

# Grass and legume breeding matching the future needs of European grassland farming

Odd Arne Rognli<sup>1</sup>  | Luciano Pecetti<sup>2</sup> | Mallikarjuna Rao Kovi<sup>1</sup> | Paolo Annicchiarico<sup>2</sup>

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

<sup>2</sup>Council for Agricultural Research and Economics (CREA), Research Centre for Animal Production and Aquaculture, Lodi, Italy

## Correspondence

Odd Arne Rognli, Department of Plant Sciences, Faculty of Biosciences, Norwegian University of Life Sciences, NO-1432 Ås, Norway.  
Email: oddro@nmbu.no

## Abstract

Plant breeding can be pivotal to produce new grassland varieties with better adaptation to changes in seasonal pattern and increased variability of temperature, precipitation and length of the growing seasons as determined by climate change. Cultivation of legume species is expected to increase, and will make crop–livestock systems more sustainable and self-sufficient for feed proteins. In northern Europe, the higher temperatures and longer growing season will create opportunities for increasing yield, while in southern Europe greater drought tolerance and growth in the cool seasons will be indispensable to maintain crop yields. Greater intra- and interspecific diversity is recommended for grasslands in both regions. In the north, also broader genetic diversity, particularly in terms of adaptation pattern (response diversity), is needed, requiring pre-breeding to enlarge the gene pool by introgression of exotic, less adapted genetic resources as a first step. Genetic gain in forage crop breeding has been modest over time, and new breeding methods need to be implemented to speed up the development of new cultivars with proper adaptation to future climate conditions. Novel technologies such as genome-enabled selection and high-throughput phenotyping will play a major role in this context. They are rapidly evolving and increasingly adopted, albeit with technical challenges and a need for optimization of breeding schemes for optimal exploitation.

## KEYWORDS

adaptation, drought tolerance, genomic selection, phenomics, pre-breeding, species mixtures

## 1 | INTRODUCTION

The global climate change is the most important challenge for breeding of forage crops in the near future. The main climate change effects are higher average temperatures, greater climate variability with extreme events, changing precipitation patterns, drought and temperature stress, more wind, less snow cover in continental regions giving more direct frost and ice encasement, and increased CO<sub>2</sub>

content in the atmosphere (Ergon et al., 2018; Olesen et al., 2011). This will create new types of abiotic and biotic stresses, requiring increased efforts in breeding new cultivars with improved adaptation to the new climatic conditions. Warming is more rapid in northern regions than the global average, with the largest increases during late autumn, winter and spring. These climate changes will most likely improve the conditions for forage production in these regions, owing to the longer (1–3 months) growing seasons with milder and rainier autumns and winters (Olesen et al., 2011). However, it will be

An earlier version of this article was a keynote presentation at the 28th General Meeting of the European Grassland Federation hosted by Finland in 2020 ([www.europeangrassland.org/](http://www.europeangrassland.org/)).

This is an open access article under the terms of the Creative Commons Attribution-NonCommercial License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2021 The Authors. Grass and Forage Science published by John Wiley & Sons Ltd.

more challenging to harvest the increased biomass in late autumn due to the higher precipitation.

The foreseen greater incidence of drought and higher frequency of extreme climatic events in southern Europe (Alessandri et al., 2014) will affect negatively the productivity of crop–livestock systems. Drought is the main environmental factor influencing forage crop yield, persistence and germplasm × environment (GE) interaction in the Mediterranean basin (Annicchiarico, Pecetti, Abdelguerfi, et al., 2011; Annicchiarico, Pecetti, Bouzerzour, et al., 2011). The increasing costs and/or decreasing availability of irrigation water can exacerbate the negative direct effects of the climate. Therefore, there is a need for forage crops with greater tolerance to stressful conditions. In general, breeding varieties more tolerant to drought are expected to play a crucial role in climate change adaptation and mitigation strategies (Ceccarelli et al., 2010).

Other trends and policies affecting the priorities and breeding goals in the near future are the UNs Sustainable Development Goals (SDG). To meet these goals, grassland systems need to be more resilient, and sustainable intensification should provide more output with less input, lower environmental footprints, including GHG emissions, and more ecosystem services (biodiversity, pollinators, soil and water quality). A key issue is represented by greater self-sufficiency of livestock feed that is produced locally, especially for feed protein, which is largely based on imported resources (mainly soybean) today. This context places greater emphasis on the breeding and cultivation of legume species and the selection of varieties more resilient and with higher nutritive quality and digestibility.

## 2 | HOW TO ADAPT NEW CULTIVARS OF FORAGE CROPS TO THE FUTURE CLIMATE?

Requirements of plant materials and associated breeding goals for adaptation to and mitigation of climate change effects are indicated in Table 1. Greater intra- and interspecific diversity is recommended for all growing seasons in the north and south of Europe. Broader

genetic diversity with more response diversity is needed for northern Europe. Increased growth during cooler and wetter seasons and stronger summer dormancy and/or active recovery after drought for perennials, maintenance of growth under moderate drought for annuals, and more persistent seed banks for self-reseeding annuals are in sharp focus in breeding for the Mediterranean region (Table 1).

## 3 | GRASSLAND SPECIES IN NORTHERN EUROPE—CHARACTERISTICS AND ADAPTATION TO FUTURE CLIMATE AND MANAGEMENT

In contrast to southern Europe, grasslands in northern Europe consist exclusively of perennial species. The main grass species are perennial ryegrass (*Lolium perenne* L.), timothy (*Phleum pratense* L.), meadow fescue (*Festuca pratensis* Huds.), *Festulolium*s (×*Festulolium* spp.) and tall fescue (*Festuca arundinacea* Schreb.), whereas the main forage legume species are red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.). Lucerne (*Medicago sativa* L.) is currently used very little but has a potential for increased cultivation following climate change. Mäkinen et al., (2018) studied diversity in response to agroclimatic variables and the adaptive capacity of forage crop cultivars under climate change. This investigation was based on records of dry-matter yields (DMY) obtained on multi-location official variety trials in Finland in the period 2000 to 2012. They found that timothy and meadow fescue cultivars had a low adaptive capacity, thus increased genetic variation is needed, particularly for tolerance to the expected higher temperatures during the growing season. Red clover cultivars were insufficiently adapted to both warm and cold winters, while tall fescue and *Festulolium* were susceptible to drought in autumn. However, *Festulolium* hybrids showed good capacity to adapt to climate change due to high response diversity, which might be explained by their genetic diversity. Although these results were obtained by studying cultivar responses to the climate of Finland, which is rather continental,

**TABLE 1** Requirements of plant materials and associated breeding goals for adaptation to and mitigation of climate change effects (modified from Ergon et al., 2018)

Region in Europe	Growing season <sup>a</sup>	Unfavourable season <sup>b</sup>
Northern	More intra- and interspecific diversity, broader genetic material with more response diversity	More intra- and interspecific diversity, broader genetic material with more response diversity
	Higher regrowth capacity	Utilize earlier spring and later autumn without losing ability to survive winters
	Maintenance of growth in water-saturated soils and during dry spells	
Southern	More intra- and interspecific diversity	More intra- and interspecific diversity
	Greater ability to grow in cool months (e.g. alfalfa without autumn dormancy)	Stronger summer dormancy and active recovery after drought in perennials
	Maintenance of growth under moderate drought	More persistent seed bank of annuals

<sup>a</sup>Northern Europe: April–early June to mid-September–early November; Southern Europe: October–November to June

<sup>b</sup>Northern Europe: Mid-September–early November to April–early June; Southern Europe: July to September–October

they are valid for most Nordic and Baltic regions. An example of how diversity can be increased is the intra-specific diversification by introgressing exotic germplasm into the adapted gene pool to improve northern adaptation of perennial ryegrass (*Lolium perenne* L.), which is the main objective of the Nordic pre-breeding project described below (Rognli et al., 2018).

