RESEARCH ARTICLE

Characterization of native perennial ryegrasses for persistence in mediterranean rainfed conditions

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Abstract

Forage varieties of *Lolium perenne* L. (perennial ryegrass) available on the seed market generally show low persistence in rainfed Mediterranean conditions. Eleven native populations of perennial ryegrass from Corsica and Sardinia, previously chosen for their ability to re-grow after summer drought, were grown in a 3-year field experiment in Sardinia (Italy). The objective was to characterize these populations for traits linked with persistence in rainfed conditions under low input management. Their differentiation and stability over time were assessed for some morphological traits (tiller, spike and flag leaf length, number of spikelets spike^{-1}, spikelet and glume length). In addition, some agronomic traits (canopy height, dry matter and seed yield, spike emergence date) and physiological traits (canopy temperature and leaf area index) were observed to identify, using a multivariate approach, correlations with persistence. A significant variability between accessions was observed for all the morphological traits over the three years of observation; a significant Accession × Year interaction was found for tiller length, flag leaf length and number of spikelet spike^{-1} with a plastic adaptation to weather conditions of some populations, these traits being the most important contributors to persistence. The most persistent perennial ryegrass accession proved to be the most vigorous and early flowering one. Some contrasting accessions represent valuable resources to be exploited into breeding programs based on Mediterranean germplasm, in order to provide farmers with perennial ryegrass varieties adapted for different purposes and which are able to survive to more frequent hot and dry summer conditions in a context of climate change.

Additional key words: AMMI analysis; forage; *Lolium perenne;* Mediterranean agriculture; morpho-physiological traits; persistence; rainfed grasslands.

Introduction

Lolium perenne L. (perennial ryegrass) is the most important forage grass species in temperate regions of Europe, Australia and New Zealand. It is an important cool-season bunchgrass, widely used in pure stands and mixtures for forage, turf and multifunctional uses, such as soil erosion control (Elazreg *et al.*, 2011). It has a wide ecological distribution across temperate humid and Mediterranean semi-arid areas. Southern Mediterranean native populations of perennial ryegrass are present in coastal areas even with annual rainfall lower than 500 mm (Charmet *et al.*, 1990; Balfourier & Charmet, 1991; Balfourier *et al.*, 1997; Elazreg *et al.*, 2011). In Italy, native perennial ryegrass is distributed between 0 and 2,000 m a.s.l. (Pignatti, 1982). Despite this large natural adaptive diversity, only a few varieties of perennial ryegrass are suitable for Mediterranean environments (Reed *et al.*, 1987, 1999; Harris *et al.*, 2008; Lelièvre *et al.*, 2010). The inability of perennial ryegrass varieties to provide permanent pure stands or to maintain their presence beyond

Abbreviations used: AMMI (Additive Main Effects and Multiplicative Interaction); CH (Canopy Height); COV (Coverage); CT (Canopy Temperature); DMY (Dry Matter Yield); FL (Flag Leaf Length); GL (Glume Length); LAI (Leaf Area Index); SE (Spike Emergence Date); SL (Spike length); SPTL (Spikelet length); SPTN (Number of Spikelets); SY (Seed Yield); TL (Tiller Length).

the sowing year within mixtures for forage use in rainfed Mediterranean conditions has been demonstrated (Franca *et al.*, 1995, 2007). Varieties of perennial ryegrass currently marketed show lower persistence and survival to hot and dry summer conditions than varieties of other perennial grasses, such as *Festuca arundinacea* Schreb. (tall fescue), *Dactylis glomerata* L. (cocksfoot) and *Phalaris tuberosa* L. (bulbous canarygrass) (Slack *et al.*, 2000; Nie *et al.*, 2004; Lelièvre & Volaire, 2009; Annicchiarico *et al.*, 2013).

A renewed interest in perennial ryegrass is justified by some important traits that make this species more suitable than other perennials for forage uses, such as tolerance to frequent cuttings (Pontes *et al.*, 2007) and feed quality for livestock (Schubiger *et al.*, 2001; Fulkerson *et al.*, 2007; Nie, 2011) However, low persistence may hinder in taking advantage of these favourable features (Porqueddu & Sulas, 1998). From an agro-economic point of view, persistence is defined as the ability to produce sufficient forage yield (Piano & Annicchiarico, 1995; Taylor & Quesenberry, 1996) in the third or fourth growing season and is often measured as the percentage of surviving plants at this stage (Choo, 1984). If selected persistent materials would have the ability to regrow after the sowing year, to produce forage and to cover the soil permanently during 3-4 years in rainfed Mediterranean conditions, they would be particularly appreciated for multifunctional uses, such as cover crop for soil protection (Porqueddu & Maltoni, 2006), management of firebreaks and restoration of degraded areas (Masson & Gintzburger, 2000). The characterization of perennial ryegrass germplasm for traits linked with the ability to produce and persist for at least 3-4 years is a prerequisite to breed perennial ryegrass varieties for dry environments (Waller & Sale, 2001; Lelièvre & Volaire, 2009).

