



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

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

Brno

10–14 September, 2023



Palacký
University
Press

Edited by David Kopecký, Ivana Frei, Tomáš Vymyslický

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

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

Brno

10–14 September, 2023

Edited by

David Kopecký

Ivana Frei

Tomáš Vymyslický



Palacký
University
Press

Organising Institutions

- Agricultural Research Ltd. Troubsko, Czech Republic
- Institute of Experimental Botany ASCR, v.v.i., Olomouc, Czech Republic
- EUCARPIA Fodder Crops and Amenity Grasses Section
- EUCARPIA *Festulolium* Working Group



ZVT



Organising Committee

Chair: Tomáš Vymyslický

Members: Bohumír Cagaš

Ivana Frei

Josef Hakl

Stanislav Hejduk

Helena HutYROVÁ

Libor Jalůvka

David Kopecký

Joanna Majka

Radek Macháč

Jan Nedělník

Simona Raab

Oldřich Trněný

Scientific Committee

Chair: David Kopecký (CZ)

Members: Torben Asp (DK)

Stephen Byrne (IR)

Bohumír Cagaš (CZ)

Josef Hakl (CZ)

Stanislav Hejduk (CZ)

Kristina Jaškune (LV)

Bernadette Julier (FR)

David Lloyd (UK)

Hilde Muyle (BE)

Jan Nedělník (CZ)

Luciano Pecetti (IT)

Jasmina Radovic (RS)

Sabine Schulze (DE)

Tomáš Vymyslický (CZ)

Table of contents

Opening address by Jan Nedělník, Agricultural Research, Ltd., Troubsko	1
Preface	3
Miroslav Trnka, Jan Balek, Mikhail A. Semenov, Daniela Semerádová, Jørgen E. Olesen, Josef Eitzinger, Andreas Schaumberger, Pavel Zahradníček, David Kopecký Future agroclimatic conditions and implications for European grasslands.	5
Bohumír Cagaš Seed production of grasses and legumes in the Czech Republic and its impact on the agro and environmental sphere.	6
Session I: Genetic resources and natural diversity	
Phillip G.H. Nichols Use of genetic resources and natural diversity in forage breeding: a case study of annual legumes in Australia	8
Michelle M. Nay, Christoph Grieder, Lea A. Frey, Helga Amdahl, Jasmina Radovic, Libor Jaluvka, Anna Palmé, Leif Skøt, Tom Ruttink, Roland Kölliker Home sweet home – the genetic and phenotypic adaptation of red clover to European growing environments	11
Marie Pégard, Matthew C Fitzpatrick, Jean-Paul Sampaou Assessment of vulnerability to climate change in natural populations of perennial ryegrass across Europe from allele frequencies at adaptive loci.	14
Odd Arne Rognli, Trygve Sveen Aamlid, Muath Alsheik, Helga Amdahl, Sigridur Dalmannsdottir, Kristoffer Herland Hellton, Marit Jørgensen, Mallikarjuna Rao Kovi, Therese Mæland, Akhil Reddy Pashapu, Ilevina Sturite, Thordis Linda Thorarinsdottir, Susanne Windju Securing adaptation of timothy cultivars under climate change and during seed multiplication	15
Beat Boller, David Kopecký Triploid hybrids <i>Festuca apennina</i> × <i>Lolium</i> occur rarely in nature but show high colonizing potential without dominating the sward excessively	17

Bill Biligetu, Hu Wang Genetic variation and genome-environment association of alfalfa (<i>Medicago sativa</i> L.) populations originating from long-term grazing sites.	20
Philip Greenspoon, Lorena Batista, Jon Bančič, Linda Öhlund, Alf Ceplitis, Gregor Gorjanc Genetic gain from simulated forage breeding programs with genomic selection.	21
Mareike Kavka, Michael Melzer, Klaus J. Dehmer, Evelin Willner Shattering resistance in genetic resources of the fodder grasses <i>Lolium perenne</i> L. and <i>Festuca pratensis</i> Huds.	22
Monica Alexandrina Tod, Mironela Bălan Allelopathic effect of herbage water extracts on seed germination and seedling development of some perennial grassland species	24
Ana Uhlarik, Marina Čeran, Đorđe Krstić, Zlatica Mamlić, Snežana Katanski, Sanja Vasiljević, Anja Dolapčev Rakić Agronomical traits, seed color and protein content of protein pea (<i>Pisum sativum</i> L.) cultivars grown in European conditions	25
Sanja Vasiljević, Mira Mikulić, Jelena Cvejić, Zorica Nikolić, Snežana Katanski, Zlatica Mamlić, Ana Uhlarik Isoflavones profiles of some diploid and tetraploid red clover cultivars (<i>Trifolium pratense</i> L.) at flowering stage.	27
Tomáš Vymyslický, Simona Raab, Ivana Frej, Helena Hutytová Grass and forage legume genetic resources in the Czech Republic and their practical utilization	28
Yutang Chen, Roland Kölliker, Dario Copetti, Bruno Studer Assembling forage grass genomes in the long-read sequencing era: a case study with <i>Lolium perenne</i> L. and <i>L. multiflorum</i> Lam.	31
Mirjana Petrović, Vladimir Zornić, Zoran Lugić, Marija Stepić, Mladen Prijović, Tomaš Vymyslicky, Boban Andjelković An initial metabolomic study on Hungarian clover	33
Bertrand Annick, Claessens Annie, Baron Vern, Thériault Mireille, Rocher Solen, Asselin Sean Evaluation of alfalfa populations selected for reduced fall dormancy in a frequent cutting trial	35

