tolerance. Field selection for low dormancy is a tedious process that depends on unpredictable fall conditions to identify less dormant genotypes. Here we describe two screening methods for low dormancy in alfalfa which can be entirely performed indoors. Both methods rely on plant exposure to a critical photoperiod during four weeks to discriminate plants according to their FD level. One method also includes successive freezing stress to simultaneously improve freezing tolerance (TF). These approaches can be integrated in a recurrent selection program to produce populations combining reduced FD and high TF.



## **Application of multivariate analysis for genotype evaluation in soybean**

**Perić V., Srebrić M., Nikolić A., Mladenović-Drinić S.**

Maize Research Institute “Zemun Polje”, Belgrade, Serbia

E-mail: vesnaperic@yahoo.com

### **Abstract**

Multivariate analysis is a frequently used approach in breeding studies. Applied to multiple trait data, Principal Component Analysis (PCA) gives an opportunity to graphically display the relationships between important agronomic traits, evaluate the varieties on the basis of multiple traits, identify valuable breeding sources and recommend possible selection strategies. This study was conducted to evaluate agronomic performance of 31 early varieties (maturity group 0) from the soybean collection of the Maize Research Institute “Zemun Polje” (Belgrade, Serbia). PCA analysis was applied to identify the best performing genotypes considering multiple traits (seed yield, major yield components and parameters of technological quality of grain), and to determine the level of trait interdependence. Genotypes were tested in a randomized complete block design with three replications during two years (2011 and 2012) at two locations in Serbia (Zemun Polje and Pančevo). An analysis of variance (mixed model) exhibited significant effects of genotype (G), environment (E), and genotype × environment (G×E) for most of the traits. According to PCA biplot, seed yield per plant was in positive correlation with number of seeds and pods per plant, indicating that those yield components could be effective selection criteria in breeding for seed yield. Comparison of early-maturing genotypes based on multiple traits revealed two varieties with above-average seed yield per plant and four varieties with high oil and above average protein content, which could be used as potential parents in breeding for important agronomic traits in future breeding programs.

## **Polymorphism of soybean cultivars and breeding lines revealed by marker *Satt100* associated with the *E7* locus**

**Zharikova D.<sup>1</sup>, Ivanyuk S.<sup>2</sup>, Chebotar G.<sup>1</sup>, Korniychuk O.<sup>2</sup>, Chebotar S.<sup>1</sup>**

<sup>1</sup> Odessa I.I. Mechnikov National University, Odessa, Ukraine

<sup>2</sup> Institute of Feeds and Agriculture of Podillia of NAAS, Vinnitsa, Ukraine  
E-mail: s.v.chebotar@onu.edu.ua

### **Abstract**

Flowering time is critical for successful seed production of plants, and with time to maturity it determines geographic adaptation, seed quality and yield. In contrast to most cereals, soybean cultivars (*Glycine max*) are confined to comparatively narrow ranges of geographical latitudes. For every 100-150 km of changes latitude (corresponding to 1°), a new cultivar has to be developed. The aim of our work was to detect alleles of the microsatellite locus *Satt100*, which is tightly linked with the locus *E7* determining the time to flowering in soybean cultivars and breeding lines and to analyze any associations of the alleles with the time to flowering. Twenty five cultivars and breeding lines from the breeding program of the Institute of Feeds and Agriculture of Podillia of NAAS (IFAP; Ukraine) that cultivated in the Vinnitsa region of Ukraine were analyzed. Five alleles of *Satt100* locus have been detected in the plant materials studied. Only two lines KR-II No3035 and PSV-I No4001 have allele 'A' of *Satt100* that corresponds to dominant *E7* allele.

The alleles that have been detected at microsatellite locus *Satt100* for soybean breeding and parental lines of IFAP and their associations with different time to flowering is the first step for precise observations of the agronomical traits of the lines and estimations of their productivity with the purpose to find genotypes that have best adaptability to conditions of the Ukraine.