In this context, we carried out a field study with the general objective of characterizing perennial ryegrass populations from Corsica and Sardinia for traits linked with persistence under low agronomic input management. Specific objectives of the study were: i) to assess the differentiation and temporal stability of perennial ryegrass populations for morphological traits in a 3-year trial; ii) to find a relationship between persistence and some morphological, agronomic and physiological traits; iii) to identify promising populations worthy of being selected for forage or alternative uses in Mediterranean rainfed conditions. In order to achieve such objectives, we first assessed the diffe-

rentiation and temporal stability of populations for some morphological traits (tiller length, spike length, flag leaf length, number of spikelets spike^{-1}, spikelet length and glume length) throughout the 3-year period. Then, we utilized a multivariate approach to identify the correlation of persistence with these traits and some other agronomic (canopy height, growth habit, dry matter yield, seed yield), phenological (spike emergence) and physiological traits (canopy temperature, leaf area index).

Material and methods

Origin of plant materials

On July 2005, 13 native populations of perennial ryegrass were collected in the mountain peninsula of Cap Corse, Corsica, France (Sanna, 2009). Cap Corse is characterized by a North-South oriented schistose dorsal, which degrades eastward and westward from above 1000 m a.s.l. in just a few kilometres to sea level. Average annual rainfalls range from 500 mm in the northern part, to about 800 mm in the southern part of the peninsula (Ministère de l'Agriculture et de la Pêche, France, Inventaire Forestier National, 2005). Such geo-morphological peculiarities within a relatively small area generate contrasting microclimates and should promote adaptative differentiation of populations (Migliore *et al.*, 2011). The collection criteria of perennial ryegrass populations included elevation, exposure, main floristic components and presence or lack of grazing. Due to the low plant density, damage caused by the trampling of grazing animals and the average low seed production capacity of perennial ryegrass natural populations, no more than 100 g of seed per accession was collected. The 13 populations from Corsica and 14 populations from a previous collection made in Sardinia, Italy (Cunningham *et al.*, 1997) were grown from September 2005 to October 2006 under rainfed conditions to test their ability of regrowth after summer drought (Sanna, 2009). Only the populations (11) that survived after summer were considered as persistent and are the accessions surveyed in this study (Table 1). All the populations came from sites characterized by total annual precipitation of about 700-800 mm and annual mean temperatures ranging from about 10 up to 15°C, climatic conditions close to the definition of sub-humid climate by Barbero & Quezel (1982).

Table 1. List of the accessions and characteristics of the collection sites

Accession	Geographic coordinates		Altitude (m)	Exposure	Average mean annual temperature	Average mean annual rainfall (°C)	Usage (mm)
RG00*	39.510°N	8.546°E	399	Est	14.7	701	Grazed
RG01	42.814°N	9.340°E	22	West	15.2	766	Grazed
RG02	42.808°N	9.368°E	255	West	13.9	766	Grazed
RG06	42.815°N	9.398°E	908	West	11.4	787	Grazed
RG07	42.817°N	9.403°E	956	East	10.1	807	Grazed
RG09	42.817°N	9.416°E	642	East	11.7	777	Grazed
RG11	42.819°N	9.428°E	359	East	13.4	760	Grazed
RG15	42.972°N	9.369°E	365	East	13.7	791	Grazed
RG16	42.958°N	9.384°E	454	West	13.5	787	Ungrazed
RG17	42.952°N	9.398°E	484	East	13.4	785	Grazed
RG18	42.935°N	9.367°E	309	West	14.4	781	Ungrazed

* Sardinian accession. All the others were collected in Cap Corse, Corsica (France).

Field experiment and measurements

The above-mentioned 11 native populations of *L. perenne,* hereafter named accessions, were evaluated in a field trial carried out at the experimental farm of Ottava, Sassari (North-West Sardinia, 40° 53' N, 8° 29' E, 60 m a.s.l.) during three consecutive years, from autumn 2006 to spring 2009. The site has a Mediterra-