Josef Hakl, Aldo Tava, Zdeňka Kozová Variability of sapogenins among <i>Medicago falcata</i> entries	37
---	----

Mallikarjuna Rao Kovi, Odd Arne Rognli Genome assembly and annotation of timothy (<i>Phleum pratense</i> L.)	40
--	----

Snežana Babić, Jasmina Radović, Snežana Andjelković, Mirjana Petrović, Goran Jevtić, Mladen Prijović, Dejan Sokolović Heritability and variability of the most important traits of meadow fescue synthetic cultivars	41
---	----

Session II:

Advanced phenotyping and genotyping technologies

Bernadette Julier, Philippe Barre, Marie Pégard New phenotyping, genotyping and statistical tools for forage breeding	44
--	----

Jenny Kiesbauer, Christoph Grieder, Meril Sindelar, Linda Helene Schlatter, Maria Hug, Daniel Ariza Suarez, Dario Copetti, Bruno Studer, Roland Kölliker A nested association mapping population reveals candidate genes for stem rust resistance in Italian ryegrass	47
--	----

Oldřich Trněný, Jana Staveníková, Magdalena Dybová, Hana Jakešová, Tereza Vojtková, Kateřina Holušová, Jan Šafář, Jan Nedělník Red clover's genetic playground: Leveraging low-coverage resequencing for enhanced breeding programs	49
--	----

Stephen Byrne, Krishna Aroju Sai, Patrick Conaghan, Agnieszka Konkolewska, Dan Milbourne Implementation of multiple cycles of genome-wide selection for seasonal forage yield in perennial ryegrass	51
--	----

Peter Lootens, Joanna Pranga, Waldo Deroo, Tom De Swaef, Guillaume Blanchy, Tommy D'Hose, Bart Vleminckx, Sarah Garré, Isabel Roldán-Ruiz Phenotyping our future crops: in-field, non-destructive, high-throughput phenotyping of above and belowground plant traits	52
---	----

Agnieszka Konkolewska, Steffie Phang, Patrick Conaghan, Dan Milbourne, Susanne Barth, Rachel Keirse, Aonghus Lawlor, Stephen Byrne Use of NIR spectra to predict heading date in perennial ryegrass	55
---	----

Nagarjun Devabhakthini, Doerte Harpke, Mareike Kavka, Evelin Willner, Klaus J. Dehmer Exploring the genetic diversity of the IPK <i>Medicago</i> germplasm collection using GBS.	57
Luciano Pecetti, Nelson Nazzicari, Nicolò Franguelli, E. Charles Brummer, Paolo Annicchiarico Development and proof-of-concept application of genome-enabled selection for alfalfa biomass yield in Northern Italy: preliminary results	58
Girma Bedada, Yousef Rahimi, Silvana Moreno, Linda Öhlund, Alf Ceplitis, Anne-Maj Gustafsson, Anna Westerbergh, Pär K Ingvarsson Haplotype-phased genomes of timothy grasses – <i>P. nodosum</i> , <i>P. alpinum</i> <i>P. pratense</i>	60
Harkingto Harkingto, Sahameh Shafiee, Stefano Zanotto, Helga Amdahl, Luciano Pecetti, Nelson Nazzicari, Paolo Annicchiarico, Åshild Ergon Conventional and UAV-based phenotyping to characterize a broad collection of European lucerne germplasm in a Nordic environment	62
Joanna Pranga, Irene Borra-Serrano, Tom De Swaef, Mathias Cougnon, An Ghesquiere, Jonas Aper, Ivan Janssens, Greet Ruyschaert, Isabel Roldán-Ruiz, Peter Lootens Unleashing the potential of drones: how high-throughput field phenotyping can assist forage grass breeding	64
Kioumars Ghamkhar, Dongwen Lu, Kenji Irie, Michael Hagedorn, Mostafa Sharifi, Steve Gebbie, Angus Heslop, Anthony Hilditch, Will Clayton, Brian Maw, Brent Barrett Rapid forage yield and growth rate measurement using a remote-controlled LIDAR sensor in perennial ryegrass field plots	67