## Genetic diversity assessment of two *Medicago sativa* genes: *CAD* and *WXP1*

Gréard C.<sup>1,2</sup>, Barre P.<sup>1</sup>, Flajoulot S.<sup>2</sup>, Santoni S.<sup>3</sup>, Julier B.<sup>1</sup>

<sup>1</sup> P3F, INRA, 86600 Lusignan, France

<sup>2</sup> Jouffray-Drillaud, INRA, 86600, Lusignan, France

<sup>3</sup> AGAP, INRA, 34060 Montpellier, France

E-mail: camille.greard@inra.fr

### Abstract

Alfalfa (*Medicago sativa* L.) is a major perennial forage legume crop with numerous nutritional and environmental benefits. This allogamous and autotetraploid species whose varieties are synthetic populations, has a highly polymorphic genome and a short linkage disequilibrium. Allele mining strategy on targeted candidate genes is an option to select valuable parents for breeding. In this paper, we assessed allelic diversity of *CAD* and *WXP1* genes, involved in lignin biosynthesis and drought tolerance respectively, in a set of 384 individuals. For *CAD* which had two splice forms, 30 and 31 variants (out of 1077 and 906 base pairs of the coding sequences, respectively) have been observed including 37 and 52% of non-synonymous mutations. More variants were observed in *WXP1* sequence, with 157 observed in *WXP1* coding sequence (coding sequence of 1116 base pairs), including 60% non-synonymous mutations. Among the non-synonymous mutations, some probably affected protein function. Both genes were under purifying selection, especially *CAD* with dN/dS rate of 0.05 and 0.12 for both splice forms, against a rate of 0.26 for *WXP1*. Difference in variant proportion is probably explained by differential selective pressure that may be induced by contrasted expression levels. Indeed, *CAD* is highly and continually expressed whereas *WXP1* is induced in some specific conditions. Further studies assessing the impact of variants on phenotype will help to conclude on the allele mining strategy in alfalfa breeding.

## Testing for freezing tolerance in perennial ryegrass

**Aleliūnas A., Brazauskas G.**

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry,  
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania  
E-mail: andrius.aleliunas@lzi.lt

### Abstract

Perennial ryegrass (*Lolium perenne* L.) is a highly valued species both for agriculture and amenity purposes. However, it lacks winter hardiness for cultivation at certain regions. As winter hardiness is a very complex trait which is affected both by numerous environmental conditions and genetic composition of a plant, freezing tolerance is an important component of winter hardiness. The freezing tolerance at the population scale might be determined at selected fixed-temperature points or by the LT50 evaluation. Here the LT50 value evaluation has a potential to reveal substantially more information about this trait, as it involves assessment of survival at a series of freezing temperatures. Herein we present a high-throughput freezing tolerance evaluation method for perennial ryegrass populations.

### Acknowledgements

The research project was funded by the Research Council of Lithuania, grant No. MIP-064/2015 (ADAPTGENAS)



## Preliminary estimation of variation of alkaloids content in white lupin (*Lupinus albus* L.) collection

Rybiński W.<sup>1</sup>, Kroc M.<sup>1</sup>, Świącicki W.<sup>1</sup>, Wilczura P.<sup>1</sup>, Kamel K.<sup>1</sup>,  
Barzyk P.<sup>2</sup>, Mikulski W.<sup>1</sup>

<sup>1</sup> Institute of Plant Genetics, Polish Academy of Sciences. Poznań, Poland

<sup>2</sup> Poznań Plant Breeders Ltd, Wiatrowo Plant Breeding Branch, Wągrowiec, Poland