The future climate will be more maritime also in continental regions in the north. Under these conditions, active photosynthesis during warmer autumns before snowfall and freezing may be critical for overwintering (Østrem et al., 2018). Cultivars of adapted species, e.g., timothy and meadow fescue, which cease growth early due to shorter photoperiods and reduce photosynthetic activity in the autumn, will be less winter hardy (Dalmannsdottir et al., 2017); this may be related to increased respiration rates and depletion of reserves during the expected warmer autumns and winters. Growth cessation of plant materials of non-adapted species, such as perennial ryegrass and *Festulolium*, is less controlled by photoperiod. Thus, in the future longer and warmer autumns, these plant types will keep on growing in the autumn under low light intensities. Although this may help compensate the increased respiration rates, this has been shown to delay cold acclimation and reduce winter survival (Østrem et al., 2015).

#### 4 | GRASSLAND SPECIES IN SOUTHERN EUROPE—CHARACTERISTICS AND ADAPTATION TO FUTURE CLIMATE AND MANAGEMENT

Livestock systems of southern Europe have largely relied upon natural grasslands. However, cultivated annual legumes such as berseem clover (*Trifolium alexandrinum* L.) and Persian clover (*T. resupinatum* L.) in pure stand or in mixture with grasses have gained importance as short-term rain-fed forage crops. Likewise, common vetch (*Vicia sativa* L.) and pea (*Pisum sativum* L.) can be valuable for forage production, particularly in moderately favourable environments and in association with cereals (Annicchiarico et al., 2017). Although the growth of these species occurs largely during the cool and moist season, increased tolerance to spring drought will be increasingly important under the changing climate. Some level of cold tolerance is needed as well for adaptation to areas prone to low-temperature winters, such as inland areas of Italy (Piano & Pecetti, 2010). Recently, annual self-reseeding legumes have also raised an agronomic interest in southern Europe. Adapted cultivars of subterranean clover (*T. subterraneum* L.) have been selected from local germplasm to exploit pasture productivity and durability thanks to the peculiar plant's morphophysiology and tolerance to grazing (Nichols et al., 2013). The main selection criteria included suitable maturity for the target environments, high forage and seed yield, good seed-burying capacity and high grazing tolerance. Selection goals for key adaptive traits were derived from ecological studies assessing the relationships between trait variability and climatic conditions of germplasm at collection sites (Piano et al., 1996).

Compared to annuals, perennial species allow to extend and regularize the feeding season because of prompt regrowth upon

the autumn rains and better exploitation of the residual moisture in late spring. A reduction in the water consumption by adopting rain-fed cropping or limited irrigation can be pursued by selecting adapted germplasm. While high water-use efficiency provided by extensive growth during the cool rainy season is a desirable trait for annuals, perennials should possess drought tolerance/avoidance mechanisms enabling them to survive through repeated dry summers (Annicchiarico, Pecetti, Bouzerzour, et al., 2011; Annicchiarico et al., 2013). Different plant species and types, relying on different adaptation strategies, can be useful to cope with different drought stress levels and patterns. Multi-site evaluation across a range of increasingly stressful conditions and subsequent modelling of cultivar yield responses can allow to predict possible shifts of top-performing species and cultivars and useful adaptive traits as a consequence of climate change (Annicchiarico, Pecetti, Abdelguerfi, et al., 2013).

Lucerne is the main forage crop in southern Europe, where it is usually grown under irrigation in low-rainfall areas. Reducing or withholding lucerne irrigation in summer (when the crop water-use efficiency is lowest and water may be precious for alternative uses), or adopting rain-fed cropping, can produce remarkable water savings when using tolerant germplasm, as reported in Annicchiarico, Pecetti, Abdelguerfi, et al., (2011) for the western Mediterranean basin. Remarkable crossover GE interaction between top-yielding cultivars occurred across moisture-contrasting environments as a consequence of different and partly incompatible adaptive traits (Annicchiarico, Pecetti, & Tava, 2013), emphasizing the importance of selecting distinct varieties for relatively favourable and severely drought-stress conditions. Breeding lucerne for specific adaptation to irrigated or rain-fed cropping exhibited distinctly greater genetic gains than breeding for wide adaptation, even for a relatively favourable region such as northern Italy (Annicchiarico, 2007a), also because farm landraces (which may still represent a core genetic base for forage crop breeding) displayed specific adaptation to conditions under which they evolved (Annicchiarico & Piano, 2005).

Cocksfoot (*Dactylis glomerata* L.) and tall fescue are the most important perennial grass species in southern Europe. For long, these species have only been bred for temperate-climate regions, displaying misadaptation to environments of southern Europe (Annicchiarico, Pecetti, Bouzerzour, et al., 2011). Summer dormancy (i.e., the complete aerial senescence at the end of spring irrespective of the water availability: Volaire et al., 2009) is especially useful for cocksfoot plant survival under severe drought (Annicchiarico, Pecetti, Bouzerzour, et al., 2011), whereas incomplete dormancy (Voltaire et al., 2009) and dehydration tolerance (by which sufficient moisture is maintained in leaf basal tissues even at low soil water potential: Voltaire, 2008) favour plant adaptation to moderate drought and can be useful in breeding drought-tolerant material that is responsive to possible water availability in summer (Piano et al., 2004). Mediterranean germplasm of tall fescue relies on dehydration delay related with water uptake from deep roots (Voltaire & Lelièvre, 2001), although mechanisms of dehydration tolerance can be present (Norton et al., 2006). Selection of tall fescue germplasm with wide adaptation across diversified Mediterranean drought

stress levels seemed feasible (Pecetti et al., 2011). Mediterranean germplasm of tall fescue tended to be better yielding than that of cocksfoot (Annicchiarico, Pecetti, Abdelguerfi, et al., 2013), likely because of greater cool season growth with subsequent higher water-use efficiency and deeper and larger root system (Lelièvre et al., 2011), thereby displaying an optimal combination of endurance to summer drought and high growth under favourable moisture availability.

## 5 | PRE-BREEDING

In general, increased intra- and interspecific diversity is needed in order to adapt to and mitigate the effects of climate change (Table 1). Therefore, more emphasis should be devoted to pre-breeding activities. Pre-breeding is defined as *all activities designed to identify desirable characteristics and/or genes from unadapted materials that cannot be used directly in breeding populations and to transfer these traits to an intermediate set of materials that breeders can use further in producing new varieties for farmers* (The Global Partnership Initiative for Plant Breeding Capacity Building [GIPB]).

### 5.1 | Northern Europe

With respect to breeding for climate change in northern Europe, focus is justified on increasing genetic diversity by introgressing exotic material in species that lack sufficient variation for key traits conferring adaptability, e.g., winter hardiness (freezing and ice-encasement tolerance), timing of growth cessation and utilization of longer growing seasons, and resistance to fungal diseases which either cause more damage in the new climate or are moving further north as the climate changes. One example is the Nordic Public-Private Partnership (PPP) on pre-breeding (Nilsson et al., 2016), running since 2012 with one of its projects on perennial ryegrass. This species has superior feed quality and productivity, and is especially well adapted to the coastal climate of Western Europe (Humphreys et al., 2010). With climate change, we expect its cultivation area to expand further north and east, which implies crop improvement for tolerance to snow mould, better control of growth cessation in the autumn in order to improve winter survival and persistence and increased resistance to crown rust (Østrem et al., 2015, 2018). Ryegrass genetic diversity is restricted, since the species is not native to the northern and continental regions (Rognli et al., 2013). Field testing of Nordic and Central European cultivars across five locations, spanning from continental Estonia to maritime Iceland, found that cultivars developed in the north in general are best adapted albeit showing large GE interactions (Helgadóttir et al., 2018). Thus, a major objective of the pre-breeding project in perennial ryegrass is to introgress and recombine exotic materials with existing breeding populations, to serve as new genetic resources to breed cultivars for the future climate of these regions.