Youth Session

Chloé Manzanares, Marius Rohner, Steven Yates, Maurice Bosch, Daniela Kupper, Daniel Thorogood, Bruno Studer Exploiting new discoveries on self-incompatibility for forage grass breeding ...	70
Agnieszka Konkolewska, Steffie Phang, Michael Dineen, Patrick Conaghan, Dan Milbourne, Susanne Barth, Rachel Keirse, Aonghus Lawlor, Stephen Byrne Establishing multi-trait genomic selection for forage improvement.	72

Ágnes Áldott-Sipos, Eszter Csepregi-Heilmann, Tamás Spitkó, János Pintér, Csaba Szőke, Tamás Berzy, Anett Kovács, János Nagy, Csaba L. Marton Evaluation of silage and grain yield of different maize (<i>Zea mays</i> L.) genotypes in ecological and conventional conditions	74
Marlies K.R. Peeters, Isabelle Maryns, Leen Leus, Joost Baert, An Ghesquiere, Mathias Cougnon, Aurélie Tredé, Sabine van Glabeke, Tom Eeckhout, Katrijn Van Laere, Yves Van de Peer, Tom Ruttink Optimized tetraploidization strategies in tissue culture for <i>Lolium</i> and <i>Festuca</i>	76
Ferez Sustek-Sánchez, Anete Boroduške, Madara Balode-Sausiņa, Erki Eelmets, Sanda Astra Bērziņa, Olav Kasterpalu, Merike Sõmera, Nils Rostoks, Cecilia Sarmiento Optimized <i>Lolium perenne</i> L. protoplasts isolation and transformation for CRISPR-Cas9 downstream applications.	78
Session III:	
Qualitative and quantitative traits	
E. Charles Brummer Thirty years of alfalfa genetic markers.	82
Hilde Muylle, Tim Vleugels, Reena Dubey, Aamir Saleem, Isabel Roldán-Ruiz GWAS for drought tolerance in red clover (<i>Trifolium pratense</i> L.)	84
Heathcliffe Riday, Neal Tilhou, Lisa Kissing Kucek, Shahjahan Ali, Brandon Carr, Annie Young-Mathews, Joel Douglas, Suresh Bhamidimarri, Mark Azevedo, Sarah Krogman, Maria Monteros, Steven B. Mirsky, Ryan Hayes, John Englert Breeding Soft-seeded Hairy Vetch	86
Stanislav Hejduk, Ivana Koláčková, Barbora Smolková Differences between di- and tetraploid red clover cultivars. A review.	88
Jenny Kiesbauer, Maria Hug, Meril Sindelar, Linda Helene Schlatter, Jonathan Ohnmacht, Roland Kölliker, Christoph Grieder Higher seed yield through targeted selection for reduced seed shattering in Italian ryegrass (<i>Lolium multiflorum</i> Lam.)	89
Annie Claessens, Mireille Thériault, Annick Bertrand, Julie Lajeunesse, Solen Rocher Genetic selection for nonfiber carbohydrates in alfalfa (<i>Medicago sativa</i> L.) stem	91

Session IV: Biotic and abiotic stresses

Susanne Barth, Lena Foerster, Linda Moloney-Finn, Carl Ng, Sheila Alves Differential growth of a panel of perennial ryegrass accessions following excess water treatment over the winter under field conditions	94
Tim Vleugels, Aamir Saleem, Reena Dubey, Hilde Muylle, Irene Borra-Serrano, Peter Lootens, Tom De Swaef, Isabel Roldán-Ruiz Phenotypic characterisation of drought tolerance in red clover (<i>Trifolium pratense</i> L.)	95
Eszter Csepregi-Heilmann, Ágnes Áldott-Sipos, Anett Kovács, Tamás Spitkó, Csaba Szőke, János Pintér, Tamás Berzy, Adrienn Széles, Csaba L. Marton Cold stress study during emergence of maize (<i>Zea mays</i> L.) inbred lines	98
Mladen Prijović, Dejan Sokolović, Snežana Babić, Mirjana Petrović, Marija Stepić, Đorđe Lazarević, Aneta Sabovljević Morpho-biochemical response perennial ryegrass (<i>Lolium perenne</i> L.) populations to water shortage	99
Gražina Statkevičiūtė, Kristina Jaškūnė, Cecilia Sarmiento, Odd Arne Rognli, Nils Rostoks Fv:Fm and RWC measurements as indicators of drought stress response in perennial ryegrass	101
Miguel Loera-Sánchez, Damian Käch, Bruno Studer, Roland Kölliker Towards an efficient detection of genetic diversity in multispecies grassland ..	103
Akhil Reddy Pashapu, Sigridur Dalmannsdottir, Marit Jørgensen, Marian Schubert, Odd Arne Rognli, Mallikarjuna Rao Kovi Surviving under ice: Insights into gene expression changes during ice-encasement in perennial grasses	104
Filip Bekčić, Marija Stepić, Snežana Anđelković, Jordan Marković, Nina Vučković, Ivana Vico, Nataša Duduk Wilt of red clover caused by <i>Fusarium oxysporum</i>	105
Bernadette Julier, Zineb El Ghazzal, Sabrina Delaunay, Béatrice Wolff, Gaëtan Louarn Early morphological traits condition the performance of lucerne plants in different competitive situations	107