nean-type climate, with mild winters, dry summers and average annual rainfall of 547 mm. The soil was shallow and calcareous with a N content of 2 g kg^{-1} (Kjeldahl) and P$_2$0$_5$ content of 0.053 g kg^{-1} (Olsen). Soil depth decreased from 80 cm to 30 cm over a calcareous rock layer. Fluctuations of rainfall during the three years of the experiment were recorded (Fig. 1). As a year, we have considered the annual growing cycle of perennial
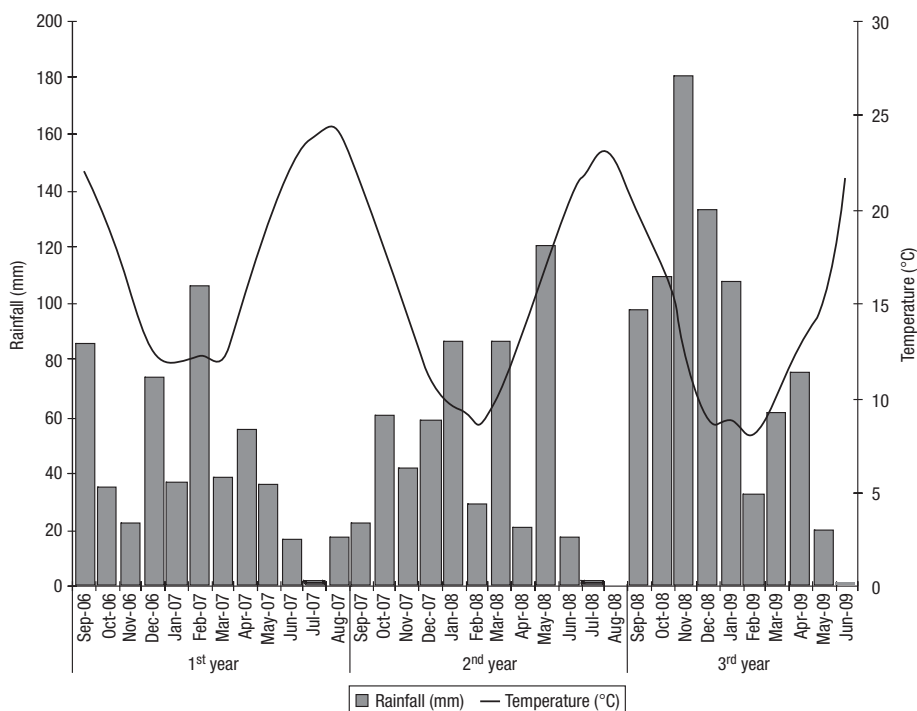

Figure 1. Experimental site climatic diagram, according to Walter & Lieth (1967), during the 3-year trial.

ryegrass in the rainfed conditions of the experimental field, approximately from September to August of the following year. In the 1st year (September 2006-August 2007), total annual rainfall (523 mm) was similar to the long-term average (547 mm). In the same period of the 2nd year (2007-2008), a total annual rainfall of 542 mm was recorded, equally distributed between winter and spring, with a peak of 120 mm in May, after a dry April. The 3rd year was unusually wet, with 832 mm of total rainfall, 532 of which concentrated between October and January. Autumn and winter were milder during the 1st year: the average temperature of the September 2006-February 2007 period resulted almost 2°C higher than temperatures of the same period in the following years.

After the collection process, seeds were stored following the standard protocol of short-term seed storage (18-20°C and 30-40% RH). A germination test was performed before the field assessment. The field was ploughed and fertilized with 36 kg ha^{-1} of N and 92 kg ha^{-1} of P_2O_5, then rolled. Pure stands of each accession were sown in 2.5 m × 1.2 m plots. Plots were arranged in a randomized complete block design, with three replicates. The blocks were arranged based on the gradient of soil depth. To avoid the small plot size blurring the assessment of traits related to biomass production, plots were separated by a buffer, sown with four 20 cm-spaced rows of *Dactylis glomerata*, to reduce the border effect. Six 20 cm-spaced rows per plot were sown at a seed density of 50 germinable seeds m^{-1}. The low-input management of plots was carried out trying to satisfy their minimum requirements (in water and nutrients) for growth and to achieve their annual cycle, in order to observe their ability to survive to the summer drought. Thus, throughout the trial duration, irrigation and fertilization were never applied and weeds were controlled by hand pulling when necessary. Plots were neither grazed nor cut during the vegetative growth. A cut was performed in July 2008 to determine total aerial dry matter yield (DMY) and seed yield (SY). In late summer 2007 and 2009, plots were cleared by cutting the dry residuals at ground level, simulating the usual summer grazing, carried out to meet the minimum feed requirements of dairy sheep and for fuel biomass reduction purposes.

The following measurements related to plant morphology were made in the three consecutive years, when inflorescences were fully expanded (spring 2007, spring 2008 and spring 2009), on 15 random tillers per plot: tiller length (TL, unit of measurement = cm), considered as the distance from the base of the stem to the tip of the apical spikelet; spike length (SL, cm), considered as the distance from the base of the last internode to the tip of the apical spikelet; flag leaf length (FL, cm), number of spikelets spike^{-1} (SPTN, number), spikelet length (SPTL, cm) and glume length (GL, cm). Spikelet and glume lengths were measured by using a vernier calliper (resolution = 0.02 mm).