### 5.2 | Southern Europe

The current knowledge on useful plant types of forage crops for European Mediterranean regions is likely to be modified by the requirements imposed by the predicted greater incidence of drought. Breeders ought to intercept information on novel germplasm or unexploited adaptive mechanisms and transfer it into their crop improvement programmes, as shown by the following examples. Results on cocksfoot suggested different genetic resources, plant types and adaptation patterns for North Africa and southern Europe. The completely summer-dormant germplasm of *D. glomerata* subsp. *hispanica* currently has prevalent interest for North Africa but will gain adaptive potential for Mediterranean-climate regions of Europe to ensure summer survival under increasing drought severity (Annicchiarico, Pecetti, Bouzerzour, et al., 2011). Piano et al., (2005) reported a coevolutionary specificity between the native Mediterranean tall fescue germplasm and its associated *Neotyphodium* endophyte, which could enable the selection of novel endophyte strains for targeted infection aimed at increasing the drought tolerance of the host crop (Malinowski et al., 2005). Irrespective of their endophyte status, however, Mediterranean native populations proved more drought tolerant than 'continental' varieties (Pecetti et al., 2007), and their introgression into the latter could be envisaged to enhance the crop's drought tolerance in southern Europe. In lucerne, single traits or combinations of traits (plant architectures) associated with specific adaptation to drought stress were identified that can be exploited for selection (Annicchiarico, 2007b; Annicchiarico, Pecetti, & Tava, 2013). The integration of adaptive trait-based and yield-based selection may increase the selection efficiency and enable the detection of the best-performing material at early selection stages. In some cases, the relevance of adaptive traits for selection is modulated through the soil type (Annicchiarico, 2007b). The use of managed environments with an accurate control of drought stress levels can be an asset to anticipate the needs that would arise due to climate change and minimize the effect on selection of year-to-year rainfall variation (Annicchiarico, 2007a). Prior knowledge borrowed from the relationships between the features of native populations and their environments of origin can provide valuable guidelines for selecting adapted varieties, as in Piano et al., (1996), who reported an adjustment of flowering time, seed size and hardseededness for subterranean clover populations that can help define distinct ideotypes for contrasting environments of Sardinia.

## 6 | BREEDING FOR MIXTURES

Forage crops are frequently grown in mixed stand (MS), annual legumes with cereals and perennial legumes with forage grasses, either in binary or complex mixtures. In general, grass-legume mixtures produce higher yields than the pure stands (PS) of each species, and better nutritive quality than the grass PS (Annicchiarico et al., 2019), while contributing to more sustainable cropping systems in various

respects (Lüscher et al., 2014). In addition, legumes benefit more from higher atmospheric CO<sub>2</sub> concentration than non-N-fixing species, and this could compensate for the decline in protein content that is expected to occur in grasses in a future scenario with elevated CO<sub>2</sub> and higher temperatures (Thivierge et al., 2016).

Forage species are usually selected in PS, with seed for mixtures pooled later on by the seed companies. However, some cultivar combinations are more compatible than others, and direct selection under target MS conditions can be preferable in various cases. In particular, a recent review of research on breeding for mixed cropping (Annicchiarico et al., 2019) indicated that: (a) averaged across several studies, selection in PS exhibited about 40% lower predicted yield gains than selection in MS; (b) selection under MS tends to be particularly important for species undergoing severe competitive stress because the genetic correlation for genotype yield response across PS and MS tends to increase as a function of the biotic environment occupied by the associated species in MS—as shown for forage crops by the study of Annicchiarico and Piano (1994); and (c) breeding for compatibility with a wide range of plant companions is encouraged by the larger size of general compatibility (alias general mixing ability or ecological combining ability) effects relative to specific compatibility effects.

Hill (1990) and Sampoux et al., (2020) proposed recurrent selection schemes aimed to improve the general compatibility of populations bred for two species aimed to be intercropped, a procedure predictively more efficient than selection in pure stand on the one hand and selection of specific pairs of components on the other (Sampoux et al., 2020). Selection of just one species for general compatibility is also possible and proved highly successful in white clover, where direct selection in MS performed simultaneously with two highly competing grass varieties led to distinctly improved clover compatibility and competitive ability in association with several grass cultivars of different species (Annicchiarico & Proietti, 2010). However, selection under MS may imply greater evaluation costs than in PS, and its convenience ought to be weighed against the expected increase of breeding progress. Upon identification of morphophysiological traits conferring relatively better performance in MS, selection for MS could also be performed by selection in PS based on these traits pooled into a selection index (Annicchiarico, 2003). There is a need for exploring the efficiency of novel and relatively low-cost breeding strategies, among which genomic selection and evolutionary breeding emerge as the most promising ones (Annicchiarico et al., 2019).

## 7 | THE PROSPECTS OF IMPLEMENTING HIGH-THROUGHPUT PHENOTYPING IN BREEDING FORAGE CROPS

Breeding forage crops is probably the most complex and costly type of breeding. Phenotyping a range of traits over several seasons, with biomass yield measurements taken over several cuts each season, is laborious, time consuming and costly. In addition, heterogeneous populations are tested over several locations often with large GE

interactions, making precise phenotyping very important in order to select superior genetic materials.

Genomic resources with high-throughput molecular markers are fast developing for forage crops (Byrne et al., 2015; De Vega et al., 2015). High-throughput phenotyping (HTP) of important traits can be important to capitalize timely on the genomic information to develop genome-enabled breeding in forage crops. NIRS (Near Infrared Reflectance Spectroscopy) has long been used to rapidly phenotype forage quality traits, but in-field measurements are difficult to develop with this technology (Walter et al., 2012). Nevertheless, Smith et al., (2019) used a field spectrometer to collect canopy spectra of individual ryegrass plants to develop predictive models for nutritive value traits (ADF, ash, CP, IVVDM, IVVOMD, NDF and WSC) relevant for breeding. The results were promising and could make breeding for nutritive value high-throughput and more efficient. What is needed in a plant breeding context is real-time, non-destructive and in-field high-throughput measurement of yield and other key traits. Recent years have seen a rapid development and application of various sensors (multispectral, hyperspectral, thermal and RGB), and laser scanning (LiDAR) as part of various UAS (Unmanned Aerial Systems) plant phenotyping platforms (see reviews by Gebremedhin et al., 2019a and Yang et al., 2020).

Reliable HTP would be highly valuable also to reduce the phenotypic selection costs, thereby allowing for evaluating more material and applying greater selection intensities. For perennial forages, which typically feature much greater within-population than among-population variation for agronomic traits of landraces or varieties (Annicchiarico et al., 2015), HTP would provide greater opportunities to exploit within-population diversity of genetic resources.

The question is whether precise phenotyping of complex traits with crucial importance for adaptation can be established using HTP technologies. The most important and costly trait to phenotype is biomass yield. At different stages during the breeding scheme, breeders need to phenotype both single plants, row plots, simulated swards and sward plots. Visual scoring is used to phenotype many traits, also yield, especially on single plants and row plots early in the breeding process. Dry-matter yield (DMY) is best estimated by weighing families tested in sward plots. A challenge for phenotyping yield is the low correlation between yield estimated on single plants and row plots and sward plots (Annicchiarico, Barrett, et al., 2015). Nevertheless, Ghamkhar et al., (2019) used LiDAR to estimate yield traits in single and paired-row plots of perennial ryegrass with high level of accuracy. Significant but highly variable correlations ( $r = 0.12-0.93$ ) were reported between visual scores of biomass yield and normalized difference vegetation index (NDVI) values on spaced plants, and between biomass yield and NDVI in row plot and sward trials in perennial ryegrass (Wang et al., 2019). NDVI obtained from multispectral sensors and UAS can replace visual scoring in spaced plant trials. Gebremedhin, Badenhorst, Wang, Giri, et al., (2019) developed a promising method for high-throughput phenotyping of herbage yield of spaced plants by combining NDVI and ultrasonic sonar estimates of plant height. Viljanen et al., (2018) used machine learning to integrate various multispectral remote

sensing features to accurately estimate biomass of grass swards; this could be relevant both for practical farming and breeding. Østrem, Rapacz, et al., (2018) compared chlorophyll fluorescence (Chl-*a*) with traditional whole-plant freezing tests in selection for freezing tolerance in a *Festulolium* population. They found that both methods were effective but gave selections with rather different adaptations. Selection by Chl-*a* gave populations with improved winter survival by maintaining active photosynthesis during the expected warmer autumns, especially in coastal climates, while selection by traditional freezing tests creates populations with early growth cessation and reduced photosynthetic activity. These populations show a typical northern continental adaptation but will be less winter-hardy possibly due to increased respiration and depletion of reserves during the warmer autumns and winters. NDVI measurements on sward plots in the field confirmed the differential adaptation patterns created by the two selection methods. Higher temperatures and associated droughts are expected to increase in prevalence in Europe, especially in the south.

