Gintaras Brazauskas
Gražina Statkevičiūtė
Kristina Jonavičienė *Editors*

Breeding Grasses and Protein Crops in the Era of Genomics

EUCARPIA

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Preface

The 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 took place in Vilnius, Lithuania, on September 11–14, 2017. A total of 142 participants from 24 countries attended the meeting hosted by the Lithuanian Research Centre for Agriculture and Forestry (LAMMC).

The meeting was subdivided into four sessions according to the thematic relevance to promote the discussion between both sections of Eucarpia. The first day had started with an opening session and the first scientific session Utilization of Genetic Resources and Pre-breeding, co-chaired by Roland Kölliker and Ulf Feuerstein. The key-note presentation was given by Odd Arne Rognli where he discussed utilization of genebank accessions to improve adaptation of perennial ryegrass to northern environmental conditions. The key-note presentation was then followed by seven further oral presentations and ten flash (max 5 min) presentations with subsequent "meet-and-greet" discussion during the coffee break. Session 2: Genetic Improvement of Quality and Agronomic Traits (co-chaired by Piet Arts and James P. Muir) opened the second day of the meeting. The key-note presentation was given by Bernadette Julier where she presented how the breeding of forage and grain legumes could contribute to increased protein self-sufficiency in the EU and China. Five further oral presentations were given in this session. Session 3: Breeding for Enhanced Stress Tolerance was co-chaired by Odd Arne Rognli and Diego Rubiales, while Torben Asp presented population genetics and GxE interaction analysis of the Eucarpia multi-site rust evaluation trial as a key-note presenter. Four oral presentations and eleven flash presentations followed by discussion concluded the second day of the meeting.

The third day was devoted to research visits to the Lithuanian Research Centre for Agriculture and Forestry (LAMMC), agri-business company Dotnuva Baltic, fertilizer plant Lifosa, and Feliksas Vaitelis organic beef cattle farm. Attendees of the meeting had possibility to see the breeding nurseries of LAMMC, seed production at Dotnuva Baltic, fertilizer production facilities at Lifosa and discuss farmers' perspective on forage and protein crops cultivated in Lithuania.

The fourth day of the meeting comprised Session 4: Implementation of Phenomic and Genomic Tools in Breeding. Two key-note presentations were given in this session. Kioumars Ghamkhar presented the use of LIDAR for forage yield measurement of perennial ryegrass field plots, while Steven Yates talked about the use of genetics and genomics to advance forage grass breeding. Two oral presentations and ten flash presentations concluded the meeting.

We gratefully acknowledge the efforts of the members of the scientific committee: Mario O. Aguilar, Paolo Annicchiarico, Torben Asp, Joost Baert, Philippe Barre, Susanne Barth, Didier Bazile, Beat Boller, Antonio M De Ron, Jean J. Drevon, Ulf Feuerstein, Deidré Fourie, Áslaug Helgadóttir, Christian Huyghe, Mike Humphreys, Hans J. Jacobsen, Bernadette Julier, Žydrė Kadžiulienė, Danutė Karčauskienė, Roland Kölliker, David Kopecký, Jan Nedělník, Liv Østrem, Roberto Papa, Izolda Pašakinskienė, Dirk Reheul, Isabel Roldán-Ruiz, Diego Rubiales, Tom Ruttink, Kadambot H. M. Siddique, Leif Skøt, Dejan Sokolović, Frederick Stoddard, Bruno Studer, Wojciech Święcicki, Lina Šarūnaitė, Vita Tilvikienė, Hari D. Upadhyaya, Bülent Uzun, Rajeev K. Varshney, Maria Carlota Vaz Patto, and Margarita Vishnyakova for the critical review of the offered papers. Their selfless efforts made it possible to have this meeting with the exciting scientific program and high-quality contributions to the proceedings of the meeting.

We also thank members of the local organizing committee: Agnė Jankauskienė, Andrius Aleliūnas, Ardas Kavaliauskas, Dangira Šidlauskienė, Eglė Norkevičienė, Giedrius Petrauskas, Irena Pabrinkienė, Kęstutis Tamošiūnas, Lina Šarūnaitė, Nijolė Lemežienė, Olakunle Kelvin Akinroluyo, Radvilė Nagrockaitė-Lelešienė, Vilma Kemešytė, Vita Tilvikienė, and Žydrė Kadžiulienė for making this meeting happen in its best form.

Finally, we thank all the participants for their active participation, desire to share their expertise, and discuss cutting-edge achievements of breeding grasses and protein crops in the era of genomics.

Gintaras Brazauskas
Gražina Statkevičiūtė
Kristina Jonavičienė

Contents

Genetic Improvement of Quality and Agronomic Traits

Breeding for Enhanced Stress Tolerance

Utilization of Genetic Resources and Pre-breeding

Utilization of Genebank Accessions to Improve Northern Adaptation of Perennial Ryegrass (*Lolium perenne* L.)