E-mail: wryb@igr.poznan.pl

### Abstract

Grain legume crops are crucial elements of global agriculture and nutrition, both as food and feed, since they are major sources of plant protein. Of the many species of genus *Lupinus*, *L. angustifolius*, *L. luteus* and *L. albus* (characterized by high protein content) are particularly important from the agricultural point of view. Among them, white lupin (*L. albus*) has unexploited potential in Poland as a crop plant due to its high seed yield as well as protein and oil content in the seeds. In white lupin breeding, the reduction of alkaloid content in seeds is important. The paper presents an assessment of the variability in total alkaloid content and qualitative composition of alkaloids in seeds of white lupin. Plant material constituted 150 accessions from domestic collections, divided into five classes: wild collected material, landraces, induced mutants, breeding lines, and cultivars. Total alkaloid content was analysed along with qualitative composition expressed by four major alkaloids, and broad variation was found. The total alkaloid content varied from 0.008 to 11.1 % of seed dry weight. An apparent decrease in alkaloid content was shown in breeding lines and cultivars, indicating a clear influence of domestication. The four major alkaloids (abundance > 1%) were lupanine (mean 71.3%), 13-hydroxylupanine (10.46%), multiflorine (6.8%) and angustifoline (3.8%). The lower alkaloid content of breeding lines and cultivars, as compared to wild lines and landraces, was associated with higher relative contents of 13-hydroxylupanine and angustifoline and lower relative contents of lupanine and multiflorine.

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## **The multifunctional role of legumes in vineyards and orchards**

**Vymyslický T., Lang J., Knotová D.**

Agricultural Research, Ltd., Troubsko, Czech Republic  
E-mail: vymyslicky@vupt.cz

### **Abstract**

In frame of the research project TA04020464 "Different ways of vineyard greening and management and their influence on reduction of soil erosion and quality of production", various species of legumes have been studied as cover-crop mixtures. The main aim of the research is to recommend the best mixtures for the use in vineyards and orchards in both organic and integrated crop production. Based on two years evaluation of five mixtures in six localities, it can be concluded that the best results were obtained by winter mixture, and perennial mixture for dry conditions, and annual mixture for fast greening. These three mixtures comply with the requirement of fast vegetation-cover establishment after sowing, and thus significantly reduce the erosion.



## Yields and quality of some perennial legumes in the Czech Republic

Knotová D.<sup>1</sup>, Pelikán J.<sup>1</sup>, Skládanka J.<sup>2</sup>, Knot P.<sup>2</sup>

<sup>1</sup> Agricultural Research, Ltd. Troubsko, Czech Republic

<sup>2</sup> Department of Animal Nutrition and forage Production, Mendel University in Brno,  
Czech Republic

E-mail: knotova@vupt.cz

### Abstract

In 2013 an experiment with perennial fodder crops (alfalfa, red clover 2n and 4n, interspecific hybrid between *Trifolium pratense* and *Trifolium medium* Pramedi, Hungarian clover and goat's rue) was established. The evaluation took place in 2014 and 2015 at two locations. The yields of green mass, hay, number of cuts and quality were compared.

## **Hydroponic evaluation of growth and nutrient uptake in a *Lolium/Festuca* introgression series**

**Gasior D., Harper J., Thomas A., Evans C., Mathews R., Allen D., Humphreys M., Armstead I.**

Institute of Biological, Environmental and Rural Sciences, Aberystwyth University, UK  
E-mail: dkg@aber.ac.uk

### **Abstract**

Introgression breeding represents a useful method for generating new germplasm resources which combine positive attributes from different species. Within forage grasses, the interfertility of *Lolium* and *Festuca* spp. represents a viable route for achieving this end. In order to evaluate the architecture of trait inheritance within this introgression context, a complete chromosome introgression series of *F. pratensis* (Fp; meadow fescue) in the *L. perenne* (Lp; perennial ryegrass) background, and derived progeny, were evaluated for shoot and root biomass and N and P uptake in a hydroponic experiment. The results indicated that the Fp parent and the monosomic introgressions relating to Fp chromosomes 3 and 4 showed enhanced shoot and root biomass. However, there was no clear relationship between these phenotypes of the Fp chromosome 3 and 4 monosomic introgressions and that of their derived, respective, progenies. Thus, the interaction between the Lp and Fp genomes in determining phenotype is likely to be complex.



