Genetic Resources

GENEBANK REPORT

Genetic Resources (2025), (S2), 106–118
DOI: 10.46265/genresj.CWLJ2580
https://www.genresj.org
ISSN: 2708-3764

The INRAE *Prairies* genebank for *ex situ* conservation of forage and turf species in France

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Abstract: In France, INRAE (Institut national de recherche pour l'agriculture, l'alimentation et l'environnement - French National Research Institute for Agriculture, Food and Environment) maintains a genebank of grass and legume perennial species for forage and turf usages in the research unit P3F located in Lusignan (region Nouvelle-Aquitaine). This genebank is a component of the plant pillar (BRC4Plants) of the French National Research Infrastructure RARe. Collections comprise natural populations collected across France and other countries, landraces, cultivars removed from registration lists and some scientific materials. 967 accessions are currently available for distribution, among which 852 are available in the frame of the Multilateral System of Access and Benefit-sharing of the UN Food and Agriculture Organization (FAO). The genebank policy has been to set up core collections of the main forage and turf species diversity and to apply a high standard of conservation and regeneration to collections of relatively small size. During past decades, genetic resources held by the genebank highly contributed to the breeding of forage and turf cultivars in France and to various scientific studies. Recently, high-throughput genotyping of accessions has provided unprecedented means to discover phylogeographic patterns and genomic adaptation in natural populations of perennial ryegrass (*Lolium perenne* L.) and to understand the breeding history of lucerne (*Medicago sativa* L.). Such approaches open promising prospects for future genetic adaptation of forage and turf species to changing environmental conditions and new usages.

Keywords: cultivar, grass, grassland, landrace, legume, natural population, perennial species

Citation: Sampoux, J. P., Barre, P., Durand, J. L., Ghesquière, M., Julier, B., Pégard, M., Priet, A. (2025). The INRAE *Prairies* genebank for *ex situ* conservation of forage and turf species in France. *Genetic Resources* (S2), 106–118. doi: 10.46265/genresj.CWLJ2580.

Introduction

Sown temporary grasslands cover around 2.47 million hectares in France (Huyghe *et al*, 2014). They provide grazed or cut forage for livestock but also various other ecosystemic services such as soil fertility improvement, carbon sequestration and biodiversity shelter (Martin *et al*, 2020). Sown grasslands are seeded with improved cultivars from various perennial grass and legume species. Most of these species commonly grow as natural populations in permanent and natural grasslands covering 9.8 million hectares in France (Huyghe *et al*, 2014) and exhibit a diversity of ecotypes adapted to various conditions of soil, climate and use. Only few species, e.g. lucerne (*Medicago sativa* L.) and Italian ryegrass (*Lolium multiflorum* Lam.), were bred as landraces before the onset of modern plant breeding. Natural populations and landraces were the starting material to develop continuously improved cultivars adapted to various pedoclimatic conditions and forage systems, e.g. Sampoux *et al* (2011) for perennial ryegrass (*Lolium perenne* L.). Grown as grass–legume binary associations, or as mixtures involving several species (up to ten or more), temporary grasslands can provide a high yield of good quality forage without artificial nitrogen and herbicide inputs (Surault *et al*, 2024). Furthermore, some grass species have also been

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Received: 03.11.2024 | Accepted: 24.01.2025 | Published online: 11.03.2025

bred for small size, high density and slow growth to create turf cultivars improved for either sport or lawn requirements (e.g. Sampoux *et al* (2012)). The breeding of turf-type genotypes of legume species, e.g. white clover (*Trifolium repens* L.), lucerne (*M. sativa*), birdsfoot trefoil (*Lotus corniculatus* L.), has also recently begun. Finally, all grassland and lawn areas contribute to providing pleasant landscapes for recreational activities.

The INRAE multidisciplinary research unit for grasslands and forage species (UR P3F) in Lusignan hosts the *Prairies* genebank targeted to the conservation of genetic resources of the main grass and legume species sown in France for either forage or turf usage (Figure 1).

Genetic materials maintained by the *Prairies* genebank

Collections of natural populations from grass and legume species of the *Prairies* genebank are the outcome of collection trips undertaken by scientists of INRA (Institut National de la Recherche Agronomique, former acronym of INRAE) in France and other countries. Some of these collection trips were undertaken by INRA staff alone and others together with academic research institutes from other countries (e.g. Spain, Portugal, Morocco, Algeria, USA) or breeding companies. Most of the seed-collecting trips were carried out from 1970 to 1990 to gather samples of genetic resources necessary to start or diversify breeding programmes. Since then, the long-term conservation and characterization of collected accessions have been progressively implemented (Prospéri and Sampoux, 2005). A more recent collecting campaign carried out in the southern part of France in 2014 gave the opportunity to collect new accessions from the natural diversity of grass and legume species that were afterwards included in the genebank besides already existing collections.

The genebank also maintains some lucerne landraces, as well as some grass and legume historical cultivars formerly bred by INRAE or other breeders, all removed from national lists. As a matter of fact, in France and other European countries, the commercialization of new cultivars is allowed only after their registration on national lists, which depends on sufficient value for cultivation and use (VCU) and distinctiveness, uniformity and stability (DUS) requirements. After a certain duration (usually ten years), cultivars are removed from national lists unless they have not been outpaced by more recent cultivars (see for examples rules in France at www.geves.fr/variety-seed-expertise/field-forage/registration-of-field-and-forage-varieties-in-the-french-catalogue).

Collection of orchard grass

Natural populations

A breeding programme for orchard grass (*Dactylis glomerata* L.) started in 1962 at INRA in Lusignan. The programme focused on the improvement of forage performances in the temperate climate areas of Europe. An early start of vegetative growth in spring and a relatively late spike emergence date were targeted, as they were expected to provide a long annual period of forage production. Thus, collection campaigns from 1962 to 1982 were directed towards oceanic regions of Europe with mild winter and cool summer conditions, i.e. Brittany and Cotentin in France, northwestern Spain and northern Portugal (Mousset, 2000). Populations from the widespread autotetraploid (4x = 28) taxon *D. glomerata* subsp. *glomerata* were collected in France, Spain and Portugal. Populations of the rare diploid taxon (2x = 14) *D. glomerata* subsp. *lusitanica* Stebbins & D. Zohary were found in Portugal and populations of Galician type (Ortiz and Rodriguez-Oubiña, 1993), either diploid or tetraploid, were found in Spain and Portugal. The autotetraploid materials thus collected were used by the INRA plant breeding unit of Lusignan to create several successive innovative cultivars: 'Lully' (1977), 'Lude' and 'Lutétia' (1978), 'Lupré' (1992), 'Ludac' (1997), 'Luron' and 'Ludovic' (1998). Many orchard grass cultivars currently available from private breeding companies for oceanic climates in Europe still stem from these original collections.