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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.

Keywords: *Lolium perenne* · Pre-breeding · Genebank · Accessions Phenotyping · Genotyping

1 Introduction

Perennial ryegrass (*Lolium perenne* L.) has superior feed quality and productivity, and it is especially well adapted to the coastal climate of Western Europe (Humphreys et al. 2010). In Northern Europe, the expected climate changes will most likely improve the conditions for forage production because the growing season will be longer (1–3 months) with milder and rainier autumns and winters (Olesen et al. 2011). Thus, we expect perennial ryegrass to be grown commercially further north and east. However, the main challenges for perennial ryegrass is its susceptibility to low-temperature pathogens, and inadequate growth cessation in the autumn to allow for sufficient cold hardening and winter survival, with low persistency as the result (Solberg et al. 1994; Østrem et al. 2015). This will be exacerbated by the higher temperatures in the new climate changing the temperature-photoperiod regimes affecting plant phenology, winter survival and seasonal yield distribution. The genetic diversity available in the current Nordic/Baltic perennial ryegrass germplasm is probably restricted since the species is not native to the northern and continental regions (Rognli et al. 2013). Therefore, exotic materials should be identified, introgressed and recombined with existing breeding populations to serve as new genetic resources for development of cultivars for the future climate in these regions. This article describes the structure and activities of the Nordic Public-Private Partnership (PPP) on pre-breeding (Nilsson et al. 2016) of perennial ryegrass. The project aims at improving winter hardiness, persistence and other important traits for northern Europe by: (i) investigating the current adaptation potential of commercial cultivars; (ii) collecting and documenting plant material with large genetic variation from several parts of the world; (iii) creating breeding populations with large variation and populations selected for extremes of different traits; and (iv) developing tools for using genomic based selection in further breeding programmes.

2 Materials and Methods

2.1 Plant Material, Phenotyping and Pre-breeding

Breeding perennial forage grasses is a long-term activity taking 10–15 years until cultivars are released. This pre-breeding project was designed to run from 2012 to 2020, and the project started by obtaining seed samples of 393 accessions from gene banks worldwide (Table 1). These accessions were seed multiplied to obtain enough seed for regular field trials, and at the same time established as replicated single plant experiments for detailed phenotyping in Denmark (DK), Sweden (SE), Norway (NO) and Finland (FI). Based on this phenotyping, 22 contrasting trait-based local populations have been composed, Syn-1 and Syn-2 seeds are produced, and field testing of these will be established in 2019. In addition, the accessions were sown as single rows in DK for proper determination of heading date in dense stand.

A broad-based diploid breeding population was constructed based on 350 populations using 10 plants from each population completely randomized within each of two crossing blocks, one in DK and one in NO. Crossing blocks in different

Table 1. Geographic origin and types of gene bank accessions used in this project.

Country	Wild	Cultivars/breeding lines	Unknown	Total
Austria		1	1	2
Belgium		1		1
Canada			1	1
Czech Rep	9	2	5	16
Denmark		27	1	28
Estonia	2	7		9
Finland		4	1	5
France	33	5		38
Germany	32	7		39
Hungary	12	2	2	16
Ireland		2		2
Italy	6			6
Japan		3		3
Kyrgyzstan[a]		1		1
Latvia	2	15	2	19
Lithuania	16	15		31
Moldova	1			1
Norway	10	13		23
Poland	29	11	2	42
Romania	15	1		16
Russia	2	7	3	12
Switzerland	8	3		11
Slovakia	2	1		3
Slovenia		1		1
Sweden	11	17	2	30
The Netherlands		6		6
Turkey	9			9
UK	5	6		11
Ukraine	4		2	6
USA		2	1	3
Unknown			2	2
Total	208	160	25	393

[a]Later confirmed to be *L. multiflorum* based on genotyping.

environments were used to reduce the effect of selection due to differences in response to photoperiod and other climatic factors. One ear per plant was harvested and used to establish one plant per ear for production of the 2nd generation seed. Half of each crossing block was harvested early, the other late, and balanced bulks constructed.

In 2016, the broad-based breeding population was sown on large plots at 1–2 locations per country (a total number of 13 plots) for natural selection to create locally adapted germplasms. In addition, new tetraploid genotypes were made by chromosome

doubling of 1000 plants of the broad-based population, these are being intercrossed in NO and DK to create a new broad-based tetraploid breeding population using similar procedures as with the diploid population. Detailed phenotyping of 250 tetraploids is performed by the Latvian (LV) partner.