In 2007-2008 (2nd year), spike emergence date (SE, days), canopy height (CH, cm), canopy temperature (CT, °C) and leaf area index (LAI) were determined. SE was measured in days from 5 October 2007, date at which all the plots reached a minimum 30% coverage at autumn re-establishment, up to the spring date at which the 50% of the inflorescence emerged. CH was determined by means of a falling plate meter (Bransby *et al.*, 1977), making four measurements per plot in the vegetative phase prior to heading (April 24th 2008). When used on perennial ryegrass swards, CH combines both estimate of vigour and production (Douglas & Crawford, 1994), and it is useful to differentiate potential destination of ecotypes. CT has been associated with dehydration avoidance in previous studies on grasses (Blum *et al.*, 1982; Franca *et al.*, 1998; Lopes & Reynolds, 2010). Furthermore, Franca *et al.* (1998) reported that, at the insurgence of summer drought conditions, the coldest canopies of *Lolium rigidum* L. (annual ryegrass) accessions showed dehydration avoidance as one of the clear drought-resistance traits. Thus, in order to study the dehydration avoidance of the observed populations, we made three series of measurements of CT, respectively, on 23 May, 5 June and 26 June 2008, using an handheld infra-red thermometer (Model 112*,* Everest Interscience). Measurements were made on all plots at midday, in the absence of wind and clouds, at a stage when the plots were completely covered by canopies, with a similar structure and phenological stage (Blum *et al.*, 1982; Franca *et al.*, 1998).

With the aim of evaluating the regrowth capability of the observed populations after summer drought, we estimated the leafiness of plants at the end of autumn (mid December 2007) through LAI measurements. LAI was estimated using the Inclined Point Quadrat method (Warren, 1963), from the formula LAI = 1.1 × N (32.5), where N (32.5) is the number of leaf contacts with a needle at the tip of a rod with an inclination angle of 32.5°. Ten point quadrats per plot were sampled. The agronomic data collected in early summer (July

2008) were dry matter yield (DMY, t ha^{-1}) and seed yield (SY, g m^{-2}). DMY was evaluated only once, at complete senescence of all the accessions. This way of managing perennial ryegrass swards deals with the low agronomic input management needed for the multifunctional use of pasture/forage species in Mediterranean rainfed conditions: marginal growth environments such as not arable rangeland, in many cases without livestock grazing (*i.e.,* in firebreaks in protected areas or cover crop in steep areas), where the main goal is to achieve a persistent coverage during the annual cycle from autumn to late spring, when the dried herbage mass is cut or grazed to control fuel and reduce the risk of fires. DMY was estimated by cutting the biomass at ground level within two sample quadrats of 50×40 cm per plot. Each sample quadrat comprised two row portions 50 cm long. Samples were dried in a ventilated oven at 65°C for 48 h. Seed yield was estimated by manually harvesting the spikes within two sample areas per plot as for DMY evaluations.

Finally, the persistence of the accessions was evaluated considering the coverage (COV, %) for each plot in the third year (early February 2009). The coverage percentage was visually assessed considering the perennial ryegrass covering ranging from 0 (bare soil) to 100 (total covering of the plot).

Statistical analyses

We first implemented a two-way fixed model of analysis of variance (ANOVA) to assess the variability of morphological traits within each year, with "Accession" (A) and "Block" (B) as fixed effects. We then investigated the stability of accessions over the experimental years by implementing a mixed model ANOVA including the interaction Accession × Year (A × Y), it being assumed that each year would represent a different environmental condition. "Block" (B) and "Accession" (A) were considered as fixed effects, whereas "Year" (Y) was considered as a random effect, assuming that climatic conditions during each year were random uncontrolled effects. All ANOVAs were performed using two-way and GLM procedure of Statgraphics Centurion XVI (StatPoint, Inc., USA). The Additive Main Effects and Multiplicative Interaction (AMMI) analysis (Gauch & Furnas, 1991) was used to further investigate the A × Y interaction and to categorize the experimental years into Mega-environments (Gauch *et al.*, 2008). With the biplot facility

from AMMI analysis, accessions and years occur both on the same scatter plot and inferences about their interactions can be made. Linear correlation coefficients were used to determine the relationships between morphological, agronomic and physiological traits and between these traits and climate data at collection sites. There was substantial intercorrelation between variables, which prompted further exploration of the data by principal component analysis (Manly, 1994). K-means clustering (of standardized data) (Hawkins, 1982) was performed using scores of accessions on the first principal components of the PCA (Principal Component Analysis) to analyse the morphological data. Five clusters were chosen because, by so doing, the highest number of significant differences between clusters for each of the variables was obtained.

The Multifactor ANOVA, linear correlations and cluster analyses were made by means of the software Statgraphics Centurion XVI (StatPoint Inc., USA). AMMI analysis was performed using Matmodel 3.0 software (Gauch, 2007).