Later, from 1987 to 1992, new collections were undertaken to collect Mediterranean types of orchard grass offering some winter growth potential and more or less substantial summer dormancy in southern France, southern Portugal, southern Spain and Algeria (Mousset, 2000). Thereby, populations were collected from the widespread autotetraploid taxon *D. glomerata* subsp. *hispanica* (Roth) Nyman and from the diploid taxa *D. glomerata* subsp. *castellata* Borrill & Parker and *D. glomerata* subsp. *mairei* Stebbins & D. Zohary. The autotetraploid collected materials were used by the INRA plant breeding unit in Montpellier to select the cultivar 'Medly' (1996), which presents adaption to summer drought and heat stresses of Mediterranean areas.

A total of 702 orchard grass natural populations were collected between 1962 and 1992. A core collection of 172 populations sampling the variability within the different taxa was extracted from this large set and is currently available from the *Prairies* genebank.

The new collection campaign undertaken in 2014 in the southern part of France enabled the collection of 39 new natural populations of the autotraploid *D. glomerata* subsp. *glomerata*. These new entries added to the previously mentioned core collection to make the set of orchard grass natural accessions currently available from the *Prairies* genebank (Figure 2 and Table 1).

Other genetic materials

Accessions publicly available from the genebank also include the *D. glomerata* subsp. *glomerata* cultivars 'Floréal' (1957), 'Lully' and 'Lutetia', formerly bred by INRA.

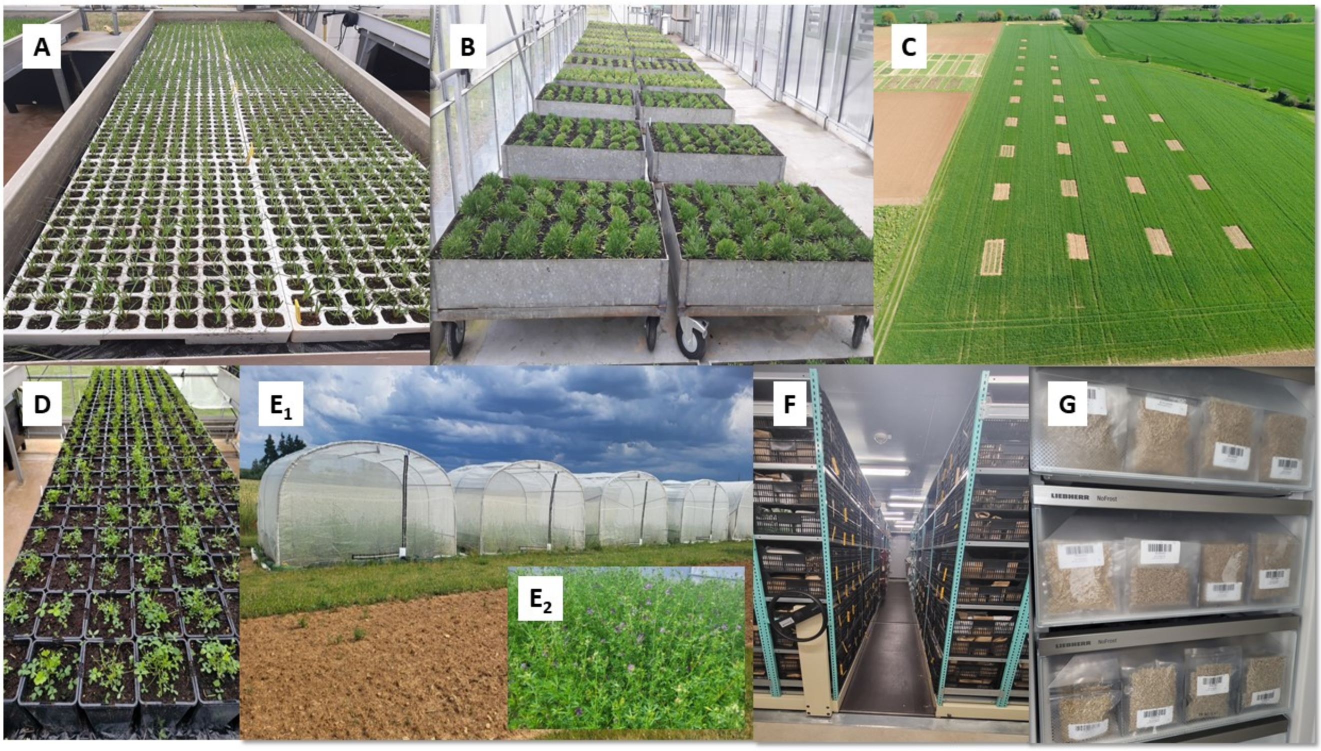

Figure 1. Implementation of different tasks in the facilities of the INRAE *Prairies* genebank. A, Growing grass plantlets in the greenhouse prior to planting accessions in spaced spots in a triticale (x *Triticosecale*)–rye (*Secale cereale* L.) field; B, Growing grass plants in trays prior to transfer in confined compartments of a partitioned greenhouse; C, Grass accessions planted in spaced spots within a triticale–rye crop which acts as a pollen barrier; D, Growing lucerne (*M. sativa*) plants in pots prior to planting under insect proof tents; E1, Insect-proof tents used to perform legume cross-pollinations; E2, A lucerne accession under an insect-proof tent (pollen transport inside the tent is ensured by bumblebees); F, Storage of active seed lots in a cold room (5°C and 30% hygrometry); G, Storage of safety seed lots in a freezer (-20°C).

Collection of tall fescue

Natural populations

Several hundred natural populations of tall fescue (*Festuca arundinacea* Schreb.) were collected by scientists of INRA Lusignan from 1969 to 1992 (Ghesquière and Jadas-Hécart, 1995). Collection campaigns focused towards southern France and the Mediterranean basin (northern Africa, Portugal). The collected populations were then characterized at INRA Lusignan from 1985 to 1995 and a core collection of 128 accessions sampling the diversity of the different taxa was drawn up. This core collection included accessions from the common allohexaploid (6x = 42) tall fescue (*F. arundinacea* subsp. *arundinacea*) collected in France, Portugal and Switzerland (77 accessions) and in northern Africa (34 accessions). Sources of *Festuca arundinacea* subsp. *arundinacea* from temperate areas and the Mediterranean part of France were discovered to give deeply sterile hybrids with Mediterranean sources from southern Portugal, southern Spain and northern Africa (see Jadas-Hécart and Gillet (1978) for the production of temperate x Mediterranean amphiploid cultivars). The core collection was also supplemented with accessions from two other northern African types, three from *F. arundinacea* var. *atlantigena* (St.-Yves) Auquier (octoploid, 8x = 56) and 14 from *F. arundinacea* var. *letourneuxiana* (St.-Yves) Torrecilla (decaploid, 10x = 70).