- Charlotte Jones, Matthew Lowe, Rhys Kelly, Denholm Bramble, Leif Skøt, David Lloyd**
 Recurrent molecular selection for improved field resistance to crown rot (*Sclerotinia trifoliorum*) in red clover (*Trifolium pratense*) 109
- Matthew Carl Graham, Lesley Suzanne Johnston, Alan Gordon, Gillian Kathleen Young**
 Evaluating root characteristics under field conditions in perennial ryegrass for potential application in commercial breeding programmes 110
- Shridhar Jambagi, Linda Öhlund, Christina Dixelius**
 Red clover root microbiota and interaction of root rot and clover rot pathogens 113
- Reah Gonzales, Steven Yates, Stéphane Charrier, Bruno Studer**
 Divergent responses of perennial ryegrass and tall fescue to drought stress. . . 114
- Silvana Moreno-Vallejo, Girma Bedada, Yousef Rahimi, Pär Ingvarsson, Anna Westerbergh**
 Response to waterlogging and drought in wild and domesticated accessions of timothy (*Phleum pratense*) and its wild relatives *P. alpinum* and *P. nodosum* . . 115

Festulolium **Working Group Workshop**

- Vladimír Černoč**
 History of *Festulolium* breeding in Czech Republic and future prospects 118
- Tom Ruttink, Marlies Peeters, Isabelle Maryns, Leen Leus, Joost Baert, An Ghesquiere, Mathias Cougnon, Aurélie Tredé, Sabine van Glabeke, Tom Eeckhaut, Katrijn Van Laere, Yves Van de Peer**
 Genome dominance in interspecific and intergeneric hybrids of *Lolium*, *Festuca* and *Festulolium*. 121
- Joanna Majka, Marek Glombik, Alžběta Doležalová, Jana Kneřová, Marco Tulio Mendes Ferreira, Zbigniew Zwierzykowski, Martin Duchoslav, Bruno Studer, Jaroslav Doležel, Jan Bartoš, David Kopecký**
 Deciphering genome dominance in *Festulolium* 124
- Philippe Barre, Sebastian Blugeon, Sabrina Delaunay, Lydia Jaffrelo, Brenadette Julier, Marie Pegard, Charles Poncet, Marc Ghesquière**
 New insights of interspecific genetic variability within and between 4x *Festulolium* hybrid cvs by using KasPar markers. 126

Vilma Kemešytė, Gražina Statkevičiūtė, Kristina Jaškūnė Development and characterization of productive and resilient <i>Festulolium</i> hybrids for future climate	129
Dawid Perlikowski, Adrianna Czapiewska, Izabela Pawłowicz, Włodzimierz Zwierzykowski, Eugeniusz Paszkowski, Katarzyna Szwarz, Katarzyna Kłodawska-Pęcińska, Dariusz Rydzyński, Łukasz Wańkiewicz, Marcin Rapacz, Arkadiusz Kosmala Physiological and molecular indicators of winter-hardiness and frost tolerance in <i>Lolium perenne</i> × <i>Festuca pratensis</i> hybrids	131
List of participants	133

Programme

EUCARPIA Brno 2023 conference – Preliminary programme “Novel technologies, strategies and crops to sustain forage production in future climate”	140
---	-----

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

Sanja Vasiljević¹, Mira Mikulić², Jelena Cvejić², Zorica Nikolić¹, Snežana Katanski¹, Zlatica Mamlić¹, Ana Uhlarik¹

¹Institute of Field and Vegetable Crops, Novi Sad, National Institute of the Republic of Serbia

²University of Novi Sad, Medical Faculty, Serbia

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

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

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

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

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

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

David Kopecký, Ivana Frei, Tomáš Vymyslický (eds.)

Managing editor Otakar Loutocký
Graphic design, DTP and pre-press Petr Jančík

Published and printed by Palacký University Olomouc
Křížkovského 8, 779 00, Olomouc
First edition
Olomouc 2023

DOI: 10.5507/vup.23.24463414
ISBN 978-80-244-6341-4 (print)
ISBN 978-80-244-6342-1 (online: iPDF)
VUP 2023/0278, 0279
Publication not for sale



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