Root phenotyping relevant for field conditions is exceedingly difficult, and systems for such high-throughput root phenotyping were apparently never reported for forage crops. Yates et al., (2019) established a non-destructive and largely automated phenotyping platform to collect leaf growth data under various water-limiting conditions. They were able to precisely quantify the genotypic response of water deficit on leaf growth.

## 8 | GENOME-ENABLED BREEDING IN FORAGE CROPS

The rate of breeding progress for forage crop yields has been low. For perennial ryegrass, Sampoux et al., (2011) reported an annual rate of 0.32% (with hardly any improvement for spring growth), whereas McDonagh et al., (2016) reported rates of 0.52% under conservation and 0.35% under simulated grazing. For perennial legumes, annual genetic gains ranged between nil and 0.26% for lucerne, while ranging between 0.53% and 0.60% for red or white clover (Annicchiarico, Barrett, et al., 2015). Hence, new breeding technologies are needed to raise the breeding progress of these crops. Most perennial forage species are outbreeding, frequently with a polyploid, large and highly heterozygous genome. Lack of reference genomes makes it challenging to develop informative and good molecular marker systems. The value of phenotyping single plants/parents is much less than in selfing species since the relevant unit of evaluation is half-sib or full-sib families (with possible selection of cloned parent plants or combining inter- and intra-family selection). Currently it may take 15–20 years to place a new forage crop cultivar on the market. Part of this time lag is progeny testing of families in sward plots, which may take up to 7–8 years (including progeny development and recombination of selected parents). Shortening the breeding cycle is of utmost importance, also to cope with variety needs for the changing climate. Genomic selection (GS) has potential for substantially ease these challenges. It was developed to raise the genetic gain for

complex quantitative traits by shortening the selection cycles and reducing expensive, time-consuming phenotyping. In GS, a germplasm sample (training population) that represents a genetic base (breeding population) is phenotyped and genotyped to construct a statistical model for prediction of breeding values (GEBVs) which, upon successful validation, is used to predict GEBVs of genotyped independent individuals belonging to the breeding population (Meuwissen et al., 2001). Precise phenotypic data and appropriate modelling are crucial requirements for accurate genomic predictions (Bernal-Vasquez et al., 2014). Simple sequence repeat (SSRs) markers, which played a role in traditional marker-assisted selection, are not appropriate for GS because of their small number. Genotyping by sequencing (GBS) (Elshire et al., 2011), by which thousands of genome-wide single nucleotide polymorphic (SNP) markers are produced, is a promising cost-effective approach. GBS can also be used on population and family basis for estimation of allele frequencies of SNPs at genome-wide positions (Byrne et al., 2013). A range of statistical (linear and non-linear) regression models are being applied in GS to predict GEBV (see Table 2.). Recent machine learning techniques like deep multi-layered neural network (DNN) have been developed to improve genomic predictions (Montesinos-López et al., 2019) and showed an advantage over traditional GS models in some studies on inbred crops (González-Camacho et al., 2018), although their ability to also capture a portion of non-additive genetic effects (Lin et al., 2014) suggests some caution for use in synthetic variety breeding, unless selecting quite narrow-based cultivars.

Genomic selection in forage crops was first outlined by Hayes et al., (2013). Annicchiarico, Barrett, et al., (2015) recommended to evaluate phenotypes of genotyped plant material based on sward plots of their half-sib or selfed progenies rather than as cloned plants, in order to model the additive genetic variation (the only relevant for synthetic variety improvement), represent more faithfully actual production environments, and ease the preservation of mapping/reference populations and the implementation of multi-environment experiments. GS application to half-sib progeny-based selection would be consistent with the greater efficiency of this selection scheme relative to other schemes involving clonal or selfed progeny-based evaluations in a recent comparison of nine lucerne breeding methods (Annicchiarico & Pecetti, unpublished data). An example of this strategy was reported for lucerne biomass yield in Annicchiarico, Nazzicari, et al., (2015), where predicted yield gains by GS clearly exceeded predicted gains by phenotypic selection because of the low narrow-sense heritability of the yield trait and the longer cycle of phenotypic selection. These results were encouraging also in view of the fact that they were obtained by treating this autotetraploid species as if it was a diploid for SNP data-based predictions, i.e., distinguishing one heterozygote class (pooling the actual Aaaa, AAaa and AAAa classes) and two homozygote classes, because of lack of sufficient number of reads for reliable allele dosage estimation. Greater sequencing efforts allowed for by decreasing genotyping costs may allow to increase the GS predictive accuracy in the future by expressing predictions as a function of estimated allele dosage values.

**TABLE 2** Overview of genomic prediction studies in forage crops

Species	Material	No. of SNP markers	Statistical models	Traits	Accuracy	Reference	
Perennial ryegrass	Breeding populations (365)	3K SNP chip	GBLUP, KNN, RF, GBM	Biomass yield	0.01–0.22 <sup>b</sup>	Grinberg et al., (2016)	
				DM digestibility	0.07–0.41 <sup>b</sup>		
				WSC	0.27–0.59 <sup>b</sup>		
	F <sub>2</sub> families (1515)	137,191 <sup>a</sup>	185,297	188,832	Biomass yield	0.34 <sup>c</sup>	Guo et al., (2018)
					Heading date	0.77 <sup>c</sup>	
					Crown rust resistance	0.55 <sup>c</sup>	
	Half-sib families (517)	1,023,011	GBLUP, KGD-BLUP, RR, RF	GBLUP, KGD-BLUP, Bayes C $\pi$	Heading date	0.40–0.52 <sup>b</sup>	Faville et al., (2018)
					18 nutritive traits	0.16–0.45 <sup>b</sup>	
	F <sub>2</sub> families (1757)	144,7,122	GBLUP	Heading date	0.9 <sup>b</sup>	Gebremedhin, Badenhorst, Wang, Giri, et al., (2019))	
	Single plants (1,800)	217,563	rrBLUP, Bayes B, Bayesian Lasso, RF	Crown rust	0.49–0.52 <sup>b</sup>	Arojju et al., (2018)	
Lucerne	Genotypes (322)	44,757	Bayes A, B and C $\pi$	ADF	0.18 <sup>d</sup>	Jia et al., (2018)	
				Crude protein	0.05 <sup>d</sup>		
				Biomass yield	0.12 <sup>d</sup>		
				Plant regrowth	0.50–0.51 <sup>d</sup>		
	Genotypes (154)	11,450	rrBLUP, Bayes B, Bayesian Lasso	Leaf protein content	0.40 <sup>b</sup>	Biazzi et al., (2017)	
	NDF digestibility at 24 hr	0.30 <sup>b</sup>					
	Genotypes–subcontinental climate (124)	7,000	rrBLUP, RR, Bayes A, B, Bayesian Lasso	Biomass yield	0.32 <sup>b</sup>		Annicchiarico, Nazzicari, et al., (2015))
	Genotypes–Mediterranean climate (154)	11,000	rrBLUP, RR, Bayes A, B, Bayesian Lasso	Biomass yield	0.35 <sup>b</sup>		
Cloned genotypes	10,000	rrBLUP	Biomass yield	0.22–0.40 <sup>b,e</sup>	Li et al., (2015)		

Abbreviations: GBLUP, genomic best linear unbiased prediction; GBM, gradient boosting machine KGD-BLUP, kinship using GBS with depth adjustment–best linear unbiased prediction; KNN, k-nearest neighbour; RF, random forest; RFF, random forest fitness; RR, random regression; rrBLUP, ridge-regression best linear unbiased prediction; SNP, single nucleotide polymorphism.

<sup>a</sup>Marker type in all studies are GBS: SNP markers derived by genotyping by sequencing.

<sup>b</sup>Pearson's correlation coefficients between genomic estimated breeding values (GEBVs) and observed phenotypes.