Field trials in five countries (Iceland (IS), SE, NO, Estonia (EE) and FI) for testing winter hardiness and phenotypic stability of 22 perennial ryegrass cultivars was completed (Helgadottir et al. 2018), and regional-specific populations (5 diploid, 5 tetraploid) created based on crossing surviving plants of diploid and tetraploid cultivars at each location (Syn-1 and Syn-2 seed produced by 2019). Forage quality of all cuts of the 2nd harvest year from the field trials in NO, IS, FI and EE has been analysed. In addition the freezing tolerance (LT_{50}) of the cultivars was determined in artificial freezing tests by the Lithuanian partner. In 2014, field experiments with seeds multiplied from the original accessions were established in seven countries (IS, SE, NO, FI, DK, EE and Lithuania (LT)) for testing using regular plot size. A core set of 138 accessions is present at all locations, and yield and other traits have been recorded for 2–3 years (2015–2017). In addition, forage quality have been measured in FI and DK using NIRS. An artificial freezing test of 150 accessions, including the core set, have been performed by the Lithuanian partner (Aleliunas et al. 2017).

Partners in the project and sites for field trials are depicted in Fig. 1.
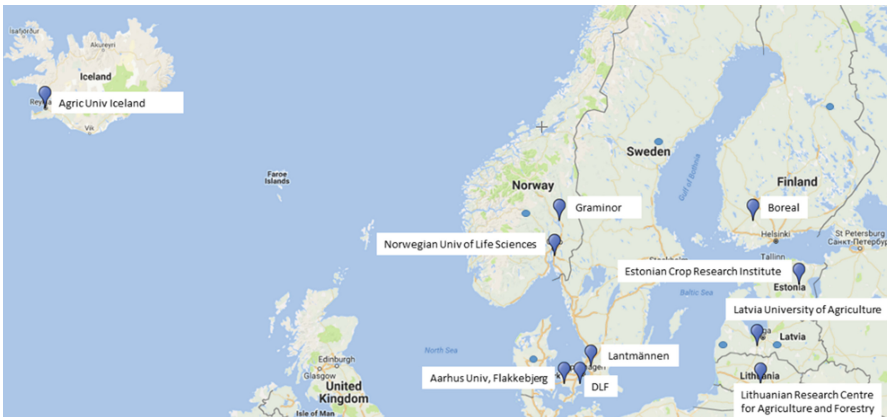
Fig. 1. Nordic and Baltic partners in the PPP perennial ryegrass project indicate field testing sites in addition to the main sites of the partners.

2.2 Genotyping, Ploidy Testing

Pooled leaf samples from plants of the original seed lots of the 380 gene bank accessions (Table 1) were genotyped by sequencing (GBS) to estimate genome wide SNP allele frequencies (see Byrne et al. 2013). Similar genotyping has been or is being performed on the 22 cultivars tested in field trials, the 10 location-specific 2x and 4x populations created from surviving plants of these cultivars (syn-1 and syn-2), and the 22 diverse trait-based local populations created from the single plant phenotyping trials

(syn-1, syn-2 and the parents). The latter genotyping data is being used to study the differential parental contributions when synthetic populations are generated by open pollination. In addition, leaf samples of about 6,700 single plants have been collected from the single plant phenotyping trials (NO, SE and FI) and stored for genotyping in a later phase of the project. These genomic resources are powerful resources for association studies and eventually, development of genomic selection schemes. It is important for characterization and further use of gene bank accessions to have exact knowledge about the ploidy level of each accession. Thus, a full flow-cytometric analysis of all accessions was carried out in 2015.

3 Results and Discussion

The results from the multi-site field trials confirm the need for more robust and stable cultivars with wider adaptations (Helgadottir et al. 2018). Cultivars, locations and years showed large interactions, and local cultivars were generally better adapted than cultivars from Central Europe. Diploid cultivars were consistently more frost tolerant than tetraploid cultivars. The Norwegian cultivar 'Falk', which was found to be a diploid/tetraploid mixture (about 50/50) by flow-cytometry, turned out to be the most stable cultivar across environments. This is interesting and indicate that using ploidy-mixtures could be a strategy to improve persistency of perennial ryegrass grown in these northern regions.

The preliminary results from the multi-site field testing of accessions indicate that none of the accessions is better than the best locally adapted cultivar. Thus, it seems like our initial assumption that introgression and recombination of exotic materials followed by natural/artificial selection is needed to create novel germplasm. Similar results were reported by Ghesquiere and Baert (2013) when they evaluated families from pair-crosses between elite breeding materials and 30 core-collection accessions. Also, most of the accessions seem to be susceptible to rust attack. Freezing tests of accessions confirmed the results from freezing tests of the cultivars, i.e. diploids are consistently more freezing tolerant than tetraploids (Aleliunas et al. 2017).

Flow-cytometric analyses of the ploidy level of the accessions showed that as much as about 16% of the accessions were tetraploids while they were classified as diploids in the genebank.

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Wild and Primitive Common Bean Populations in the Germplasm Collection at the MBG-CSIC

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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.