Results

Variability and stability of morphological traits

Although the collection sites of populations had close geographical proximity, a high variability of morphological traits was noted, as revealed by significant differences found by multiple range tests among accessions and years for all traits. On average, RG00 plants had tillers longer than the other accessions (Table 2). The accessions RG06 and RG07 had TL about 30% shorter than the average value in the 1st year and 20% shorter in the other two years. RG00 was the accession with the longest spike over the three years considered, while RG06 and RG07 showed the shortest spikes. Maximum values of FL were just over 17 cm in all years, but for different accessions: RG01 and RG00, respectively in 1st and 2nd year, RG16 and RG18 in the 3rd. Regarding reproductive traits (Table 3), SPTN ranged between 20.1 (RG00 in 3rd year) and 30.6 (RG01 in 1st year) spikelets spike^{-1}. The accession RG17 maintained high values of SPTL and GL throughout the 3-year period.

Among the main effects, A did not affect FL and SPTN, while Y significantly affected all the morphological traits (Table 4). As expected by the arrange-

Table 2. Averages and multiple range test of morphological traits for the eleven perennial ryegrass accessions of perennial ryegrass, at full elongation of the spike, in each of the three trial years

Accession	TL (cm)			SL (cm)			FL (cm)		
	2007	2008	2009	2007	2008	2009	2007	2008	2009
RG00	73.2[ab]	61.0[a]	62.9[a]	45.5[a]	39.0[a]	34.6[a]	14.9[abc]	17.2[a]	16.0[abc]
RG01	69.8[abcd]	54.3[b]	58.1[abcd]	36.2[bc]	29.3[b]	27.9[bcd]	17.2[a]	15.6[a]	16.0[ab]
RG02	60.1[ef]	47.7[dc]	54.6[cd]	35.1[bc]	26.0[bcd]	27.4[bcd]	14.7[abc]	12.2[cd]	15.8[abc]
RG06	45.1[hg]	35.1[e]	44.7[e]	27.7[de]	19.4[f]	24.6[cd]	12.8[cd]	11.2[d]	16.3[ab]
RG07	41.5[h]	35.0[e]	40.7[e]	26.7[e]	20.1[ef]	19.5[e]	12.4[cd]	11.3[d]	14.8[bc]
RG09	71.3[abc]	49.7[bcd]	56.0[bcd]	39.2[ab]	28.7[b]	28.5[bcd]	16.5[ab]	14.8[ab]	15.3[acd]
RG11	68.9[bcde]	50.6[bc]	59.0[abc]	39.6[ab]	24.5[cd]	30.3[ab]	16.0[ab]	13.7[bc]	13.7[c]
RG15	62.9[cde]	53.9[b]	61.5[ab]	33.6[bcd]	27.8[bc]	29.7[bc]	13.7[bcd]	13.7[bc]	15.8[abc]
RG16	62.3[de]	44.4[d]	58.1[abcd]	36.9[abc]	23.4[de]	30.8[ab]	13.9[bcd]	11.7[d]	17.0[a]
RG17	78.1[a]	34.1[e]	56.4[bcd]	39.5[ab]	23.0[def]	27.5[bcd]	11.5[d]	6.8[e]	16.0[ab]
RG18	53.6[fg]	38.1[e]	52.8[d]	30.9[cde]	19.9[f]	24.6[d]	13.8[bcd]	12.4[cd]	17.1[a]

TL: tiller length, SL: spike length, FL: flag leaf length. Means followed by the same letter within a column are not significantly different ($p \le 0.05$) as determined by the Bonferroni multiple comparison test.

ment of blocks along a soil depth gradient, Block (B) had a significant effect on TL, SL and SPTL. The impact of effect Y on the total variance was similar to that of effect A for TL, SL and FL (on average accounting respectively for 33.5, 36.0 and 22.5% of total sum of squares). The percentage of sum of squares (SS) explained by A × Y interactions ranged from 6% for SL to 22% for SPTN. The study of the interaction A × Y for each morphological trait allows us to point out traits showing variability in the plastic response of accessions to inter-annual variations of environmental conditions. It was then possible to check whether populations with the best persistence were more or less

plastic than average for such traits. Populations showing a high level of plasticity for one or more traits, have to be considered as less stable for these traits. Significant A × Y interactions were found for TL, FL and SPTN. These traits showed variability in the plastic response of populations to the weather fluctuations. On the other hand, SL, SPTL and GL, were not affected by significant A × Y interactions. The main effect of A was larger on TL, SL, SPTL and GL than were the A × Y interaction effects. The A × Y interaction for FL accounted for 22% of the total variance, a percentage similar as for the main effects A and Y. A dominant contribution of A × Y variation over the accession