<sup>c</sup>Pearson's correlation coefficients between GEBVs and phenotypes by squared root of heritability.

<sup>d</sup>Predictive accuracy measured as the correlation between the EBVs and GEBVs.

<sup>e</sup>Training in one generation for prediction in the following generation.

There is a paucity of results on GS of perennial forages, all relative to perennial ryegrass and lucerne, which are summarized in Table 2. Prediction accuracies ranged from low to good (considering the reproductive systems) for DMV (0.01–0.40) and nutritive value traits (0.05–0.45), confirming in most studies the potential interest of GS. For perennial ryegrass, high predictive abilities were observed for mineral traits like S, Na and Mg (0.44–0.45), while the lowest predictive abilities were found for P (0.16), digestibility (0.22) and high

molecular weight WSC (0.23) (Arojju et al., 2020). Predictive ability estimates for most nutritive traits were retained when marker number was reduced from one million to as few as 50,000. For lucerne, the moderate GS predictive ability of NDF digestibility is comforting, in view of the crucial impact of this trait on cattle dry-matter intake and milk yield (Oba & Allen, 1999). These results highlight another potential advantage of GS relative to phenotypic selection, namely easier and more cost-efficient simultaneous selection for

several traits (e.g. yield and one or more forage quality traits), as GS implies the same genotyping cost when targeting one or several traits, whereas phenotypic selection implies raising costs as a function of the number of evaluated traits. Preliminary GS results for dry-matter yield in timothy, relative to a training population of 720 full-sib (FS) families tested in 3-year field trial sown twice at 2–3 locations of Norway, are quite promising (Rognli *et al.*, unpublished data). GEBV predictions for DMV exhibited outstanding predictive accuracies (>0.90) detected by both gBLUP and machine learning models (RKHS). Validation of the prediction models in unrelated FS families (213 FS families, tested 3 years at two locations) showed good prediction accuracies for a couple of biomass yield recordings (0.45 for DMV first cut second year; 0.29 for total DMV second year). However, predictions of other yield recordings and traits gave very low correlations, in the same range as detected by Grinberg *et al.*, (2016) in perennial ryegrass.

GS may be applied directly to the breeding population, or to a subset of elite candidate parents selected from the breeding population by a preliminary stage of stratified mass selection. Such a stage, envisaged of short duration (16 month), proved cost-efficient for lucerne biomass improvement prior to half-sib progeny-based selection under field conditions, suggesting that it may be useful also for GS at current phenotyping and genotyping costs (Annicchiarico & Pecetti, unpublished data).

Applying GS to a narrow-based breeding population can increase the GS prediction accuracy, but the inference space of the resulting GS model is expected to be particularly narrow, thereby requiring extensive phenotyping and genotyping efforts to develop GS models for several different breeding populations. Breeding populations with moderately large genetic variation may be more convenient in the absence of large budgets for phenotyping because GS predictions, albeit of lower accuracy, may apply to a large portion of a breeder's genetic base. This option is supported by the only moderate loss of predictive accuracy (25%–30%) passing from intra-population predictions to cross-population predictions of biomass yield for sub-continental and Mediterranean germplasm of lucerne in Annicchiarico, Nazzicari, *et al.*, (2015).

High phenotyping costs may lead to suboptimal training population sample size for GS of forage crops. However, GS predictions of practical value for biomass selection could be obtained for a broadly based breeding population of alfalfa using a training set of just 124 genotypes (Annicchiarico, Nazzicari, *et al.*, 2015). Also, a modest increase in prediction accuracy was expected beyond the training population threshold of 200 individuals, in a simulation study targeting open-pollinated crops that assumed low trait heritability and 5,000 SNP markers available (Viana *et al.*, 2016).

GS models for cultivars adapted to future climates could be developed by combining historical data from multi-location yield trials with climate, soil and genomic data. Prediction of the effects of changing environments on performance will help breeders to compare the results over multiple years, to gain information about how experimental varieties will likely perform in a target environment.

Genome editing could be of interest for specific mono- or oligogenic traits of key importance, if genome-edited material could avoid the challenges for commercialization faced by transgenic varieties. For example, transgenic lucerne cultivars bred for higher digestibility as a consequence of modified lignin composition (Guo *et al.*, 2001) are likely to be obtainable also via genome editing.

## 9 | CONCLUSIONS

The combination of new HTP and GS methods may require some changes to future breeding schemes for optimization. For example, if HTP could be applied to the evaluation of a very large number of genotypes grown as non-replicated, relatively close-spaced plants according to a stratified mass selection layout (Annicchiarico, 2004), this evaluation stage would be used for preliminary phenotypic selection of fairly high numbers of genotypes (on the basis of biomass production and other traits that could meaningfully be recorded on individual plants). The selected material could undergo GS for biomass production and a few other key traits (e.g. one or two forage quality traits) by exploiting GS models that were defined at an earlier stage for the same genetic base, selecting genomically a set of plants used as parents of a new synthetic variety. This candidate variety would finally be assessed under field conditions against top-performing commercial varieties before registration. As anticipated, GS could be particularly useful to decrease the cost of breeding for mixed cropping, thereby reversing whenever convenient the current practise of selection in pure stand. Cross-population predictive ability results will be essential to understand the size, genetic diversity and number of genetic bases (ranging from only one, large and highly diversified to several relatively narrow based) used by a breeding programme for variety selection.

### AUTHOR CONTRIBUTION

**Odd Arne Rognli:** Conceptualization (lead); Writing-original draft (lead); Writing-review & editing (equal). **Luciano Pecetti:** Writing-original draft (supporting); Writing-review & editing (supporting). **Mallikarjuna Rao Kovi:** Writing-original draft (supporting); Writing-review & editing (supporting). **Paolo Annicchiarico:** Writing-original draft (equal); Writing-review & editing (equal).

### DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analysed in this study.

### ORCID

Odd Arne Rognli  <https://orcid.org/0000-0002-5787-752X>

### REFERENCES

Alessandri, A., De Felice, M., Zeng, N., Mariotti, A., Pan, Y., Cherchi, A., Lee, J. Y., Wang, B., Ha, K. J., Ruti, P., & Artale, V. (2014). Robust