Keywords: Domestication · *Phaseolus vulgaris* · Phenotypic traits Variability

1 Introduction

The common bean is the most important grain legume for direct human consumption on a global scale. Among the main food crops, the common bean shows the greatest variation in growth habit, seed characteristics (size, shape and colour) and maturation time. This variability enables its production in a wide range of cropping systems and agrosystems in different continents. Andean domesticated common bean germplasm is remarkably diverse in plant and seed morphology and agroecological adaptation, but it has a narrow genetic base according to the molecular analysis (Beebe et al. 2001) as compared to Mesoamerican germplasm.

Northwestern Argentina (NOA) has been considered as an unique segment of the Andean gene pool, isolated from other bean germplasm since and intermediate or weedy types have been described in this area (Beebe et al. 2001). In this area there is still a considerable amount of genetic diversity in many wild populations and also in the primitive cultivated landraces since traditional agriculture in small farms is still practised in isolated valleys that include the use of primitive or unimproved landraces of "porotos" or beans often intercropped with maize (Parodi 1953). The wild forms grow in Argentina through the provinces of Salta, Jujuy and Tucumán, along the valleys of the Eastern Andean Mountain Range.

The aim of this study was to describe some characteristics of the wild and primitive common bean populations and their origin in the Andean region.

2 Material and Methods

Plant material

The common bean collection at the MBG-CSIC includes 2014 accessions from Europe (17 countries), The Americas (15), Asia (4), Africa (1) and Oceania (1). This collection incorporated wild and primitive populations from the NOA in South America that is one of the domestication centers (together with Mesoamerica) of the species (Singh et al. 1991).

3 Results and Discussion

The Table 1 shows the origin of the wild and primitive populations maintained in the germplasm collection at the MBG-CSIC.

Table 1. Origin of the wild and primitive populations maintained in the germplasm collection at the MBG-CSIC.

Code	Code2	Type	Location	Province	Altitude (masl)	Year
PHA-0725	VAV6388	Wild	Chicoana	Salta	1450	1991
PHA-0726	VAV6389	Wild	Chicoana	Salta	1400	1991
PHA-0727	VAV6368	Wild	Trancas	Tucumán	2600	1986
PHA-0728	VAV6395	Wild	Chicoana	Salta	1400	1993
PHA-0729	VAV6382	Wild	Salta	Salta	1800	1991
PHA-0730	VAV6396	Wild	La Caldera	Salta	1300	1993
PHA-0731	VAV6385	Wild	Chicoana	Salta	1450	1991
PHA-0732	VAV6387	Wild	Chicoana	Salta	1400	1991
PHA-0733	VAV6397	Wild	Santa Victoria	Salta	1630	1993
PHA-0733	VAV6406	Wild	Salta	Salta	1690	1993
PHA-0734	MCM168	Wild	San Carlos	Salta	1600	1994
PHA-0736	VAV6398	Wild	Valle Grande	Jujuy	1470	1993
PHA-0737	VAV6362	Wild	Tumbaya (route)	Jujuy	1850	1986
PHA-0738	VAV6363	Wild	Tiraxi (route)	Jujuy	1670	1986
PHA-0739	VAV6364	Wild	Santa Victoria	Salta	2340	1986
PHA-0740	VAV6365	Wild	Santa Victoria	Salta	2600	1986
PHA-0741	VAV6369	Wild	Trancas	Tucumán	1310	1986
PHA-0742	VAV6370	Wild	Trancas	Tucumán	1520	1986
PHA-0743	MCM69	Primitive	Iruya	Salta	2900	1986
PHA-0744	MCM132	Primitive	Iruya	Salta	2900	1986
PHA-0746	VAV5674	Primitive	Tilcara	Jujuy	2400	1977
PHA-0747	VAV5873	Primitive	Tilcara	Jujuy	2400	1978
PHA-0748	VAV5868	Primitive	Santa Victoria	Salta	2400	1978
PHA-0749	VAV5882	Primitive	Santa Victoria	Salta	2400	1978

(*continued*)

Table 1. (*continued*)