Table 3. Averages and multiple range test of spike traits for the eleven perennial ryegrass accessions, at full elongation of the spike, in the three trial years

Accession	SPTN (n°)			SPTL (cm)			GL (cm)		
	2007	2008	2009	2007	2008	2009	2007	2008	2009
RG00	29.8[ab]	28.8[a]	20.1[c]	1.08[bc]	1.07[ab]	1.00[a]	0.83[bc]	0.72[ab]	0.75[a]
RG01	30.6[a]	27.6[ab]	23.4[ab]	1.03[cd]	0.91[cd]	0.87[bc]	0.78[cde]	0.63[cd]	0.67[abc]
RG02	27.3[bcd]	28.2[a]	24.2[a]	0.93[ef]	0.95[bcd]	0.88[bc]	0.73[de]	0.58[d]	0.68[abc]
RG06	25.0[de]	25.1[abc]	23.2[ab]	0.87[f]	0.89[cd]	0.91[ab]	0.71[e]	0.62[cd]	0.66[abc]
RG07	24.3[e]	24.7[c]	21.1[bc]	0.86[f]	0.88[cd]	0.76[c]	0.70[e]	0.61[cd]	0.59[c]
RG09	27.9[bc]	29.5[a]	23.7[ab]	0.94[def]	1.01[bc]	0.91[ab]	0.71[e]	0.66[bcd]	0.67[abc]
RG11	29.5[ab]	27.0[abc]	23.0[ab]	1.15[ab]	0.95[bcd]	0.90[ab]	0.86[b]	0.62[cd]	0.64[bc]
RG15	27.1[bcd]	27.6[ab]	23.1[ab]	1.09[bc]	1.00[bc]	0.87[bc]	0.86[bc]	0.66[bc]	0.69[abc]
RG16	25.9[cde]	24.7[c]	25.1[a]	1.02[cde]	0.92[cd]	0.94[ab]	0.80[bcd]	0.66[bcd]	0.71[ab]
RG17	27.6[bc]	20.6[d]	23.8[a]	1.20[a]	1.18[a]	0.95[ab]	0.96[a]	0.78[a]	0.72[ab]
RG18	28.8[ab]	24.5[c]	23.7[ab]	0.98[de]	0.86[d]	0.92[ab]	0.74[de]	0.65[bcd]	0.72[ab]

SPTN: number of spikelets spike^{-1}, SPTL: spikelet length, GL: glume length. Means followed by the same letter within a column are not significantly different ($p \le 0.05$) as determined by the Bonferroni multiple comparison test.

variation was only clearly detected for SPTN, trait for which the Y effect and A × Y interaction amounted for 56% of the total variance.

As the mixed model ANOVA, the AMMI analysis reported a significant A × Y interaction for TL, FL and SPTN, but it was limited to the first interaction principal component axis (IPCA1). IPCA1 explained a percentage of the variance in the A × Y interactions ranging from 59% (SPTN) to 86% (TL). The AMMI Biplot displaying IPCA1 scores and adjusted means of accessions or years provided the pattern of A × Y interaction for each trait (Figs. 2a, 2b and 2c). The components of the biplot for each trait explained most of the sum of squares of treatments, which ranged from 88.3% for SPTN to 99.6% for FL; thus, the biplot was suitable to interpret the A × Y interaction and the main effects. RG17 showed the highest absolute IPCA1 scores for all traits, thus turning out to be the most unstable population.

The results of the Mega-environment analysis for the AMMI1 model (Table 5) showed that in the driest year with mild autumn-winter (1st year), RG01 took advantage for FL and SPTN, and RG17 for TL. Sardinian accession RG00 benefited from weather conditions in the second year, exhibiting a positive plastic response of TL, FL and SPTN in relative dry conditions and low autumn-winter average temperature. In the third year, RG00 also won for TL when autumn-winter temperatures were the coldest. In the same year, in sub-humid conditions, other accessions adapted their morphology with different mechanisms: RG17 increasing SPTN and RG16 showing the longest flag leaf.

Relationship between persistence and some morphological, agronomic and physiological traits