Five accessions of *F. arundinacea* var. *glaucescens* Boiss. are also maintained in the genebank. They result from the pooling of various natural populations of this taxon collected around lake Embrun in the French Alps in 1982. This taxon, which is autotetraploid (4x = 28), has been recognized as one of the two progenitors of the hexaploid tall fescue *F. arundinacea* subsp. *arundinacea* together with the diploid (2x = 14) *Festuca pratensis* Huds. after natural amphiploidization (Humphreys *et al*, 1995). *F. arundinacea* var. *glaucescens* was widely used by the INRA plant breeding unit of Lusignan from the 1980s onwards in hybridization with autotetraploid ryegrasses to create the first x *Festulolium* varieties (Ghesquière *et al*, 2010).

The collection campaign carried out in France in 2014 provided 36 new natural populations of the tall fescue *F. arundinacea* subsp. *arundinacea*. The historical core collection of 128 accessions, supplemented by accessions from *F. arundinacea* var. *glaucescens* and accessions collected in 2014 make the set of tall fescue natural populations available from the *Prairies* genebank (Figure 3 and Table 1).

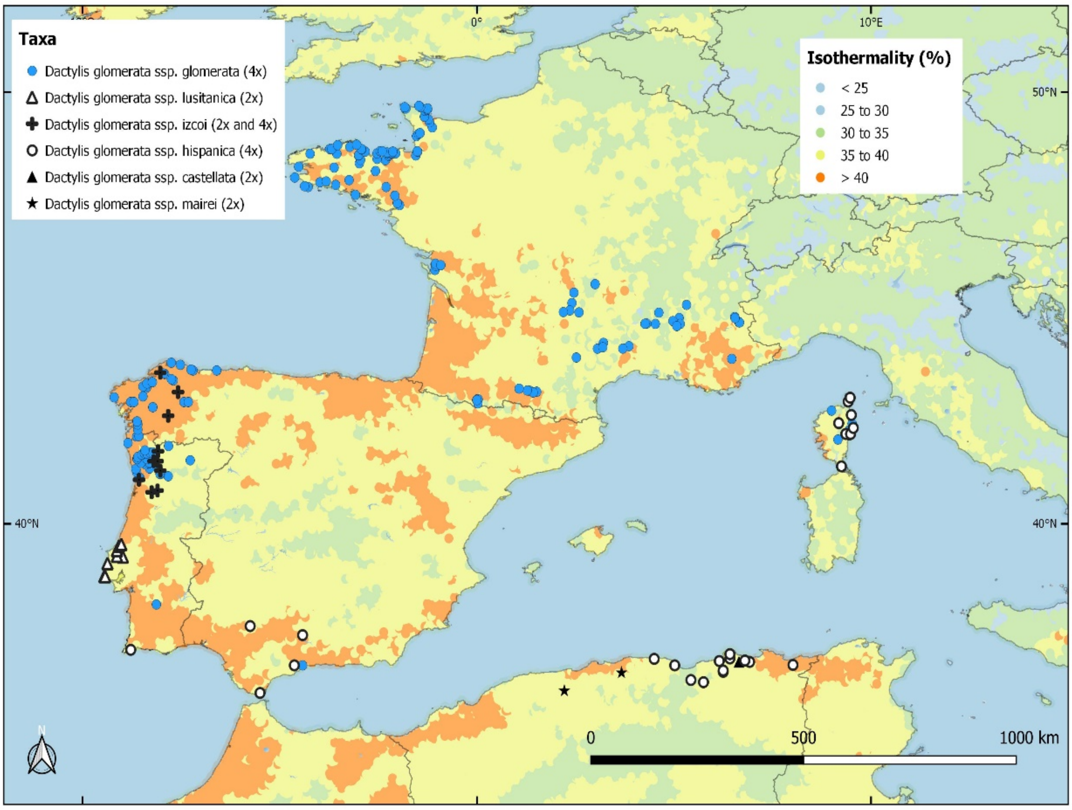

Figure 2. Geographical distribution of *D. glomerata* natural populations maintained by the *Prairies* genebank. In the background, isothermality is the ratio 'temperature diurnal range/temperature annual range' in percentage. Values are computed from 1989–2010 climate norms. High isothemality values are typical of oceanic temperate climate whereas low values are typical of continental climate. Credit: Fabien Sampoux, project ColNat_Fourrage+ (AAC RPG 2018-2 CTPS GNIS)

Other genetic materials

Several x *Festulolium* accessions created by the INRAE plant breeding unit of Lusignan are maintained. This includes three amphiploid cultivars created after hybridization between *F. arundinacea* var. *glaucescens* genotypes and *Lolium multiflorum* tetraploid genotypes, namely 'Lueur' (2007), 'Luxane' (2008) and 'Lusilium' (2008). This also includes several pools obtained from the backcross of amphiploid (*F. arundinacea* var. *glaucescens* × *L. multiflorum*) material into tetraploid *L. multiflorum* or *Lolium perenne* materials.

Accessions publicly available from the genebank also include the *F. arundinacea* subsp. *arundinacea* cultivars 'Gloria' (1976), 'Lubrette' (1981) and 'Ludical' (2002) bred by INRA.

Collection of ryegrasses

Natural populations

In 1983 and 1984, the INRA plant breeding unit of Clermont-Ferrand, together with the breeding companies of ACVF (*Association des Créateurs de Variétés Fourragères* – Society of plant breeding companies involved in the breeding of forage and turf cultivars in France), undertook a dense and even collection of more than 500 natural populations (diploid, 2x = 14) of perennial ryegrass (*L. perenne*) across France. These populations were thoroughly characterized at INRA Clermont-Ferrand during the following years (Charmet *et al*, 1990), providing data to reveal informative relationships between characterization traits and ecogeographical variables at the sites of origin of the populations (Balfourier and Charmet, 1991). The collected materials provided the sources to create the cultivar 'Clerpin' (1996) by the INRA plant breeding unit of Clermont-Ferrand and were the root of a substantial leap in the release of perennial ryegrass cultivars with late heading date and improved crown rust resistance by private breeding companies. A core collection of 160 accessions was finally set up using a clustering based on characterization data combined with a geostatistics contiguity constraint (Charmet and Balfourier, 1995). In 2008, this core collection was transferred to the *Prairies* genebank in Lusignan which is maintaining it.