- assessment of the expansion and retreat of the Mediterranean climate in the 21st century. *Scientific Reports*, 4, 7211.
- Annicchiarico, P. (2003). Breeding white clover for increased ability to compete with associated grasses. *Journal of Agricultural Science*, 140, 255–266. <https://doi.org/10.1017/S0021859603003198>
- Annicchiarico, P. (2004). A low-cost procedure for multi-purpose, large-scale field evaluation of forage crop genetic resources. *Euphytica*, 140, 223–229. <https://doi.org/10.1007/s10681-004-3782-y>
- Annicchiarico, P. (2007a). Wide- versus specific-adaptation strategy for lucerne breeding in northern Italy. *Theoretical and Applied Genetics*, 114, 647–657. <https://doi.org/10.1007/s00122-006-0465-1>
- Annicchiarico, P. (2007b). Lucerne shoot and root traits associated with adaptation to favourable and drought-stress environments and to contrasting soil types. *Field Crops Research*, 102, 51–59.
- Annicchiarico, P., Barrett, B., Brummer, E. C., Julier, B., & Marshall, A. H. (2015). Achievements and challenges in improving temperate perennial forage legumes. *Critical Reviews of Plant Science*, 34, 327–380.
- Annicchiarico, P., Collins, R. P., De Ron, A. M., Firmat, C., Litrico, I., & Hauggaard-Nielsen, H. (2019). Do we need specific breeding for legume-based mixtures? *Advances in Agronomy*, 147, 141–215.
- Annicchiarico, P., Nazzicari, N., Li, X., Wei, Y., Pecetti, L., & Brummer, E. (2015). Accuracy of genomic selection for alfalfa biomass yield in different reference populations. *BMC Genomics*, 16, 1020. <https://doi.org/10.1186/s12864-015-2212-y>
- Annicchiarico, P., Pecetti, L., Abdelguerfi, A., Bouzigaren, A., Carroni, A. M., Hayek, T., M'Hammadi, B. M., & Mezni, M. (2011). Adaptation of landrace and variety germplasm and selection strategies for lucerne in the Mediterranean basin. *Field Crops Research*, 120, 283–291. <https://doi.org/10.1016/j.fcr.2010.11.003>
- Annicchiarico, P., Pecetti, L., Abdelguerfi, A., Bouzerzour, H., Kallida, R., Porqueddu, C., Simões, N. M., & Volaire, F. (2013). Optimal forage grass germplasm for drought-prone Mediterranean environments. *Field Crops Research*, 148, 9–14. <https://doi.org/10.1016/j.fcr.2013.03.024>
- Annicchiarico, P., Pecetti, L., Bouzerzour, H., Kallida, R., Khedim, A., Porqueddu, C., Simões, N. M., Volaire, F., & Lelièvre, F. (2011). Adaptation of contrasting cocksfoot plant types to agricultural environments across the Mediterranean basin. *Environmental and Experimental Botany*, 74, 82–89. <https://doi.org/10.1016/j.envexpbot.2011.05.002>
- Annicchiarico, P., Pecetti, L., & Tava, A. (2013). Physiological and morphological traits associated with adaptation of lucerne (*Medicago sativa*) to severely drought-stressed and to irrigated environments. *Annals of Applied Biology*, 162, 27–40.
- Annicchiarico, P., & Piano, E. (1994). Interference effects in white clover genotypes grown as pure stands and binary mixtures with different grass species and varieties. *Theoretical and Applied Genetics*, 88, 153–158. <https://doi.org/10.1007/BF00225891>
- Annicchiarico, P., & Piano, E. (2005). Use of artificial environments to reproduce and exploit genotype x location interaction for lucerne in northern Italy. *Theoretical and Applied Genetics*, 110, 219–227.
- Annicchiarico, P., & Proietti, S. (2010). White clover selected for competitive ability widens the compatibility with grasses and favours the optimization of legume content and forage yield in mown clover-grass mixtures. *Grass and Forage Science*, 65, 318–324.
- Annicchiarico, P., Thami, A. I., Abbas, K., Pecetti, L., Melis, R. A. M., & Porqueddu, C. (2017). Performance of legume-based annual forage crops in three semi-arid Mediterranean environments. *Crop and Pasture Science*, 68, 932–941. <https://doi.org/10.1071/CP17068>
- Arojju, S. K., Cao, M., Zulfi Jahufer, M. Z., Barrett, B. A., & Faville, M. J. (2020). Genomic predictive ability for foliar nutritive traits in perennial ryegrass. *G3: Genes|genomes|genetics*, 10(2), 695–708. <https://doi.org/10.1534/g3.119.400880>
- Arojju, S. K., Conaghan, P., Barth, S., Milbourne, D., Casler, M. D., Hodkinson, T. R., Michel, T., & Stephen, B. (2018). Genomic prediction of crown rust resistance in *Lolium perenne*. *BMC Genetics*, 19, 35. <https://doi.org/10.1186/s12863-018-0613-z>
- Bernal-Vasquez, A. M., Möhring, J., Schmidt, M., Schönleben, M., Schön, C. C., & Piepho, H. P. (2014). The importance of phenotypic data analysis for genomic prediction—a case study comparing different spatial models in rye. *BMC Genomics*, 15, 646. <https://doi.org/10.1186/1471-2164-15-646>
- Biazzi, E., Nazzicari, N., Pecetti, L., Brummer, E. C., Palmonari, A., Tava, A., & Annicchiarico, P. (2017). Genome-wide association mapping and genomic selection for alfalfa (*Medicago sativa*) forage quality traits. *PLoS One*, 12, e0169234. <https://doi.org/10.1371/journal.pone.0169234>
- Byrne, S., Czaban, A., Studer, B., Panitz, F., Bendixen, C., & Asp, T. (2013). Genome wide allele frequency fingerprints (GWAFs) of populations via genotyping by sequencing. *PLoS One*, 8, e57438. <https://doi.org/10.1371/journal.pone.0057438>
- Byrne, S. L., Nagy, I., Pfeifer, M., Armstead, I., Swain, S., Studer, B., Mayer, K., Campbell, J. D., Czaban, A., Hentrup, S., Panitz, F., Bendixen, C., Hedegaard, J., Caccamo, M., & Asp, T. (2015). A synteny-based draft genome sequence of the forage grass *Lolium perenne*. *Plant Journal*, 84, 816–826.
- Ceccarelli, S., Grando, S., Maatougui, M., Michael, M., Slash, M., Hagharast, R., Rahmanian, M., Taheri, A., Al-Yassin, A., Benbelkacem, A., Labdi, M., Mimoun, H., & Nachit, M. (2010). Plant breeding and climate changes. *Journal of Agricultural Science*, 148, 627–637. <https://doi.org/10.1017/S0021859610000651>
- Dalmanndottir, S., Jørgensen, M., Rapacz, M., Østrem, L., Larsen, A., Rødven, R., & Rognli, O. A. (2017). Cold acclimation in warmer extended autumns impairs freezing tolerance of perennial ryegrass (*Lolium perenne*) and timothy (*Phleum pratense*). *Physiologia Plantarum*, 160, 266–281.
- De Vega, J. J., Ayling, S., Hegarty, M., Kudrna, D., Goicoechea, J. L., Ergon, Å., Rognli, O. A., Jones, C., Swain, M., Geurts, R., Lang, C., Mayer, K. F., Rössner, S., Yates, S., Webb, K. J., Donnison, I. S., Oldroyd, G. E., Wind, R. A., Caccamo, M., ... Skøt, L. (2015). Red clover (*Trifolium pratense* L.) draft genome provides a platform for trait improvement. *Scientific Reports*, 5, 17394. <https://doi.org/10.1038/srep17394>
- Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PLoS One*, 6, e19379. <https://doi.org/10.1371/journal.pone.0019379>
- Ergon, Å., Seddaiu, G., Korhonen, P., Virkajärvi, P., Bellocchi, G., Jørgensen, M., Østrem, L., Reheul, D., & Volaire, F. (2018). How can forage production in nordic and Mediterranean Europe adapt to the challenges and opportunities arising from climate change. *European Journal of Agronomy*, 92, 97–106. <https://doi.org/10.1016/j.eja.2017.09.016>
- Faville, M. J., Ganesh, S., Cao, M., Jahufer, M. Z. Z., Bilton, T. P., Easton, H. S., Ryan, D. L., Trethewey, J. A. K., Rolston, M. P., Griffiths, A. G., Moraga, R., Flay, C., Schimidt, J., Tan, R., & Barrett, B. A. (2018). Predictive ability of genomic selection models in a multi-population perennial ryegrass training set using genotyping-by-sequencing. *Theoretical and Applied Genetics*, 131, 703–720. <https://doi.org/10.1007/s00122-017-3030-1>
- Fè, D., Cericola, F., Byrne, S., Lenk, I., Ashraf, B. H., Pedersen, M. G., Roulund, N., Asp, T., Janss, L., Jensen, C. S., & Jensen, J. (2015). Genomic dissection and prediction of heading date in perennial ryegrass. *BMC Genomics*, 16, 921. <https://doi.org/10.1186/s12864-015-2163-3>
- Gebremedhin, A., Badenhorst, P. E., Wang, J., Giri, K., Spangenberg, G. C., & Smith, K. F. (2019). Development and validation of a model to combine NDVI and plant height for high-throughput phenotyping of herbage yield in a perennial ryegrass breeding program. *Remote Sensing*, 11, 2494. <https://doi.org/10.3390/rs11212494>