The average values of the agronomic and physiological traits included in the multivariate analysis are reported in Table 6 (the average values of morphological traits for previous years have been already reported in Table 2). Significant differences were found between accessions for all the traits. LAI differed significantly only between RG11 and RG07. Coverage (COV) was up to 70% in seven out of the eleven accessions, with the lowest value for RG17 (40.5%). High variability was found for CH, with particularly low mean values for RG17. Accessions RG17, RG06 and RG07 were significantly more prostate than all the other accessions. Spike emergence date (SE) was about
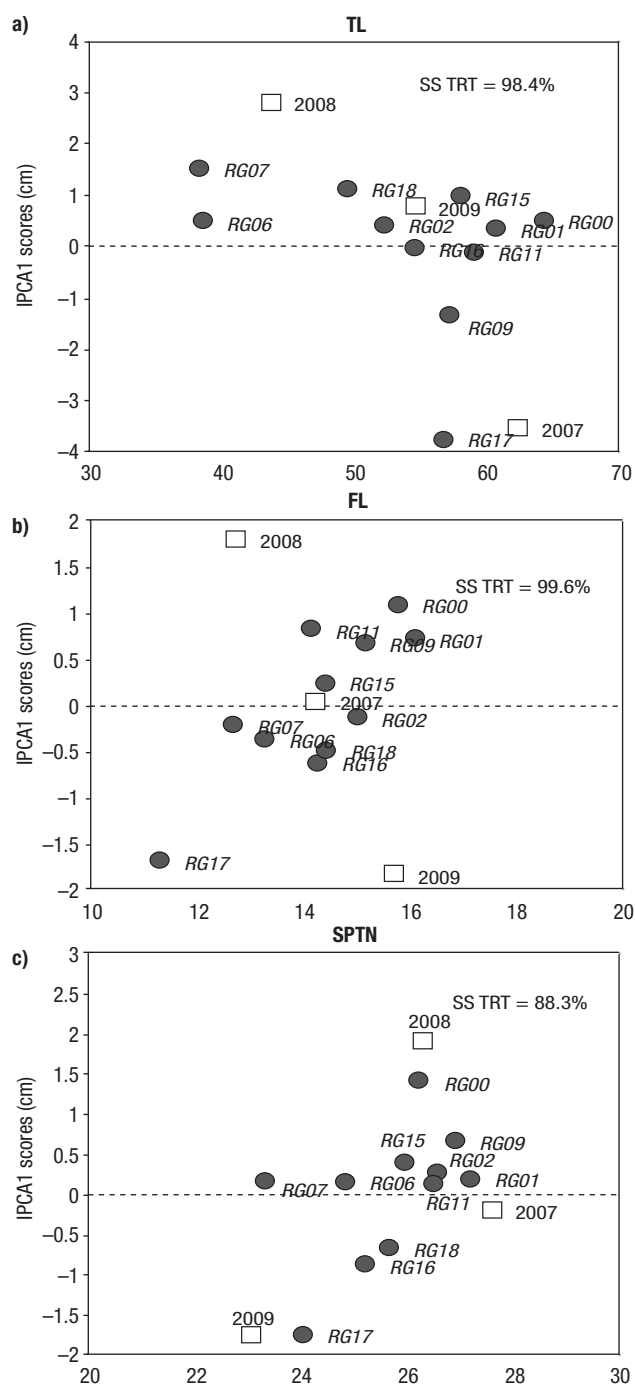

Figure 2. Biplot of Accession × Year interaction for tiller length (a), flag leaf length (b) and number of spikelets spike^{-1} (c) of the 11 perennial ryegrass populations (RG) in each of the examined periods (2007: from September 2006 to August 2007; 2008: from September 2007 to August 2008; 2009: from September 2008 to June 2009). Accessions (points) and years (squares) are positioned by the mean value of each trait and the first interaction principal component (IPCA1) score. The percentage of the treatment sum of squares (SS TRT) accounted by the AMMI1 model for each trait is reported (top right).

Table 4. Results of mixed model ANOVA applied to morphological traits measured over the three years of the experiment, performed considering Year (Y) as random factor and Accession (A) and Block (B) as fixed factors. Statistics for the first Interaction Principal Component Axis (IPCA1) from AMMI analysis are reported only for traits significantly influenced by IPCA1

Source	df	TL (cm)				SL (cm)				FL (cm)				SPTN				SPTL (cm)				GL (cm)			
		SS%	MS[b]	F-ratio	P	SS%	MS	F-ratio	P	SS%	MS	F-ratio	P	SS%	MS	F-ratio	P	SS%	MS	F-ratio	P	SS%	MS	F-ratio	P
Total	95																								
Accession (A)	10	38	562.8	6.2	***	35	172.7	11.4	***	24	16.8	2.1	n.s.	13	13.0	1.2	n.s.	33	0.048	4.28	**	22	0.024	4.8	**
Year (Y)	2	29	2,209.7	24.2	***	37	902.7	59.4	***	20	68.9	8.5	**	34	173.4	15.3	***	14	0.101	9.08	**	32	0.175	35.2	***
Block (B)	2	2	165.0	3.6	*	5	113.8	8.1	***	1	1.8	0.5	n.s.	0	2.3	0.5	n.s.	4	0.028	2.81	*	2	0.012	1.9	n.s.
A × Y	20	12	91.7	2.0	*	6	15.2	1.1	n.s.	23	8.2	2.2	*	22	11.4	2.2	**	15	0.011	1.22	n.s.	9	0.005	0.8	n.s.
Residual	61	19	45.8			17	14.1			33	3.7		**	31	5.1			35	0.009			35	0.006		
IPCA1	11	8	204.7		*	—	—			18	11.9		**	14	13.6		**	—	—			—	—		

TL: tiller length. SL: spike length. FL: flag leaf length. SPTN: number of spikelets spike^{-1}. SPTL: spikelet length. GL: glume length. SS%: percentage of sum of squares. MS: mean squares. n.s.: not significant. * Significant for $p \le 0.05$. ** Significant for $p \le 0.01$. *** Significant for $p \le 0.001$.