The whole set of perennial ryegrass natural populations maintained by the genebank also includes 27 other natural populations collected in France at diverse times as well as 36 natural populations collected during the 2014 collection campaign in southern France (Figure 4 and Table 1). The 2014 collection campaign also gave the opportunity to collect seven spontaneous popula-

Table 1. Number of accessions per species made publicly available by the *Prairies* genebank in 2024. *, The whole set of 852 accessions proposed for notification by France as Contracting Party to the Multilateral System (MLS) of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) comprises three different subsets: 1) a subset of 531 accessions maintained by INRAE (*Prairies* genebank) and notified to the MLS in 2014, 2) a subset of 146 accessions maintained by INRAE (*Prairies* genebank) and 3) a subset of 175 accessions maintained by INRAE (*Prairies* genebank) and ACVF (society of companies breeding forage and turf species in France), 2) and 3) proposed in 2024 to the French Ministry of Agriculture for notification to the MLS. The subsets of 531 and 175 accessions can already be browsed on the public databases EURISCO, Florilège and Siregal. The subset of 146 accessions is currently in the process of addition to these databases. ^{a}, Accessions whose occurrence data were forwarded to the Global Biodiversity Information Facility (GBIF) database are a subset of the set of accessions proposed for notification to the MLS of the ITPGRFA. ^{b}, 52 perennial ryegrass cultivars, 27 *Phalaris* accessions and 3 x *Festulolium* cultivars are currently in the process of seed regeneration and will be afterwards added to the public databases Siregal, Florilège and EURISCO.

Species in collection		**Number of accessions**					
Latin name	**Vernacular name**	**Natural pop.**	**Landraces**	**Cultivars**	**EURISCO Florilège Siregal**	**Proposed to MLS of TIRPAA***	**GBIF^{a}**
Dactylis glomerata	Orchard grass	211		3	175	214	172
Lolium perenne	Perennial ryegrass	223		54^{b}	193	206	191
Lolium multiflorum	Italian ryegrass	7		2	2	9	
Lolium hybridum				1	1	1	
Festuca arundinacea	Tall fescue	169		3	128	164	
Festuca pratensis	Meadow fescue	11				11	
x *Festulolium*				3^{b}			
Festuca rubra aggr.	Red fescues	139		3	142	142	139
Festuca ovina aggr.	Sheep fescues	35			35	35	35
Phleum pratense	Timothy			2	2	2	
Phalaris arundinacea	Phalaris	27^{b}					
Phalaris aquatica							
Medicago sativa	Lucerne	12	17	25	41	48	
Trifolium pratense	Red clover	2		3	3	5	
Trifolium repens	White clover	6		4	4	10	
Onobrychis viciifolia	Sainfoin			2	2	2	
Vicia sativa	Vetch			3	3	3	
Total		**842**	**17**	**108**	**731**	**852**	**537**

tions (diploid, 2x = 14) of Italian ryegrass (*L. multiflorum*) in natural grasslands.

Other genetic materials

Accessions publicly available from the *Prairies* genebank also include the perennial ryegrass cultivars ‘Primevère’ (1957) and ‘Clerpin’ and the Italian ryegrass cultivars ‘Lutil’ (1972) and ‘Rina’ (early 1960s), all bred by INRA. Furthermore, a set of 52 perennial ryegrass cultivars from different breeding origins was gathered in 2005 to represent the history of modern breeding in this species for forage and turf usages. This set was used to experimentally assess the genetic improvement for these two usages (Sampoux *et al*, 2011, 2012) and it is currently in the process of seed regeneration in order to make it publicly available.

Collection of fine-leaved fescues

Natural populations

An extensive collection was performed for fine-leaved fescues by the INRA plant breeding unit of Lusignan and breeding companies of ACVF in 1993 and 1994. More than 500 natural populations from red fescue (Stace, 1980) and sheep fescue (Wilkinson and Stace, 1991) taxa were collected across France. During the following years, they were characterized, evaluated for turf performances in dense swards and regenerated. Sampoux and Huyghe (2009) showed that the summer water balance, soil texture and land use were the main environmental variables differentiating the realized niches of the inland taxa, i.e. the caespitose red fescue taxon *Festuca nigrescens* Lam. (hexaploid, 6x = 42), the strong creeping (abundant long rhizomes) red fescue taxa *Festuca rubra* subsp. *fallax* (Thuill.) Nyman (octoploid, 8x = 56) and *F. rubra* L. subsp. *rubra* (hexaploid, 6x = 42) and various *Festuca ovina* L. taxa with karyotype varying from diploids (2x = 14) to octoploids (8x = 56). Sampoux and Huyghe (2009) also highlighted that the differentiation of inland taxa for adaptive traits contributed more than their ploidy level variation to the diversity of their realized niches (Hutchinson, 1957). Coastal populations of red fescues were also collected along the Channel coast. Hexaploid (6x = 42) slender creeping (few short rhizomes) red fescues were found on salt marshes (green *F. rubra* subsp. *litoralis* (G.Mey.) Auquier) and