Code	Code2	Type	Location	Province	Altitude (masl)	Year
PHA-0750	VAV6198	Primitive	Santa Victoria	Salta	2400	1981
PHA-0752	VAV5867	Primitive	Santa Victoria	Salta	2400	1978
PHA-0753	MCM121	Primitive	Santa Victoria	Salta	2400	1986
PHA-0754	MCM123	Primitive	Santa Victoria	Salta	2400	1986
PHA-0755	MCM122	Primitive	Santa Victoria	Salta	2400	1986
PHA-0756	MCM128	Primitive	Santa Victoria	Salta	2400	1986
PHA-0757	MCM109	Primitive	Iruya	Salta	3200	1986
PHA-0758	MCM116	Primitive	Iruya	Salta	2800	1986
PHA-0759	MCM110	Primitive	Iruya	Salta	3020	1986
PHA-0760	MCM130	Primitive	Iruya	Salta	2800	1986
PHA-0761	MCM79A	Primitive	Santa Victoria	Salta	2400	1986
PHA-0762	MCM32	Primitive	Santa Victoria	Salta	2600	1986
PHA-0763	MCM75	Primitive	Santa Victoria	Salta	2400	1986
PHA-0764	MCM14	Primitive	Santa Victoria	Salta	2400	1986
PHA-0765	MCM85	Primitive	Santa Victoria	Salta	2400	1986
PHA-0766	MCM44	Primitive	Santa Victoria	Salta	2700	1986
PHA-0767	MCM88	Primitive	Santa Victoria	Salta	2400	1986
PHA-0768	MCM15	Primitive	Santa Victoria	Salta	2400	1986
PHA-0769	MCM2	Primitive	Iruya	Salta	2900	1986
PHA-0770	MCM78	Primitive	Santa Victoria	Salta	2400	1986
PHA-0772	VAV5883	Primitive	Santa Victoria	Salta	2500	1978
PHA-0773	VAV5878	Primitive	Tilcara	Jujuy	2400	1978
PHA-0774	VAV5874	Primitive	Tilcara	Jujuy	2400	1978
PHA-0775	VAV5881	Primitive	Santa Victoria	Salta	2400	1978
PHA-0777	MCM90	Primitive	Santa Victoria	Salta	2400	1986
PHA-0780	VAV3716	Primitive	Iruya	Salta	2900	1971
PHA-0782	VAV5672	Primitive	Tilcara	Jujuy	2400	1973
PHA-0852	MCM18	Primitive	Santa Victoria	Salta	2400	1986
PHA-0855	MCM194	Primitive	Santa Victoria	Salta	2600	1996
PHA-0856	MCM201	Wild	Santa Victoria	Salta	2500	1996
PHA-0858	MCM207	Wild	Chicoana	Salta	1540	1996
PHA-0860	VAV3733	Primitive	Tilcara	Jujuy	2400	1972
PHA-0867	VAV5675	Primitive	Santa Victoria	Salta	2400	1977
PHA-0875	VAV6366	Wild	Jujuy	Jujuy	1720	1986
PHA-0876	VAV6367	Wild	Jujuy	Jujuy	1650	1986
PHA-0878	VAV6381	Wild	Jujuy	Salta	1800	1991
PHA-0880	MCM87	Primitive	Santa Victoria	Salta	2400	1986
PHA-0882	MCM1	Primitive	Iruya	Salta	2900	1986
PHA-0883	MCM7	Primitive	Iruya	Salta	2900	1986
PHA-0884	MCM9	Primitive	Iruya	Salta	2900	1986

Table 1. (*continued*)

Code	Code2	Type	Location	Province	Altitude (masl)	Year
PHA-0885	MCM16	Primitive	Santa Victoria	Salta	2400	1986
PHA-0886	MCM26	Primitive	Santa Victoria	Salta	2400	1986
PHA-0887	MCM73	Primitive	Santa Victoria	Salta	2400	1986
PHA-0888	MCM82	Primitive	Iruya	Salta	2900	1986
PHA-0889	MCM84	Primitive	Iruya	Salta	2900	1986
PHA-0890	MCM89	Primitive	Santa Victoria	Salta	2400	1986
PHA-0891	MCM99	Primitive	Santa Victoria	Salta	2400	1986
PHA-0892	MCM100	Primitive	Santa Victoria	Salta	2400	1986
PHA-0893	MCM131	Primitive	Iruya	Salta	2900	1986
PHA-0894	MCM133	Primitive	Iruya	Salta	2900	1986
PHA-0895	MCM199	Primitive	Tumbaya	Jujuy	1900	1996
PHA-0896	MCM200	Wild	Tumbaya	Jujuy	1900	1996
PHA-0897	MCM202	Wild	Tumbaya	Jujuy	1900	1996
PHA-0898	MCM206	Wild	Chicoana	Salta	1400	1996
PHA-1400	VAV6404	Primitive	Los Toldos	Salta	1600	1993

Code - MBG-CSIC accession name, *Code2* - original accession name.

Wild beans of South America are very small seeded compared with their corresponding domesticated forms. Common bean has evolved during domestication from small- to large-seeded forms, from extreme indeterminate climbing types to determinate bush types, from seed dormancy and water impermeability of the seed coat to lack of dormancy and water permeable seed coat and from highly fibrous pod wall and shattering forms to lack of fibres and no shattering types. There was also a loss of seed coat pigmentation in some populations and a reduction of the content in toxic materials, which have probably improved the flavour and caused the loss of seed protection against predators (Smartt 1988).