- Gebremedhin, A., Badenhorst, P. E., Wang, J., Spangenberg, G. C., & Smith, K. F. (2019). Prospects for measurement of dry matter yield in forage breeding programs using sensor technologies. *Agronomy*, 9, 65. <https://doi.org/10.3390/agronomy9020065>
- Ghamkhar, K., Irie, K., Hagedorn, M., Hsiao, J., Fourie, J., Gebbie, S., Hoyos-Villegas, V., George, R., Stewart, A., Inch, C., Werner, A., & Barrett, B. (2019). Real-time, non-destructive and in-field foliage yield and growth rate measurement in perennial ryegrass (*Lolium perenne* L.). *Plant Methods*, 15, 72. <https://doi.org/10.1186/s13007-019-0456-2>
- González-Camacho, J. M., Ornella, L., Pérez-Rodríguez, P., Gianola, D., Dreisigacker, S., & Crossa, J. (2018). Applications of machine learning methods to genomic selection in breeding wheat for rust resistance. *Plant Genome*, 11, 170104. <https://doi.org/10.3835/plantgenom.e2017.11.0104>
- Grinberg, N. F., Lovatt, A., Hegarty, M., Lovatt, A., Skøt, K. P., Kelly, R., Blackmore, T., Thorogood, D., King, R. D., Armstead, I., Powell, W., & Skøt, L. (2016). Implementation of genomic prediction in *Lolium perenne* breeding populations. *Frontiers in Plant Science*, 7, 133.
- Guo, D., Chen, F., Wheeler, J., Winder, J., Selman, S., Peterson, M., & Dixon, R. A. (2001). Improvement of in-rumen digestibility of alfalfa forage by genetic manipulation of lignin O-methyltransferases. *Transgenic Research*, 10, 457–464.
- Guo, X., Cericola, F., Fè, D., Pedersen, M. G., Lenk, I., Jensen, C. S., Jensen, J., & Janss, L. L. (2018). Genomic prediction in tetraploid ryegrass using allele frequencies based on genotyping by sequencing. *Frontiers in Plant Sciences*, 9, 1165. <https://doi.org/10.3389/fpls.2018.01165>
- Hayes, B. J., Cogan, N. O. I., Pembleton, L. W., Goddard, M. E., Wang, J., Spangenberg, G. C., & Forster, J. W. (2013). Prospects for genomic selection in forage plant species. *Plant Breeding*, 132, 133–143. <https://doi.org/10.1111/pbr.12037>
- Helgadóttir, Á., Aavola, R., Isolahti, M., Marum, P., Persson, C., Aleliūnas, A., Brazauskas, G., Kristjánsdóttir, T. A., Asp, T., & Rognli, O. A. (2018). Adaptability and phenotypic stability of *Lolium perenne* L. cultivars of diverse origin grown at the margin of the species distribution. *Journal of Agronomy and Crop Science*, 204, 493–504.
- Hill, J. (1990). The three C's – competition, coexistence and coevolution – and their impact on the breeding of forage crop mixtures. *Theoretical and Applied Genetics*, 79, 168–176. <https://doi.org/10.1007/BF00225947>
- Humphreys, M., Feuerstein, U., Vandewalle, M., & Baert, J. (2010). Ryegrasses. In B. Boller, U. K. Posselt, & F. Veronesi (Eds.), *Handbook of plant breeding*, Vol. 5 (pp. 211–260). Fodder Crops and Amenity Grasses, Springer.
- Jia, C., Zhao, F., Wang, X., Han, J., Zhao, H., Liu, G., & Wang, Z. (2018). Genomic prediction for 25 agronomic and quality traits in alfalfa (*Medicago sativa*). *Frontiers in Plant Sciences*, 9, 1220. <https://doi.org/10.3389/fpls.2018.01220>
- Lelièvre, F., Seddaiu, G., Ledda, L., Porqueddu, C., & Volaire, F. (2011). Water use efficiency and drought survival in Mediterranean perennial forage grasses. *Field Crops Research*, 121, 333–342. <https://doi.org/10.1016/j.fcr.2010.12.023>
- Li, X., Wei, Y., Acharya, A., Hansen, J. L., Crawford, J. L., Viands, D. R., Michaud, R., Claessens, A., & Brummer, E. C. (2015). Genomic prediction of biomass yield in two selection cycles of a tetraploid alfalfa breeding population. *Plant Genome*, 8, 2.
- Lin, Z., Hayes, B. J., & Daetwyler, H. D. (2014). Genomic selection in crops, trees and forages: A review. *Crop and Pasture Science*, 65, 1177–1191. <https://doi.org/10.1071/CP13363>
- Lüscher, A., Mueller-Harvey, I., Soussana, J. F., Rees, R. M., & Peyraud, J. L. (2014). Potential of legume-based grassland–livestock systems in Europe: A review. *Grass and Forage Science*, 69, 206–228. <https://doi.org/10.1111/gfs.12124>
- Mäkinen, H., Kaseva, J., Virkajärvi, P., & Kahiluoto, H. (2018). Gaps in the capacity of modern forage crops to adapt to the changing climate in northern Europe. *Mitigation and Adaptation Strategies for Global Change*, 23, 81–100. <https://doi.org/10.1007/s11027-016-9729-5>
- Malinowski, D. P., Belesky, D. P., & Lewis, G. C. (2005). Abiotic stresses in endophytic grasses. In C. A. Roberts, C. P. West, & D. E. Spiers (Eds.), *Neotyphodium in cool-season grasses* (pp. 187–199). Blackwell Publishing.
- McDonagh, J., O'Donovan, M., McEvoy, M., & Gilliland, T. J. (2016). Genetic gain in perennial ryegrass (*Lolium perenne*) varieties 1973 to 2013. *Euphytica*, 212, 187–199. <https://doi.org/10.1007/s10668-016-1754-7>
- Meuwissen, T. H., Hayes, B. J., & Goddard, M. E. (2001). Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, 157, 1819–1829.
- Montesinos-López, O. A., Martín-Vallejo, J., Crossa, J., Gianola, D., Hernández-Suárez, C. M., Montesinos-López, A., Juliana, P., & Singh, R. (2019). New deep learning genomic-based prediction model for multiple traits with binary, ordinal, and continuous phenotypes. *Genes|genomes|genetics*, 9(5), 1545–1556. <https://doi.org/10.1534/g3.119.300585>
- Nichols, P. G. H., Foster, K. J., Piano, E., Pecetti, L., Kaur, P., Ghamkhar, K., & Collins, W. J. (2013). Genetic improvement of subterranean clover (*Trifolium subterraneum* L.). 1. Germplasm, traits and future prospects. *Crop and Pasture Science*, 64, 312–346. <https://doi.org/10.1071/CP13118>
- Nilsson, A., von Bothmer, R., Johansson, T., Nybom, H., Bendevis, M. A., Bengtsson, T., & Rognli, O. A. (2016). Promoting Nordic plant breeding for the future. In: A. Hägnfelt (Ed). *PPP public private partnership for pre-breeding*, Vol. 730 (p 27). Nordic Council of Ministers.
- Norton, M. R., Volaire, F., & Lelièvre, F. (2006). Summer dormancy in *Festuca arundinacea* Schreb.: The influence of season of sowing and a simulated mid-summer storm on two contrasting cultivars. *Australian Journal of Agricultural Research*, 57, 1267–1277. <https://doi.org/10.1071/AR06082>
- Oba, M., & Allen, M. S. (1999). Evaluation of the importance of the digestibility of neutral detergent fiber from forage: Effects on dry matter intake and milk yield of dairy cows. *Journal of Dairy Science*, 82, 589–596. [https://doi.org/10.3168/jds.S0022-0302\(99\)75271-9](https://doi.org/10.3168/jds.S0022-0302(99)75271-9)
- Olesen, J. E., Trnka, M., Kersebaum, K. C., Skjelvåg, A. O., Seguin, B., Peltonen-Sainio, P., Rossi, F., Kozyra, J., & Micala, F. (2011). Impacts and adaptation of European crop production systems to climate change. *European Journal of Agronomy*, 34, 96–112. <https://doi.org/10.1016/j.eja.2010.11.003>
- Østrem, L., Asp, T., Ghesquière, M., Sanada, Y., Rognli, O. A. et al (2018). Low crown rust resistance in Norwegian material of *Lolium perenne* and × *Festulolium*. In G. Brazauskas (Ed.), *Breeding Grasses and Protein Crops in the Era of Genomics* (pp. 145–149). Springer International Publishing AG.
- Østrem, L., Rapacz, M., Larsen, A., Dalmannsdottir, S., & Jørgensen, M. (2015). Influences of growth cessation and photoacclimation on winter survival of non-native *Lolium-Festuca* grasses in high-latitude regions. *Environmental and Experimental Botany*, 111, 21–31. <https://doi.org/10.1016/j.envexpbot.2014.10.008>
- Østrem, L., Rapacz, M., Larsen, A., Marum, P., & Rognli, O. A. (2018). Chlorophyll a fluorescence and freezing tests as selection methods for growth cessation and increased winter survival in × *Festulolium*. *Frontiers in Plant Science*, 9, 1200. <https://doi.org/10.3389/fpls.2018.01200>
- Pecetti, L., Annicchiarico, P., Abdelguerfi, A., Kallida, R., Mefti, M., Porqueddu, C., Simões, N. M., Volaire, F., & Lelièvre, F. (2011). Response of Mediterranean tall fescue cultivars to contrasting agricultural environments and implications for selection. *Journal of Agronomy and Crop Science*, 197, 12–20. <https://doi.org/10.1111/j.1439-037X.2010.00443.x>