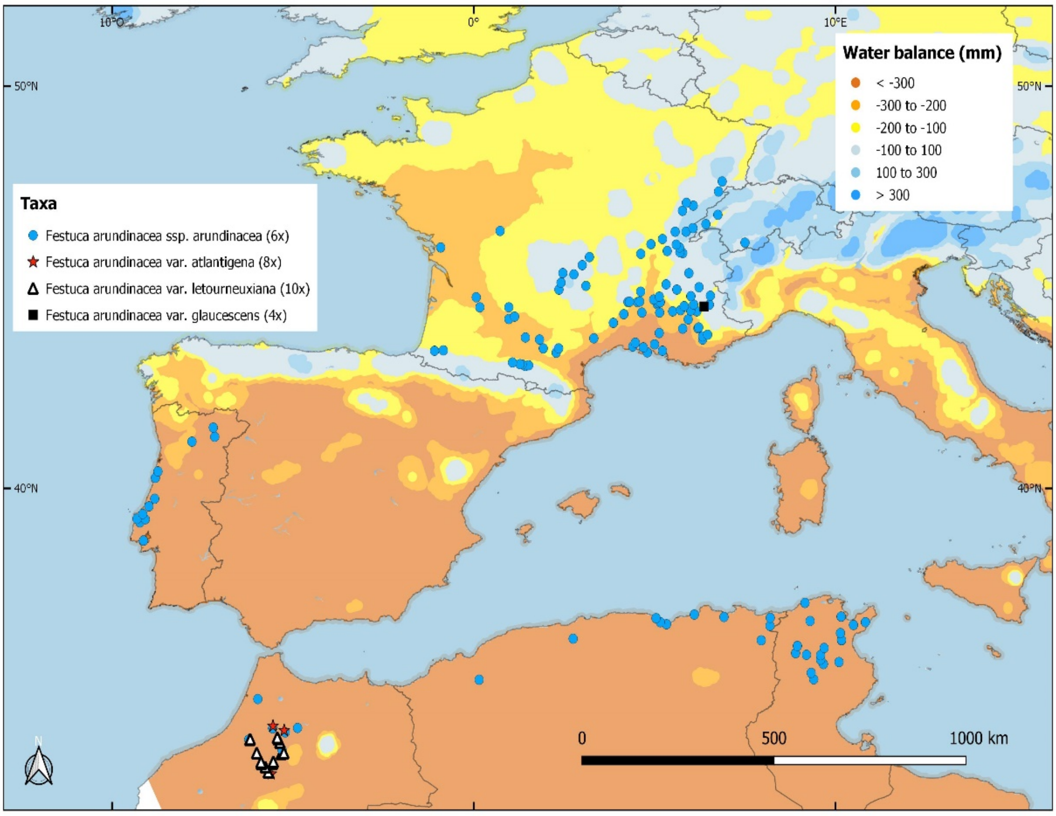

Figure 3. Geographical distribution of *F. arundinacea* natural populations maintained by the *Prairies* genebank. In the background, the water balance is the cumulated rainfall minus the cumulated evapotranspiration of June, July and August. Values are computed from 1989–2010 climate norms. Credit: Fabien Sampoux, project ColNat_Fourrage+ (AAC RPG 2018-2 CTPS GNIS)

on calcareous cliffs (glaucous *F. rubra* subsp. *pruinosa* (Hack.) Piper), and an octoploid (8x = 56) strong creeping red fescue (*F. rubra* subsp. *arenaria* (Osbeck) Syme) was found on sand dunes. During the years after collection, the populations of inland and coastal fine-leaved fescue taxa were extensively used by breeding companies in France to select new cultivars for turf usage. A core collection of 170 accessions was identified by selecting populations sampling the environmental range of each taxon (Figure 5) and it now makes the set of fine-leaved fescues natural populations available from the *Prairies* genebank (Table 1).

Other genetic materials

Accessions publicly available from the genebank also include the *F. rubra* subsp. *fallax* cultivars ‘Ludivine’ (1981) and ‘Milda’ (1975) and the *F. rubra* subsp. *pruinosa* cultivar ‘Luciole’ (2002), all formerly bred by INRA.

Collection of lucerne

History of the collection

The cultivated lucerne (*Medicago sativa* subsp. *sativa*) is not present with natural populations in France, except some feral populations escaped from sown stands. However, some wild populations of the spontaneous subspecies *Medicago sativa* subsp. *falcata* (L.) Arcang. and *Medicago sativa* subsp. *glomerata* (Balb.) Rouy can be found in northeastern and southeastern France, respectively (Julier, 1996). Lucerne as a forage crop (the subspecies *sativa*) was introduced in France in the 16th century from Spain (Michaud *et al*, 1988). Since then, traditional breeding has been applied on farm in almost all regions of France, producing landraces. Seed exchanges were frequent among different regions and with foreign countries (Julier, 1996). Hybridization between cultivated landraces of the subspecies *sativa* and wild populations of the subspecies *falcata*, naturally occurring in continental Europe, conferred frost resistance and variegated flower colour to cultivated populations. The first collections of landraces were set up during the first half of the 20th century by the plant breeding station of Versailles, a precursor of INRA, which delivered an extensive phenotypic description of them (Alabouvette and Méneret, 1935; Mayer *et al*, 1951). These studies and that of Demarly (1957) concluded that six main types of cultivated lucerne were present in France: ‘Provence’ in the south-east, ‘Poitou’, ‘Marais de Luçon’ and ‘Marais de Challans’ in the centre-west, ‘Flamande’ (or ‘Flemish’) in the north and ‘Ormelong’ in the south of the Paris Basin. These six types differentiated for traits like tap root, kidney-shaped seeds, pod coil numbers and frost resistance and for the frequency of plants with varie-

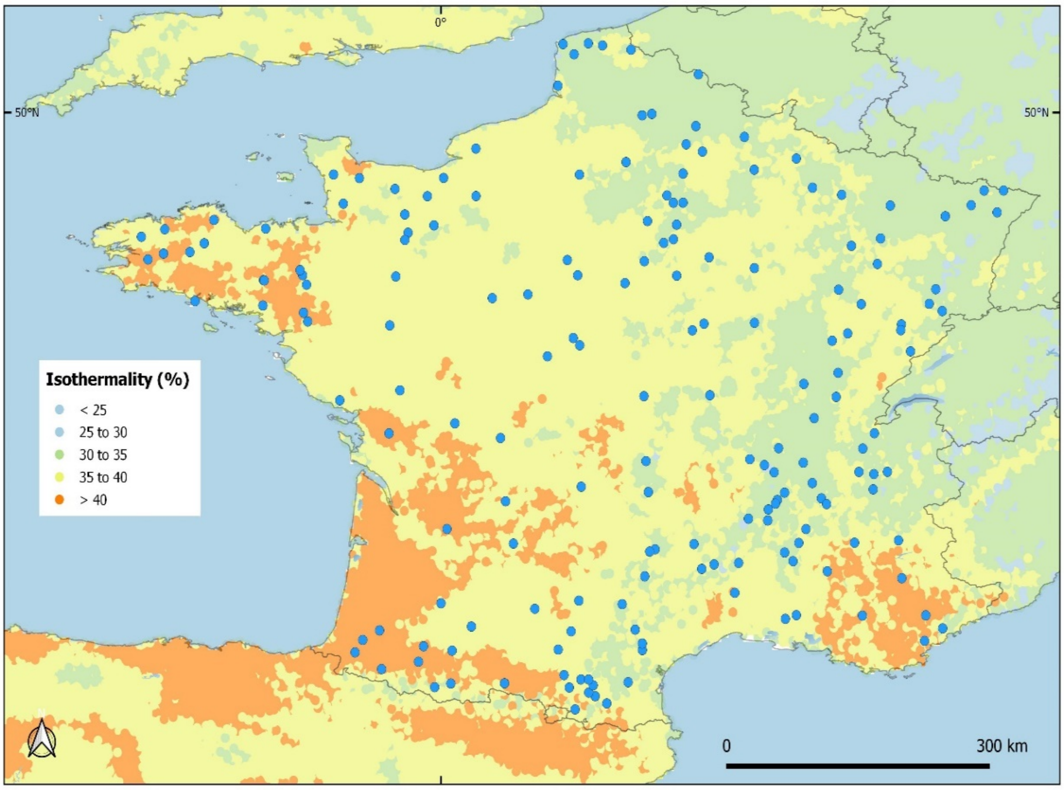

Figure 4. Geographical distribution of *L. perenne* natural populations maintained by the *Prairies* genebank. In the background, isothermality is the ratio 'temperature diurnal range/temperature annual range' in percentage. Values are computed from 1989–2010 climate norms. High isothemality values are typical of oceanic temperate climate whereas low values are typical of continental climate. Credit: Fabien Sampoux, project ColNat_Fourrage+ (AAC RPG 2018-2 CTPS GNIS)

gated flowers (Julier, 1996). They were used to initiate breeding programmes and the very first registered cultivars (e.g. 'Du Puits') were produced after a brief selection in 'Flamande'.