The missions for collect the wild bean populations from the NOA included in the present study were carried out since 1971 in the Argentinean provinces of Jujuy, Salta and Tucumán. Mostly of the studied populations grow in the Yungas ecoregion, a narrow strip in the slopes of the Andean mountains in the provinces of Salta, Jujuy, Tucumán and Catamarca reaching heights of up to 3000 masl. The climate is warm and humid with summer rains and frost in winter, annual rainfall reaches 2500 mm. It is a region of mountains, canyons and plains with forest acid soils with its three characteristic horizons: humifer, topsoil and mineral earth. The wild and primitive bean germplasm maintained at the MBG-CSIC was studied for their phenotypic (Menéndez-Sevillano 2002; De Ron et al. 2004; Santalla et al. 2004) and genetic variation (Galván et al. 2006).

4   Conclusions

As a conclusion, an efficient use of the genetic resources requires an understanding of the origin and the variation in the germplasm collections available. Variation in some quantitative traits revealing agronomic value together with other associated with regional preferences, i.e. seed coat colour and pattern, is relevant for breeding purposes. The use of wild and primitive populations (with low inputs for their growth) in breeding programs (Singh et al. 1995) along with improved varieties will allow increasing the sustainability of the bean crop. These common bean germplasm is being conserved *in situ;* the wild populations in their ecosystems and the domesticated ones in farms through a participatory program with farmers of the NOA under traditional growing methods.

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Patterns of Spring Growth and Phenology in Natural Populations of *Lolium perenne* Under Contrasting Field Conditions

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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.

Keywords: Ecotype · Heading date · Growth rate · *Lolium perenne*
Natural diversity · Spring growth · Thermal time

1 Introduction

Grasslands represent the most widespread agricultural land use in Europe. Among grassland species, *Lolium perenne* is one of the most prevalent and economically important grass species (Humphreys et al. 2010, Sampoux et al. 2013). *Lolium perenne* has undergone spontaneous in-situ differentiation into ecotypes (Boller and Greene 2010), presumably adapted to local environmental conditions. A large number of natural *L. perenne* populations are maintained in European genebanks as plant genetic resources (PGRs). With the concern of ongoing climate change, the introduction of ecotypic variability into breeding programs may contribute to develop cultivars with high ecophysiological resilience (Boller and Greene 2010), while meeting the usual

requirements for agronomic performances, related notably to forage yield and feeding quality (Conaghan and Casler 2011).

Herbage production and feeding quality consistently depend on spring growth dynamic and earliness of heading (Dent and Aldrich 1963). A thorough assessment of these traits is therefore crucial to evaluate accessions of potential interest in breeding. We report hereafter field observations of spring growth and earliness of heading in a set of *L. perenne* genebank accessions that aimed at depicting the wide range of ecotypic variability existing in this species in Europe.

2   Material and Methods

2.1   Plant Material and Phenotyping

Fourteen European genebanks contributed to the set of *L. perenne* accessions under study, which included 419 natural populations and 14 cultivars. Micro-sward trials were established in three locations at Lusignan in France "LUS" (0.08 E; 46.40 N), Melle in Belgium "MEL" (3.79 E; 50.98 N) and Poel island in Germany "POE" (11.46 E; 54.00 N). The number of sown accessions was 427, 391 and 433 at LUS, MEL and POE, respectively. In each location, accessions were sown in three complete blocks. Elementary micro-plot size was 1 m^2. LUS and POE were sown in April 2015; MEL was sown in October 2015. Trials were fertilized with mineral nitrogen (200 to 300 kg N ha^{-1} split in several dressings in each location in 2016). In 2016, spring growth was measured weekly as sward canopy height using a herbometer. This consisted of a weighted plate on a graduated pole. The measurement thus integrates over the height and the density of the vegetation layer (Holmes 1974). Sward height was measured as undisturbed height at POE holding the herbometer plate at the top of the canopy, whereas it was measured by letting the plate settle on the canopy at LUS and MEL. Sward height measurement started at the onset of growth in all locations. It was performed weekly until heading date at LUS and POE and until the 3rd of May (before completion of heading) at MEL. Heading date was recorded at LUS and POE as the date when the ears of 20 plants were visible in a micro-plot. At each location, growing degree days (GDD) were computed starting on 01/01/2016 and taking 0 °C as the base temperature.

2.2   Data Analysis

We chose the Schnute's non-linear model (Schnute 1981) to fit spring growth data. Due to its flexibility, this model is able to fit various shapes of response curves as it was necessary with our data. It is expressed as:

$$Y_i = \left(c^b + (d^b - c^b) \left(\frac{1 - \exp^{-a(X - x1)}}{1 - \exp^{-a(x2 - x1)}} \right) \right)^{\frac{1}{b}} + \in_i$$

where Y is the sward height; X is GDDs at time of measurement, and a and b are the parameters determining the shape of the curve. x1 and x2 are respectively the first and