- Pecetti, L., Romani, M., Carroni, A. M., Annicchiarico, P., & Piano, E. (2007). The effect of endophyte infection on persistence of tall fescue (*Festuca arundinacea* Schreb.) populations in two climatically contrasting Italian environments. *Australian Journal of Agricultural Research*, *58*, 893–899.
- Piano, E., Bertoli, F. B., Romani, M., Tava, A., Riccioni, L., Valvassori, M., Carroni, A. M., & Pecetti, L. (2005). Specificity of host-endophyte association in tall fescue populations from Sardinia, Italy. *Crop Science*, *45*, 1456–1463. <https://doi.org/10.2135/cropsci2004.0287>
- Piano, E., & Pecetti, L. (2010). Minor legume species. In B. Boller, U. K. Posselt, & F. Veronesi (Eds.), *Handbook of Plant Breeding*, Vol. 5 (pp. 477–500). Fodder Crops and Amenity Grasses, Springer.
- Piano, E., Pecetti, L., Annicchiarico, P., Carroni, A. M., Fornasier, F., & Romani, M. (2004). Combining drought tolerance and responsiveness to summer moisture availability in cocksfoot (*Dactylis glomerata* L.) germplasm grown in Mediterranean environments. *Australian Journal of Agricultural Research*, *55*, 1197–1204. <https://doi.org/10.1071/AR04067>
- Piano, E., Pecetti, L., & Carroni, A. M. (1996). Climatic adaptation in subterranean clover populations. *Euphytica*, *92*, 39–44. <https://doi.org/10.1007/BF00022826>
- Rognli, O. A., Aavola, R., Aleliūnas, A., Asp, T., Brazauskas, G., Gylstrøm, K. H., Helgadóttir, A., Isolahti, M., Kovi, M. R., Kristjánsdóttir, T. A., Larsen, A. S., Marum, P., Paina, C., Persson, C., & Rancāne, S. (2018). Utilization of Genebank Accessions to Improve Northern Adaptation of Perennial Ryegrass (*Lolium perenne* L.). In G. Brazauskas, G. Statkeviciute, & K. Jonaviciene (Eds.), *Breeding Grasses and Protein Crops in the Era of Genomics* (pp. 3–8). Springer International Publishing AG.
- Rognli, O. A., Fjellheim, S., Pecetti, L., & Boller, B. (2013). Semi-natural grasslands as a source of genetic diversity. In A. Helgadóttir & A. Hopkins (Eds.), *The Role of Grasslands in a Green Future - Threats and Perspectives in Less Favoured Areas, Proceedings of the 17th Symposium of the European Grassland Federation, Akureyri, Iceland, 23-26 June 2013, Grassland Science in Europe*, Vol. 18, pp. 303–313.
- Sampoux, J. P., Baudouin, P., Bayle, B., Béguier, V., Bourdon, P., Chosson, J. F., Deneufbourg, F., Galbrun, C., Ghesquiere, M., Noel, D., Pietraszek, W., Tharel, B., & Viguie, A. (2011). Breeding perennial grasses for forage usage: An experimental assessment of trait changes in diploid perennial ryegrass (*Lolium perenne* L.) cultivars released in the last four decades. *Field Crops Research*, *123*, 117–129. <https://doi.org/10.1016/j.fcr.2011.05.007>
- Sampoux, J.-P., Giraud, H., & Litrico, I. (2020). Which recurrent selection scheme to improve mixtures of crop species? Theoretical Expectations. *G3: Genes|genomes|genetics*, *10*, 89–107. <https://doi.org/10.1534/g3.119.400809>
- Smith, C., Cogan, N., Badenhorst, P., Spangenberg, G., & Smith, K. (2019). Field spectroscopy to determine nutritive value parameters of individual ryegrass plants. *Agronomy*, *9*(6), 293. <https://doi.org/10.3390/agronomy9060293>
- Thivierge, M.-N., Jégo, G., Bélanger, G., Bertrand, A., Tremblay, G. F., Rotz, C. A., & Qian, B. (2016). Predicted yield and nutritive value of an alfalfa–timothy mixture under climate change and elevated atmospheric carbon dioxide. *Agronomy Journal*, *108*, 585–603. <https://doi.org/10.2134/agronj2015.0484>
- Viana, J. M. S., Piepho, H.-P., & Fonseca, F. (2016). Quantitative genetics theory for genomic selection and efficiency of genotypic value prediction in open-pollinated populations. *Scientia Agricola*, *74*, 41–50. <https://doi.org/10.1590/1678-992x-2015-0479>
- Viljanen, N., Honkavaara, E., Näsi, R., Hakala, T., Niemeläinen, O., & Kaivosoja, J. (2018). A novel machine learning method for estimating biomass of grass swards using a photogrammetric canopy height model, images and vegetation indices captured by a drone. *Agronomy*, *8*, 70.
- Volaire, F. (2008). Plant traits and functional types to characterise drought survival of pluri-specific perennial herbaceous swards in Mediterranean areas. *European Journal of Agronomy*, *29*, 116–124. <https://doi.org/10.1016/j.eja.2008.04.008>
- Volaire, F., & Lelièvre, F. (2001). Drought survival in *Dactylis glomerata* and *Festuca arundinacea* under similar rooting conditions in tubes. *Plant and Soil*, *229*, 225–234.
- Volaire, F., Norton, M. R., & Lelièvre, F. (2009). Summer drought survival strategies and sustainability of perennial temperate forage grasses in Mediterranean areas. *Crop Science*, *49*, 2386–2392. <https://doi.org/10.2135/cropsci2009.06.0317>
- Walter, A., Studer, B., & Kölliker, R. (2012). Advanced phenotyping offers opportunities for improved breeding of forage and turf species. *Annals of Botany*, *110*, 1271–1279. <https://doi.org/10.1093/aob/mcs026>
- Wang, J., Badenhorst, P., Phelan, A., Pembleton, L., Shi, F., Cogan, N., Spangenberg, G., & Smith, K. (2019). Using sensors and unmanned aircraft systems for high-throughput phenotyping of biomass in perennial ryegrass breeding trials. *Frontiers in Plant Science*, *10*, 1381. <https://doi.org/10.3389/fpls.2019.01381>
- Yang, W., Feng, H., Zhang, X., Zhang, J., Doonan, J. H., Batchelor, W. D., Xiong, L., & Yan, J. (2020). Crop phenomics and high-throughput phenotyping: Past decades, current challenges, and future perspectives. *Molecular Plant*, *13*(2), 187–214. <https://doi.org/10.1016/j.molp.2020.01.008>
- Yates, S., Jaškūnė, K., Liebisch, F., Nagelmüller, S., Kirchgessner, N., Kölliker, R., Walter, A., Brazauskas, G., & Studer, B. (2019). Phenotyping a dynamic trait: Leaf growth of perennial ryegrass under water limiting conditions. *Frontiers in Plant Science*, *10*, 344. <https://doi.org/10.3389/fpls.2019.00344>

**How to cite this article:** Rognli OA, Pecetti L, Kovi MR, Annicchiarico P. Grass and legume breeding matching the future needs of European grassland farming. *Grass Forage Sci.* 2021;76:175–185. <https://doi.org/10.1111/gfs.12535>