The six types of French landraces were transferred to the INRA forage plant breeding unit in Lusignan at its creation in 1960. In order to widen the genetic basis of its lucerne breeding programmes, the plant breeding unit of Lusignan received various cultivars, landraces and also wild populations from different countries in Europe, North Africa and America. A few of them were included in the genetic resources made publicly available, in agreement with provider requirements and plant breeding rights. As a noticeable event, a collection trip was undertaken by the INRA plant breeding unit of Lusigan in 1985 in Lorraine (east of France) after a witness mentioned the presence of some prostrate, yellow-flowered lucerne populations in this region. Two populations, named 'Malzeville' and 'Marron', were collected and multiplied, and a smooth selection was applied to remove cultivated off-types. These populations turned out to be typical of the Eurasian continental subspecies *falcata*. Furthermore, from 1985 to 1987, scientists of the INRA plant breeding unit of Montpellier organized collection trips in Spain and Portugal to collect wild populations of lucerne, also named 'Mielga' populations (Prosperi *et al*, 1989). These populations, mainly located on roadsides and low-input grasslands, had a prostrate growth habit but their violet flower colour attested to their relatedness to the subspecies *sativa*. The phenotypic description of these wild populations showed various levels of introgression by cultivated materials (Prosperi *et al*, 2006). Some 'Mielga' genetic materials are currently available in the *Prairies* genebank (see below). Several studies, carried out by the INRAE plant breeding unit of Lusignan, provided extended analyses of the phenotypic and genetic diversity of lucerne genetic resources, including cultivars, landraces and natural populations (Julier *et al*, 1995; Crochemore *et al*, 1998; Herrmann *et al*, 2018; Pégard *et al*, 2023a).

Genetic resources currently available

The numbers of landraces, natural populations and cultivars maintained by the *Prairies* genebank are reported in Table 1. The landrace accessions include the six original French landrace types (see above) as well as several landraces from northern Africa. The natural populations comprise five *M. sativa* subsp. *falcata* accessions including the 'Malzeville' and 'Marron' populations, one *M. sativa* subsp. *glomerata* accession as well as seven *M. sativa* subsp. *sativa* accessions including

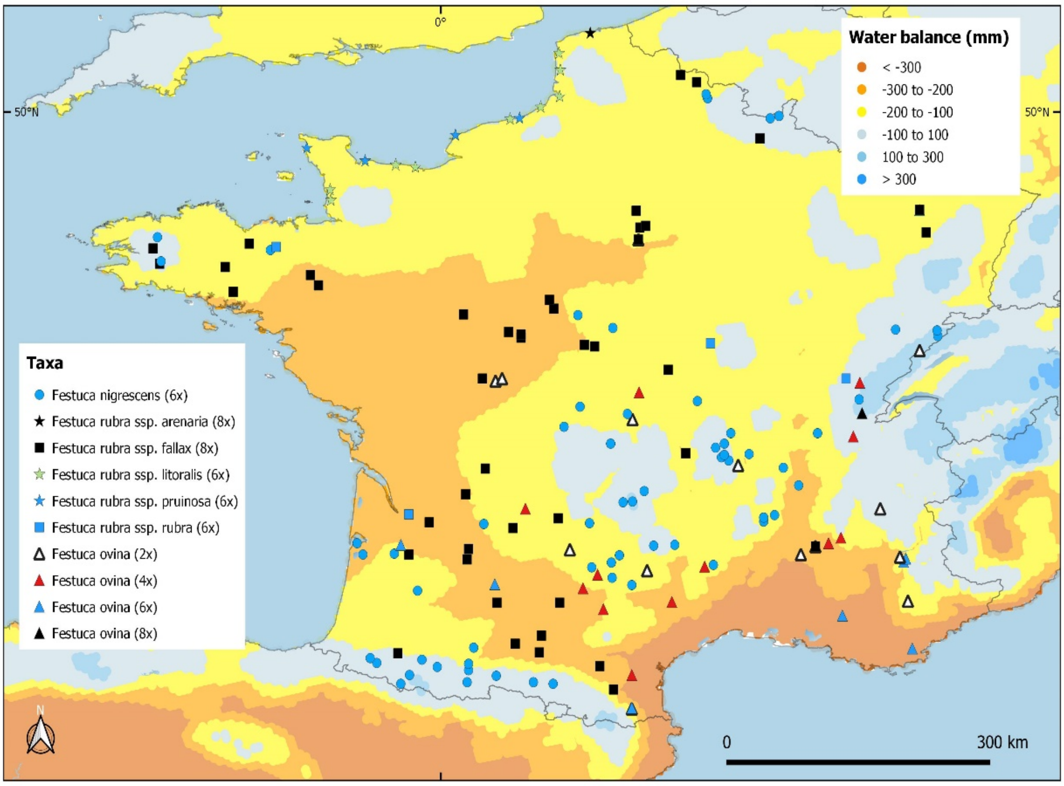

Figure 5. Geographical distribution of *Festuca rubra* aggr. and *Festuca ovina* aggr. natural populations maintained by the *Prairies* genebank. In the background, the water balance is the cumulated rainfall minus the cumulated evapotranspiration of June, July and August. Values are computed from 1989–2010 climate norms. Credit: Fabien Sampoux, project ColNat_Fourrage+ (AAC RPG 2018-2 CTPS GNIS)

five ’Mielga’ populations from Spain. Note that a large set of ‘Mielga’ accessions has recently been transferred from INRAE Montpellier to the *Prairies* genebank in Lusignan but these accessions are not currently ready for distribution.

There are 25 *M. sativa* subsp. *sativa* cultivars maintained by the genebank. They notably include several historical cultivars formerly grown in France, i.e. cultivars ‘Coussouls’ (1998), ‘Janine’ (1974), ‘Luciole’ (1965), ‘Luisante’ (1998), ‘Lutèce’ (1976), ‘Luxor ‘(1977), ‘Mireille’ (1971) bred by INRA, cultivar ‘Medalfa’ (1988) bred by INRA and ACVF, and cultivars ‘Du Puits’ (1950), ‘Gamma’ (1952), ‘Glacier’ (1975), ‘Elga’ (1964), ‘Emeraude’ (1952), ‘Etoile du Nord’ (1965), ‘Hybride de Crécy’ (1974), ‘Polder’ (1972), ‘Prima’ (1963), ‘Oméga’ (1952), ‘Orchésienne’ (1952), ‘Verneuil’ (1968) bred by private breeders.