last values of the X domain for which the model is fitted; parameters c and d are the values of Y at x1 and x2 respectively. ε_i is the experimental error of the i measurement. Parameters a and b were estimated using the Levenberg–Marquardt iterative method with automatic computation of the analytical partial derivatives (SAS 8.01, SAS Institute, Cary, NC, USA). Maximum growth rate (Growth$_{max}$) was determined as when the second derivative of the Schnute's model function equaled zero. X and Y values at this point of the modelled curve gave the thermal time when the growth rate was maximum (GDD$_{growth.max}$) and the sward height at maximum growth rate (Height$_{growth.max}$), respectively. This model was implemented using a custom R script. In cases where the Schnute model took the shape of an exponential curve, the analytical result for GDD$_{growth.max}$ and Height$_{growth.max}$ became infinite. In these cases, for comparisons purposes, we considered GDD$_{growth.max}$ to be x2 and Height$_{growth.max}$ to be d. The Schnute model was used to predict growth rates for X = 200, 400, 600 and 800 GDD (Growth$_{200–800}$), the latter corresponding to the date of the latest measurements at MEL.

Genetic correlations between heading data in thermal time (HEA$_{GDD}$), Growth$_{max}$, Height$_{growth.max}$ and GDD$_{growth.max}$ were computed for LUS and POE separately. These correlations were obtained from a multivariate mixed ANOVA model in which the accession effect was considered as random. Phenotypic correlations were also computed in LUS and POE as pairwise Pearson correlations based on the means of accessions over the three replicates.

3   Results

Spring growth dynamics contrasted between trial locations. Figure 1 shows the distribution of accession growth rates in each trial site at different thermal times. The average growth rate (mean of accessions) continuously increased from 200 to 800 GDD at MEL and LUS (Fig. 1 A-D), whereas it showed a maximum round 500 GDD at POE (Fig. 1G). Figure 2G also shows that the average growth rate increased until 1000 GDD at LUS. Furthermore, the average maximum growth rate was found higher at MEL than at LUS (Fig. 1, E) but the average sward height at maximum growth rate than at LUS a smaller value at MEL than at LUS (Fig. 1, F).

Heading occurred later at LUS than at POE in terms of GDDs (Fig. 2) but it was the reverse in terms of calendar days (heading 5 days earlier on average in LUS). Genetic correlations were fairly comparable to phenotypic correlations (Table 1), indicating small residual variation in the phenotypic means of accessions per location. In LUS, the thermal time to heading of accessions (HEA$_{GDD}$) was strongly positively correlated to their thermal time to maximum growth rate (GDD$_{growth.max}$). Meanwhile, in this location, thermal times to heading and to maximum growth rate were negatively correlated to maximum growth rate (Growth$_{max}$). However, HEA$_{GDD}$ and GDD$_{growth.max}$ exhibited moderate positive correlations with sward height at time of maximum growth rate (Height$_{growth.max}$), because of the longer growing period of late accessions. In contrast, HEA$_{GDD}$ was negatively correlated to GDD$_{growth.max}$, in POE. In this location, Growth$_{max}$ was still negatively correlated to HEA$_{GDD}$ but it was positively correlated to GDD$_{growth.max}$. In POE, Height$_{growth.max}$ was finally positively correlated with GDD$_{growth.max}$ but not with HEA$_{GDD}$.
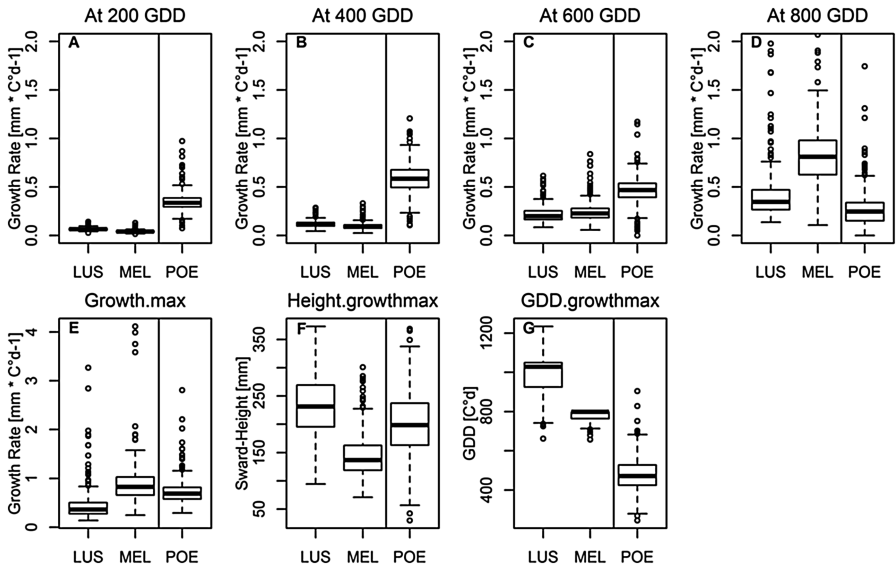
Fig. 1. A-D: Growth rates at the three locations MEL, LUS and POE from 200 to 800 growing degree-days (GDD); **E**: Maximum growth rates (Growth$_{max}$) at the three trial sites; **F**: Sward heights at time of maximum growth rate (Height$_{growth.max}$) at the three trial sites; **G**: Thermal time (growing degree-days) at time of maximum growth rate (GDD$_{growth.max}$) at the three trial sites. Figures show the distribution of accessions means over the three replicates per trial sites. Note that measurements at LUS and MEL are not formally comparable to those at POE due to different measurement techniques.
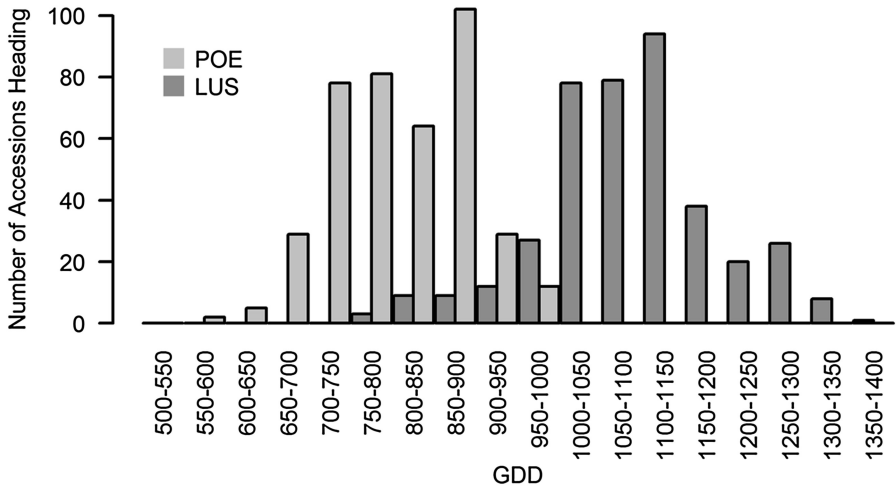