## **Use of GBS for lucerne variety distinction**

**Julier B.<sup>1</sup>, Barre P.<sup>1</sup>, Lambroni P.<sup>1</sup>, Delaunay S.<sup>1</sup>, Lafaillette F.<sup>2</sup>,  
Thomasset M.<sup>3</sup>, Gensollen V.<sup>4</sup>**

<sup>1</sup> P3F, INRA, 86600 Lusignan, France

<sup>2</sup> GEVES, Domaine de l'Anjouère, 49370 Erdre-en-Anjou, France

<sup>3</sup> BioGEVES, Domaine du Magneraud, 17700 Surgères, France

<sup>4</sup> GEVES, 711 rue JF Breton, 34090 Montpellier, France

E-mail: bernadette.julier@inra.fr

### **Abstract**

Distinctness among lucerne varieties, evaluated in DUS tests, is a frequent obstacle to variety registration. Promising varieties may fail the distinction test despite an agronomic plus-value so the genetic progress is not delivered to farmers. The Genotyping by Sequencing (GBS) technology that enables to obtain a large number of markers on pools of individuals was used to evaluate the distinction among 20 lucerne varieties of the European Catalogue. A total of 40 000 polymorphic markers was obtained. All the varieties were statistically different from the others, and the structure was consistent with knowledge on the varieties. Perspectives to use GBS in DUS testing are given.

## **Genome-wide and functional gene analysis of perennial ryegrass for improved growth under water limiting conditions (GrowGene)**

**Statkevičiūtė G., Aleliūnas A., Jonavičienė K.**

Institute of Agriculture, Lithuanian Research Centre for Agriculture and Forestry,  
Instituto al. 1, Akademija, Kėdainiai distr., Lithuania  
E-mail: kristinaja@lzi.lt

### **Abstract**

Grasslands play a major role in milk and meat production, therefore biomass yield of fodder grasses is the primary breeding target. Perennial ryegrass (*Lolium perenne* L.) exhibits poor performance under unfavourable environmental conditions such as drought, and it can substantially reduce perennial ryegrass biomass production. GrowGene project was started in 2017 to deepen the understanding of the basic mechanisms leading to drought tolerance and to improve this trait by breeding. Perennial ryegrass panel, characterised for growth parameters under water-limiting conditions using a high-throughput phenotyping platform, will be genotyped by sequencing, enabling the build-up of high-density genome profiles that can be used for genome-wide association studies. Candidate genes controlling growth traits under drought will be further evaluated for their functional role in the tolerance to changing environments process using TILLING.

### **Acknowledgements**

The research project was funded by the Research Council of Lithuania, grant No. S-MIP-17-24 (GrowGene)



## Genetics and genomics to advance forage grass breeding

Yates S., Kölliker R., Studer B.

Molecular Plant Breeding, Institute of Agricultural Sciences, ETH Zurich,  
Universitaetstrasse 2, 8092 Zurich, Switzerland  
E-mail: steven.yates@usys.ethz.ch

### Abstract

State-of-the-art genetic, genomic and bioinformatic tools have greatly advanced breeding of many major cereal crops such as maize (*Zea mays* L.), rice (*Oryza sativa* L.), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.). In contrast to these autogamous species, many of the most important forage grasses are allogamous and characterized by a highly effective self-incompatibility system, promoting crosspollination. Thereby, high levels of genetic diversity are maintained within populations, and genotypes within such populations are highly heterozygous. In order to fully capture the allelic diversity of such highly heterozygous forage grass genomes, new tools and methods are required to make use of the rich extant diversity.

Here, we first present the latest achievement towards end-to-end chromosome sequencing of a diploid heterozygous Italian ryegrass (*Lolium multiflorum* Lam.) genome. We describe the development and the results of a phased genome assembly, its annotation and outline the use of this novel resource to unlock allelic diversity for breeding. Second, a novel PCR-based method for direct haplotype determination in multiplexed samples is presented. This method takes advantage of the long read length of PacBio sequencing and is particularly useful for mutation detection in outbreeding TILLING populations. Finally, we describe a novel bioinformatics suite for DNA sequence data analysis, comprising, for example, a new tool for SNP phasing using genotyping by sequencing data.