Other genetic materials in collections

The seed-collecting campaign carried out in 2014 to collect natural populations of grass and legume species in southern France (see previous paragraphs) also enabled the collection of 11 populations of meadow fescue (*Festuca pratensis*), two populations of red clover (*Trifolium pratense* L.) and six populations of white clover (*Trifolium repens*). In 2017, another collecting trip enabled the collection of 27 *Phalaris* natural populations (*Phalaris arundinacea* L. and *Phalaris aquatica* L.) in southern France and Corsica, which are currently in the process of seed regeneration.

Finally, the genebank maintains a small number of cultivars of red and white clovers, thimothy (*Phleum pratense* L.), vetch (*Vicia sativa* L.) and sainfoin (*Onybrychis viciifolia* Scop.), all from INRA breeding.

Practical organization of the *Prairies* genebank

In 2008, the INRAE research unit UR P3F formally set up the *Prairies* genebank on its operational site in Lusignan in order to gather genetic resources of forage and turf species previously maintained in several INRAE sites across France. During the following years, substantial investments were achieved to equip the genebank with renewed facilities such as cold rooms for seed storage and a partitioned greenhouse to grow accessions in pollen isolation. Accessions were inventoried and a database was created to record all necessary information (passport data of accessions, quality and quantity of seed lots, characterization and evaluation data) and to trace the activity (seed quality control, seed regenerations, seed distributions, etc.).

For each accession, seeds harvested after a regeneration event are split into three seed lots: an active lot weighing from 200 to 800g which is stored in a cold room (+5°C and 30% hygrometry), a 20 to 30g safety lot stored at -20°C, and a 1 to 2g long-term conservation lot stored at -20°C in a different freezer than the safety lots. The genebank maintains active and safety seed lots only from the most recent regeneration event and long-term seed lots from all successive regeneration events and the initial seed batch introduced in the genebank. Germination tests are performed every three years on active lots, if they are not replaced by lots from a new regeneration event, as well as on safety seed lots if the germination percentage of active seed lots is below the established thresholds. Active and safety seed lots are regenerated when the germination percentage of active seed lots falls below 80%, i.e. every 10 to 15 years.

Species maintained in the genebank are allogamous and more or less self-incompatible. Because of this sexual reproduction biology, natural populations, landraces and cultivars (synthetic varieties) from these species are polygenotypic populations in principle at panmictic equilibrium. Therefore, the regeneration of accessions from these different kinds of genetic materials is performed by intercrossing a number of plants expected as sufficient to avoid genetic drift (i.e. 100 to 250 plants) in pollen isolation from any plant from the same species not belonging to the accession. Different means are implemented to ensure pollen isolation. In field conditions, accessions from grass species are grown in spaced spots within a triticale–rye crop which acts as a pollen barrier. Another way to achieve pollen isolation is the use of pollen-proof (for anemophilous grasses) or insect-proof (for entomophilous legumes) tents and a partitioned greenhouse with 12 confined compartments.

To date, 731 accessions made publicly available for sample delivery (Table 1) can be browsed online on the Siregal website of the Biology and Plant breeding department of INRAE (https://urgi.versailles.inra.fr/siregal), on the Florilege website (https://florilege.arcad-project.org/fr/collections) set up by the plant pillar (BRC4Plants) of the French National Research Infrastructure RARe (Bergheaud *et al*, 2025) and on the website of the European Search Catalogue for Plant Genetic Resources EURISCO (https://eurisco.ecpgr.org) with the holding institute field filled in as FRA001. These databases will be soon updated with the recently introduced accessions still missing in their records. All the species maintained in the *Prairies* genebank are listed in Annex I of the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA). Therefore, 531 of the publicly available accessions were notified in 2014 as a contribution of France to the Multilateral System of the ITPGRFA. An additional set of 321 accessions has been proposed in 2024 for further contribution to the Multilateral System (Table 1). Furthermore, passport data of 537 accessions from the natural diversity of grass species have been uploaded to the Global Biodiversity Information Facility (GBIF) database (https://www.gbif.org/publisher/0b5846f7-20b5-410a-93c5-5de83b522deb).

The *Prairies* genebank was labelled as a Biological Resource Centre by the network IBiSA (French network of biology, health and agronomy infrastructures) in 2011 and approved as a genebank of the plant pillar of the French National Research Infrastructure RARe in 2021. It was furthermore officially recognized as a curator of plant genetic resources for food and agriculture by the French Ministry of Agriculture in 2020. The *Prairies* genebank manages the French network for conservation of forage and turf species genetic resources, which includes INRAE and private companies actively breeding these species in France. In 2023, the *Prairies* genebank as well as the biotechnology and chemistry facilities of UR P3F were acknowledged for ISO9001:2015 certification.

Over the five last years, the *Prairies* genebank has distributed around 1,300 seed samples to various recipients, which were, by decreasing order of magnitude, French and foreign academic research institutions, French and foreign plant breeding companies, farmers and hobby-growers.

Uniqueness of the collections

The collections of natural populations maintained by the *Prairies* genebank originate from areas not or little represented in other genebanks. Notably, most of the grass and lucerne populations from northern Africa kept by the genebank are not maintained in their country of origin, and sometimes do not grow anymore in their site of origin, because of changes in climate and land use, excessive grazing or replacement by cultivars from modern breeding. Recent collections of natural populations undertaken by the INRAE research unit UR P3F in France and other European countries have confirmed that the *in situ* conservation of these populations is often threatened by the continuous regression of natural and permanent grasslands, climate change, and the corruption of their genetic integrity by overseeding with unrelated cultivars. The *ex situ* collections of grass and legume natural populations maintained by the *Prairies* genebank thus make a unique, although non-exhaustive, contribution to the preservation of the genetic diversity of these species. Furthermore, the *Prairies* genebank is the only repository of INRAE cultivars removed from national lists. However, the polygenotypic nature and the allogamous sexual reproduction of forage and turf species make the seed regeneration of accessions costly, even though the seed market for these species, and consequently the means for genetic resources conservation, are relatively limited. International networks of genebanks, like the Forages Working Group of the European Cooperative Programme for Plant Genetic Resources (ECPGR), are thus essential to share conservation efforts in these species. In the coming years, the *Prairies* genebank will aim to achieve a better sampling of the French heritage for genetic resources of forage and turf species in its collections. Present collections of orchard grass and tall fescue natural populations only sample a limited part of the French territory, and it will be aimed to complete the collections through new sampling campaigns in regions not visited so far. It will also be considered to sample the French natural diversity of legume species, such as white and red clover. Another important goal will be to work towards a more representative collection of cultivars, bred by INRAE and also other breeders, that were grown in France since the 1950s for all forage and turf species that had significant use.