Fig. 2. Histogramme of heading time in growing-degree-days (GDD) at POE and LUS. Light grey bars refer to POE and dark grey bars to LUS

Table 1. Correlations between spring growth traits (Growth$_{max}$, Height$_{growth.max}$, GDD$_{growth.max}$) and thermal time to heading (HEA$_{GDD}$) in the trial sites POE and LUS. Genetic and phenotypic correlations are displayed below and above the diagonal of tables, respectively. The level of significance of phenotypic correlations is displayed as "*" for p value < 0.05 and "***" for p value < 0.001.

POE	GDD$_{growth.max}$	Height$_{growth.max}$	Growth$_{max}$	HEA$_{GDD}$	LUS	GDD$_{growth.max}$	Height$_{growth.max}$	Growth$_{max}$	HEA$_{GDD}$
GDD$_{growth.max}$	1	0.791***	0.123*	–0.184***	GDD$_{growth.max}$	1	0.486***	–0.624***	0.894***
Heigh$_{growth.max}$	0,637	1	0.428***	–0.316***	Height$_{growth.max}$	0,486	1	0.032	0.278***
Growth$_{max}$	0,243	0,732	1	–0.561***	Growth$_{max}$	–0,654	0,022	1	–0.687***
HEA$_{GDD}$	–0,323	–0,465	–0,845	1	HEA$_{GDD}$	0,926	0,302	–0,725	1

4   Discussion and Conclusion

This study presents preliminary results of spring growth and phenology for a wide range of *L. perenne* ecotypes. We cannot rule out that different modes of height measurements using the herbometer might have interfered with our results. However, it is clear that the dynamics of spring growth along thermal time was different in the three locations. It is, more specifically, a matter of fact that the genetic correlation between thermal times to heading and to maximum growth rate changed from strongly positive in LUS to moderately negative in POE. This is evidence of a strong interaction between accessions and trial sites in the timing of spring growth. The late heading accessions reached their maximum growth rate at smaller amount of degree-days in POE than in LUS, whereas early accessions did at similar amount of degree-days in the two trial sites (data not shown). This indicates that late accessions suffered from a limiting factor in POE which depressed their canopy growth quite soon until the end of the canopy height measurement period. We indeed used a very wide set of accessions covering almost the full range of heading earliness of perennial ryegrass. The late accessions we used were mostly ecotypes from oceanic climate (Western Europe) with warmer winter and spring than in POE. It is thus likely that the growth of late accessions was depressed in POE by insufficient delivery of growing-degree-days and/or by cold stresses.

The data used for the reported investigations was recorded within the frame of the project *GrassLandscape* which investigates climatic adaptation in the natural diversity of *L. perenne* at phenotypic and genomic levels. Next developments in the course of this project will contribute to better understand the response of spring growth to climatic conditions in *L. perenne* and the genetic variability of this response.