Together, these tools provide a means to efficiently capture allelic diversity. They will enable higher precision association analysis, support map-based gene cloning strategies, improve diversity analyses and ultimately, will lead to increased crop productivity. These developments are synergistic with many other ongoing research projects that will bridge the gap between orphan and model crop species for research and breeding.

## Genomic selection for biomass yield of perennial and annual legumes

**Annicchiarico P., Nazzicari N., Pecetti L., Romani M.**

Research Centre for Fodder Crops and Dairy Productions (CREA-FLC),  
Lodi, Italy  
E-mail: paolo.annicchiarico@crea.gov.it

### Abstract

This study aimed to assess the predictive ability of genomic selection (GS) for biomass yield of alfalfa and pea, considering different data sets, GS models, and thresholds for genotype missing data. An additional aim was to briefly devise the incorporation of GS into breeding schemes of these crops. For alfalfa, the predictive ability of best GS models ranged from  $r = 0.18$  to  $r = 0.36$  in three data sets. The lowest value (observed in a data set with higher experimental error or lower genetic variation relative to the other data sets) may still be of practical interest, given the long selection cycle and the low narrow-sense heritability of biomass yield in this crop. For pea biomass yield, the predictive ability of best GS models averaged  $r = 0.45$  across three recombinant inbred line (RIL) populations. Predictions were less accurate for this trait than for pea straw or grain yield. GS is a promising approach but its adoption implies important modifications of alfalfa and pea breeding schemes. We identified five stages of GS-based selection schemes, whose implementation depends largely on the reproductive system of the target species.







## Breeding Grasses and Protein Crops in the Era of Genomics

## **The Palace of the Grand Dukes of Lithuania**

The Palace of the Grand Dukes of Lithuania is one of the most important projects of Lithuania's Millennium programme. It became a center for civic education, historical consciousness-raising and appreciation of material culture, for state ceremonial events and tourist information. The Palace is important for national selfconsciousness and historical memory, for the restitution of historical truths regarding Lithuania's independence and Vilnius as its historical capital, for the regeneration of the city's historic core, for illustrating Lithuania's historical and cultural roots in the Grand Duchy of Lithuania – a multinational state of Lithuanians, Poles, Belarusians, Ukrainians, Jews, Tatars, Karaims and other nationalities – and for showing the importance of Lithuania's European ties, historic and current.

For more information please visit <http://www.valdovurumai.lt/en>



## AB “Lifosa”

The history of Lifosa started in 1959 when the decision was taken to build the chemical plant in Kėdainiai, the geographical center of Lithuania. The first tons of sulphuric acid were produced in 1963. This date is officially considered the birthday of the plant. Throughout more than 50 years Lifosa went through many substantial changes. Initially build as state-owned company, it was privatized in 1996. By the y. 2000 Lifosa became the largest sulphuric acid producer in Europe. Since 2002, Lifosa has been incorporated into the Russian company EuroChem, which holds the main portfolio of the shares and provides the company with the basic raw materials. The main product manufactured at Lifosa is the nitrogen-phosphorus fertilizer diammonium phosphate (DAP). The company also produces aluminium fluoride, phosphoric acid, technical grade sulphuric acid and feed phosphates. The major part of the company’s production is exported. During the two decades of the Lithuanian independence, AB Lifosa was awarded many times for the achievements in different fields. The company has received the awards of “Successfully Working Company”, “Lithuanian Exporter of the Year”, “Environmental Achievements”, “National Responsible Business Award”, “Product of the Year”, “Investor of the Year”.