Valorization of the collections

Initially set up to have genetic resources available to start breeding programmes, collections of natural populations may provide relevant materials to study the environmental distribution of inter- and intra-specific plant diversity. In this respect, 167 scientific papers from various international institutions have to date used the occurrence data of grass species forwarded by the *Prairies* genebank to the GBIF database. The main asset of these collections is their capacity to supplement occurrence data with phenotypic and genomic information already existing or newly generated. Earlier in this paper, we reported the use of phenotypic data to document adaptive differentiation in fine-leaved fescue taxa (Sampoux and Huyghe, 2009) and adaptive variability within perennial ryegrass (Balfourier and Charmet, 1991). More recently, the FACCE-JPI ERA-NET+ (https://www.faccejpi.net/en/faccejpi/actions/core-theme-1/facce-era-net-plus-on-climate-smart-agriculture.htm) project GrassLandscape gathered INRAE, IPK (*Leibniz Institut für Pflanzengenetik und Kulturpflanzenforschung* - Institute of Plant Genetics and Crop Plant Research, Germany), IBERS (Institute of Biological, Environmental and Rural Sciences, Wales, United Kingdom) and ILVO (*Instituut voor Landbouw-, Visserij- en Voedingsonderzoek* - Flanders Research Institute for Agriculture, Fisheries and Food, Belgium) to implement phylogeographic and landscape genomics analyses on a set of 470 accessions from the natural diversity of perennial ryegrass provided by 15 genebanks from European countries and the USDA. High throughput genotyping of these accessions allowed for the reconstruction of the expansion history of perennial ryegrass across Europe (Blanco-Pastor *et al*, 2019). Then, a multivariate landscape genomics analysis retrieved 633 potentially adaptive loci associated with either winter cold or summer drought and heat stresses and pointed phenotypic traits putatively conferring adaptation to these stresses (Blanco-Pastor *et al*, 2020). The adaptive relevance of these traits was further confirmed by a functional ecology approach (Keep *et al*, 2021). Results of the project GrassLandscape were then used to set up a European core collection of perennial ryegrass natural populations within the frame of an ECPGR Activity (Keep *et al*, 2020; Sampoux and Willner, 2022). Adaptive phenotypic and genomic variations revealed by the project GrassLandscape have furthermore been used to assess the future of perennial ryegrass local populations exposed to climate change by implementing genomic offset analyses (Pégard *et al*, 2023b). For other forage species than perennial ryegrass, collections maintained in European genebanks, and beyond, could likely provide sufficiently wide sets of natural populations to set up environmental niche models of species (Sampoux and Badeau, 2009) and reveal adaptive phenotypic and genomic diversities within species (see for example Boller *et al* (2010) for genetic resources and diversity in forage species). This information could be used to implement predictive analyses, such as genomic offset analyses, to foresee spatial shifts of adaptive diversity required for adaptation to ongoing changes in climate and other environmental factors (Fitzpatrick *et al*, 2021; Gougherty *et al*, 2021; Hung *et al*, 2023; Aitken *et al*, 2024; Zou *et al*, 2024). On this basis, strategies for *in situ* conservation could be developed, taking into account ongoing environmental changes on a European scale. For most forage and turf species that can spontaneously persist and evolve in long-duration grasslands, *in situ* conservation planned in this way would be the cheapest and most sustainable means to maintain valuable functional and adaptive genetic diversity in the long term. Incorporating adaption to future environmental changes across territories in *in situ* conservation programmes would contribute to maintaining an economically viable forage production on involved grasslands that would thus help the sustainability of these programmes.

Results of the project Grasslandscape also showed that the diversity of perennial ryegrass forage cultivars currently grown worldwide represents only a small part of the natural diversity of the species (Blanco-Pastor *et al*, 2019). Moreover, the H2020 European project EUCLEG (www.eucleg.eu) led by UR P3F studied a worldwide set of lucerne landraces and cultivars, which revealed clear phenotypic and genomic differentiation related to geographic origin and evidence that the cultivar diversity grown in Europe represents a limited part of the whole genetic diversity of the species (Pégard *et al*, 2023a). These results suggest that natural diversity, landraces and old cultivars of forage and turf species may still provide potentially useful genetic variability to adapt grown cultivars to upcoming challenges. Such genetic resources may be useful sources to adapt species to new combinations of climate constraints (Blanco-Pastor *et al*, 2020) and to improve their tolerance or resistance to biotic stresses (Sampoux and Badeau, 2009; Annicchiarico *et al*, 2015). They could also be valuable sources to develop cultivars adapted to cultivation in species mixtures. During the last five decades, forage and turf species have intensively been bred for performances in pure stands and it may be assumed that phenotypes more adapted to interactions prevailing in species mixtures (Litrico and Violle, 2015; Sampoux *et al*, 2020) could be recovered from natural diversity or genetic material that experienced only limited breeding (e.g. old cultivars). Furthermore, with the transition to agroecological practices, some forage species have emerged as service crops. They could be used as intermediary cover crops, e.g. Italian ryegrass, or as perennial living mulches, e.g. lucerne, red and white clovers, sainfoin (*Onobrychis viciifolia* Scop.) and birdsfoot trefoil (*Lotus corniculatus*). Cultivars bred for forage usage are probably not the best-adapted materials for these new uses (El-Ghazzal *et al*, 2024) and genetic resources may help to breed more suitable phenotypes.

However, introgressing allele diversity from raw genetic resources to elite cultivars may have become more and more challenging as continuously improved cultivars have been released. This may especially be the case for the allogamous and partially auto-incompatible forage and turf species, for which it can be assumed that the alleviation of the genetic load has been a substantial part of the genetic improvement (Kimbeng and Bingham, 1998; Annicchiarico *et al*, 2015). Genotyping based on genome sequencing should be expected to help the efficient introgression of desirable multigenic features from genetic resources to elite germplasm within a reasonable timespan. While investigations such as the ones of the project GrassLandscape have proven that collections of natural populations can be used to reveal the signature of natural selection (loci whose allele frequency variation departs from neutral expectations), it could be expected that collections of cultivars punctuating the history of modern breeding may reveal the signature of artificial breeding (see for example Welcker *et al* (2022) for such approach in maize). Marker-assisted selection methods could then possibly be designed to optimize favourable allele frequencies at both loci involved in desirable natural adaptations and loci that determine agronomic performances in the cultivars of forage and turf species, essentially bred as synthetics. Efforts to broaden, phenotype and genotype the collections of the *Prairies* genebank are to be conceived in order to contribute in these directions.

Author contributions

The manuscript was written by JPS with contributions from BJ and MG. All authors reviewed and commented the manuscript and approved the final version.

Conflict of interest statement

The authors have no conflicts of interest to report.

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