For more information please visit <https://www.lifosa.com/en>



## **Dotnuva Baltic**

The company was established in 1996 in Dotnuva, Lithuania. Subsidiary companies were registered in Latvia and Estonia in 2010. At the moment, Dotnuva Baltic is one of the leading companies in agribusiness sector in the Baltics. The company is engaged in service and sales of agricultural machinery, production, processing and sales of seeds, design and construction of grain cleaning, drying and storage facilities and farm equipment. Dotnuva Baltic represents such worldwide known companies as Case IH, Kverneland, Cimbria, DLF, Saaten Union, Lantmännen SW Seed, Syngenta, Ragt, Rapool, Maribo Seed, Boreal and etc. The company owns one of the biggest seed processing plant in the Baltics with the capacity of 22.000 tons of certified seed per year. Forage grass and cereal varieties bred in the LAMMC Institute of Agriculture make 80 % of sales portfolio of forage grass seeds and 20 % of cereals seeds. Sales are carried out not only in Lithuania but also in Latvia, Estonia, Belorussia. Perennial ryegrass variety “Elena DS” is registered in USA and successfully distributed there by the partners.

For more information please visit <https://www.dotnuvabaltic.eu>



## Feliksas Vaitelis Family Farm

Feliksas Vaitelis family organic meat cattle farm, occupying approx. 300 ha, is located in Pernarava, Kėdainiai distr. It is one of the best examples of successful transition from collective agriculture system, forced on Lithuanian landowners under Soviet rule, to modern private farming. About half of farms' income comes from grain cereal and another half comes from beef. Mixture of red clover with *Festulolium* is grown to produce feed for the herd of 200 Limousin cattle. Most of the farms production, cereal and meat alike, is exported. The two sons of F. Vaitelis followed their fathers' footsteps; their own organic farms with similar farming system are located nearby.



## **Lithuanian Research Centre for Agriculture and Forestry (LAMMC), Institute of Agriculture**

Institute of Agriculture is an integral part of the Lithuanian Research Centre for Agriculture and Forestry and carries out fundamental and applied research in agricultural and related sciences with a specific focus on soil quality management, plant nutrition, plant pathology and plant breeding. Institute has a total staff of 190 employees of which 67 are researchers. Twenty PhD students currently carry their research projects at the Institute. Institute's plant breeding activities are distributed among three departments, namely Grass Breeding Department with breeding programs in grasses and legumes, Cereal Breeding Department where breeding programs of cereals and dry pulses are carried out and Laboratory of Genetics and Physiology which provides genotyping and biotechnological support for both breeding departments. Since the beginning of the plant breeding in Lithuania in 1922, breeders of the Institute have developed more than 300 varieties of various crops. After the restoration of Lithuania's independence in 1990, 34 cereal, 14 dry pulses and 59 grass and legume cultivars were developed, which are all listed on the National Plant Variety List and the EU Common Catalogue of Varieties of Agricultural Plant Species. Most of them are successfully grown not only in Lithuania, but also in neighbouring countries as well as in the US.

For more information please visit <https://www.lammc.lt/en>



**PARTICIPANTS LIST**

<b>Last name</b>	<b>First name</b>	<b>Country</b>	<b>E-mail</b>
1	2	3	4
Aavola	Rene	Estonia	rene.aavola@etki.ee
Akinroluyo	Olakunle Kelvin	Lithuania	akinkunle98@yahoo.com
Aleliūnas	Andrius	Lithuania	andrius.aleliunas@lzi.lt
Amdahl	Helga	Finland	helga.amdahl@graminor.no
Annicchiarico	Paolo	Italy	paolo.annicchiarico@crea.gov.it
Aper	Jonas	Belgium	jonas.aper@ilvo.vlaanderen.be
Armstead	Ian	United Kingdom	ipa@aber.ac.uk
Arts	Piet	Netherlands	parts@barenbrug.nl
Asp	Torben	Denmark	torben.asp@mbg.au.dk
Baert	Joost	Belgium	joost.baert@ilvo.vlaanderen.be
Barzyk	Pawel	Poland	pawel.barzyk@phr.pl
Bender	Ants	Estonia	ants.bender@etki.ee
Bertrand	Annick	Canada	Annick.bertrand@agr.gc.ca
Bjarup Hansen	Pernille	Denmark	pernille.bjarup@mbg.au.dk
Boerboom	Nic	Netherlands	nic.boerboom@dsv-zaden.nl
Böhm	Christof	Germany	christof.boehm@saatzucht-steinach.de
Boller	Beat	Switzerland	beat.boller@agroscope.admin.ch
Brazauskas	Gintaras	Lithuania	gintaras.brazauskas@lzi.lt
Butkutė	Bronislava	Lithuania	brone@lzi.lt
Cernoch	Vladimir	Czech Republic	vc@dlf.cz
Charrier	Stephane	France	stephane.charrier@barenbrug-recherches.fr
Claessens	Annie	Canada	Annie.Claessens@agr.gc.ca
Cnops	Gerda	Belgium	gerda.cnops@ilvo.vlaanderen.be
Cotton	Jamie	United Kingdom	jamiecotton@hotmail.co.uk
Cougnon	Mathias	Belgium	mathias.cougnon@ugent.be
De Ron	Antonio M.	Spain	amderon@mbg.csic.es
Ergon	Aashild	Norway	ashild.ergon@nmbu.no
Fe	Dario	Denmark	dfe@dlf.com
Feuerstein	Ulf	Germany	ulf.feuerstein@dsv-saaten.de
Firmat	Cyril	France	cyril.firmat@inra.fr
Flajoulot	Sandrine	France	sflajoulot@jouffray-drillaud.fr
Galiolla	Meirman	Russia	sakyshyer@mail.ru
Gasior	Dagmara	United Kingdom	dkg@aber.ac.uk
Ghamkhar	Kioumars	New Zealand	kioumars.ghamkhar@agresearch.co.nz
Ghesquiere	An	Belgium	an.ghesquiere@ilvo.vlaanderen.be
Ghesquiere	Marc	France	Marc.Ghesquiere@inra.fr
Greard	Camille	France	camille.greard@inra.fr

1	2	3	4
Grieder	Christoph	Switzerland	christoph.grieder@agroscope.admin.ch
Haase	Florian	Germany	florian.haase@julius-kuehn.de
Haland Gylstrom	Kristin	Norway	kristin.gylstrom@graminor.no
Hartmann	Stephan	Germany	Stephan.Hartmann@LfL.Bayern.de
Humphreys	Mike	United Kingdom	mkh@aber.ac.uk
Yates	Steven	Switzerland	steven.yates@usys.ethz.ch
Jacobs	Jeanne	New Zealand	jeanne.jacobs@agresearch.co.nz
Jansons	Aldis	Latvia	aldisjan@tvnet.lv
Jonavičienė	Kristina	Lithuania	kristinaja@lzi.lt
Jones	Charlotte	United Kingdom	cgj@aber.ac.uk
Julier	Bernadette	France	bernadette.julier@inra.fr
Kemešytė	Vilma	Lithuania	vilma@lzi.lt
Knotova	Daniela	Czech Republic	knotova@vupt.cz
Kölliker	Roland	Switzerland	roland.koelliker@usys.ethz.ch
Kopecký	David	Czech Republic	kopeccky@ueb.cas.cz
Kovi	Mallikarjuna Rao	Norway	mallikarjuna.rao.kovi@nmbu.no
Leyva	Maria de la O	Denmark	mleyva@mbg.au.dk
Lemežienė	Nijolė	Lithuania	nijole@lzi.lt
Lenk	Ingo	Denmark	il@dlf.com
Liatukienė	Aurelija	Lithuania	aurelija@lzi.lt
Lu	Qiongxian	Denmark	qiongxian.lu@mbg.au.dk
Luesink	Wilbert	Germany	w.luesink@npz.de
Malenica	Milka	Germany	milka.malenica@saatzucht-steinach.de
Maršalkienė	Nijolė	Lithuania	nijolemars@gmail.com
Marum	Petter	Norway	petter.marum@graminor.no
Masajada	Katarzyna	Poland	kmas@igr.poznan.pl
Mikulski	Wojciech	Poland	minari@onet.eu
Milcendeau	Jules	France	stephane.charrier@barenbrug-recherches.fr
Moen	Maria	Norway	maria.moen@nmbu.no
Muir	James P.	USA	Jim.Muir@ag.tamu.edu
Nedělník	Jan	Czech Republic	nedelnik@vupt.cz
Norkevičienė	Eglė	Lithuania	egle.norkeviciene@lzi.lt
Oehlund	Linda	Sweden	linda.oehlund@lantmannen.com
Ogrodowicz	Pior	Poland	pogr@igr.poznan.pl
Osiecka	Agnieszka	Poland	a.osiecka@coboru.pl
Østrem	Liv	Norway	liv.ostrem@nibio.no
Pecetti	Luciano	Italy	luciano.pecetti@crea.gov.it
Palme	Anna	Sweden	anna.palme@nordgen.org
Pašakinskienė	Izolda	Lithuania	izolda.pasakinskiene@gf.vu.lt



## Breeding Grasses and Protein Crops in the Era of Genomics

1	2	3	4
Pedersen	Morten Greve	Denmark	mg@dlf.dk
Perić	Vesna	Serbia	vesnaperic@yahoo.com
Petrauskas	Giedrius	Lithuania	giedrius.petrauskas@inbox.lt
Rancāne	Sarmite	Latvia	sarmite.rancane@inbox.lv
Reheul	Dirk	Belgium	dirk.reheul@ugent.be
Rybiński	Wojciech	Poland	wryb@igr.poznan.pt
Rocher	Solen	Canada	solen.rocher@agr.gc.ca
Rognli	Odd Arne	Norway	odd-arne.rognli@nmbu.no
Roschanski	Anna Maria	Germany	roschanski@ipk-gatersleben.de
Roulund	Niels	Denmark	NR@dlf.dk
Rubiales	Diego	Spain	diego.rubiales@ias.csic.es
Ruge-Wehling	Brigitte	Germany	brigitte.ruge-wehling@julius-kuehn.de
Ruņģis	Dainis	Latvia	dainis.rungis@silava.lv
Sakysh	Yerzhanova	Russia	sakyshyer@mail.ru
Schubiger	Franz Xaver	Switzerland	franz.schubiger@agroscope.admin.ch
Schulze	Sabine	Germany	sabine.schulze@saatzucht-steinach.de
Seyed ali zahir	Moulana	Sri Lanka	apsccchairman@gmail.com
Skøt	Leif	United Kingdom	lfs@aber.ac.uk
Sokolović	Dejan	Serbia	dejan.sokolovic@ikbks.com
Statkevičiūtė	Grazina	Lithuania	grazinastat@lzi.lt
Stewart	Alan	New Zealand	astewart@pggwrightsonseeds.co.nz
Stoddard	Fred	Finland	frederick.stoddard@helsinki.fi
Stuczynska	Elzbieta	Poland	e.stuczynska@coboru.pl
Studer	Bruno	Switzerland	bruno.studer@usys.ethz.ch
Tamm	Sirje	Estonia	sirje.tamm@etki.ee
Tanaka	Tsuneki	Japan	tanaka-tsuneki@hro.or.jp
Trněný	Oldrich	Czech Republic	trneny@vupt.cz
Tuna	Metin	Turkey	metintuna66@yahoo.com
van der Weijde	Tim	Netherlands	tvdweijde@barenbrug.nl
Vaz Patto	Maria Carlota	Portugal	cpatto@itqb.unl.pt
Vezis	Ivo	Latvia	vezis92@gmail.com
Vymyslický	Tomas	Czech Republic	vymyslicky@vupt.cz
Vleugels	Tim	Belgium	tim.vleugels@ilvo.vlaanderen.be
Wanjiru	Stephen	United Kingdom	ahk@aber.ac.uk
Westermeier	Peter	Germany	Peter.Westermeier@lfl.bayern.de
Wident	Mathieu	Netherlands	mathieu.wident@dlf.com
Willner	Evelin	Germany	willner@ipk-gatersleben.de
Winzeler	Michael	Switzerland	michael.winzeler@agroscope.admin.ch
Zharikova	Daria	Ukraine	daria.bigen14@gmail.com
Zwierzykowski	Zbigniew	Poland	zzwi@igr.poznan.pl