10 days earlier for RG00 than for RG06, RG07 and RG09. Canopy temperatures (CT) significantly differed between accessions only at the third date of measurements (26 June). A difference of 3.2°C in CT was found between the coldest and the warmest canopies. RG15 was the highest yielding accession. High variability was observed for SY, with particularly low mean values recorded for RG07.

Many significant correlations in Table 7 supported the implementation of the multivariate analyses reported in the next section. Of the 120 correlation coefficients, 28% were statistically significant at the 5% threshold. Soil coverage in third year (COV) was significantly and positively correlated with five traits, namely TL, FL and SPTN (morphological traits) and DMY and CH (agronomic traits), which can be considered as descriptive for the overall plant vigour. TL was the variable most frequently correlated with other traits (at least $p \le 0.05$). Among the physiological variables, CT was positively correlated with DMY. LAI did not correlate with any of the morphological characters considered during autumn regrowth. Among

Table 5. Mega-environments* revealed by the AMMI1 model analysis. Results (scores along the first interaction principal component axis – IPCA1) are reported only for traits significantly affected by A × Y interaction

Trait	Year	IPCA1 Score	AMMI1 Winner
TL			
Mega-environment 1	2008	2.81	RG00
	2009	0.75	RG00
Mega-environment 2	2007	−3.60	RG17
FL			
Mega-environment 1	2008	1.80	RG00
Mega-environment 2	2007	0.04	RG01
Mega-environment 3	2009	−1.84	RG16
SPTN			
Mega-environment 1	2008	1.98	RG00
Mega-environment 2	2007	−0.21	RG01
Mega-environment 3	2009	−1.77	RG17

* Mega-environments are environmental groupings having the same accession (AMMI1 winner) as superior for the trait under evaluation.

Table 6. Average values of the variables* included in the multivariate analysis, for each accession included in the study. Results refer to data in the second year (2008)

Accession	LAI	CH (cm)	COV (%)	SE (days)	CT (°C)	DMY (t ha⁻¹)	SY (g m⁻²)
RG00	4.7[ab]	38.5[a]	82.1[a]	195[c]	32.3[ab]	2.6[ab]	30[b]
RG01	5.6[ab]	34.9[ab]	70.0[ab]	201[b]	33.3[a]	2.6[ab]	21[bc]
RG02	4.5[ab]	27.1[cd]	71.6[ab]	203[ab]	30.1[c]	1.3[bc]	19[bc]
RG06	3.9[ab]	13.0[ef]	54.4[bc]	204[ab]	31.2[abc]	1.2[bc]	13[bc]
RG07	3.6[b]	14.8[e]	63.5[abc]	206[a]	31.6[abc]	1.2[bc]	5[c]
RG09	3.9[ab]	29.9[bcd]	77.7[ab]	205[ab]	30.9[bc]	2.5[abc]	28[b]
RG11	5.9[a]	33.1[abc]	79.1[ab]	202[ab]	31.0[bc]	2.3[abc]	29[b]
RG15	4.3[ab]	33.5[ab]	71.9[ab]	201[b]	32.7[ba]	3.5[a]	57[a]
RG16	5.2[ab]	29.8[bcd]	78.6[ab]	203[ab]	31.7[abc]	2.6[ab]	31[b]
RG17	4.5[ab]	7.6[f]	40.5[c]	206[a]	31.3[bc]	0.9[c]	19[bc]
RG18	4.6[ab]	26.3[d]	63.4[abc]	206[a]	32.3[ab]	2.6[ab]	20[bc]

* LAI: leaf area index. COV: plot coverage at third year. CH: canopy height,.SE: spike emergence date. CT: canopy temperature, DMY: dry matter yield. SY: seed yield. Means followed by the same letter within a column are not significantly different ($p \leq 0.05$) as determined by the Bonferroni multiple comparison test.

the environmental variables characterizing the collection sites, temperature was positively correlated with LAI (0.720***), cumulated annual rainfall was positively correlated with SE (r = 0.846***) and negatively correlated with TL, SL and FL (respectively with r = –0.839**, –0.717* and –0.652*) and elevation was negatively correlated with LAI (r = –0.761**) and positively with CH (r = 0.633*).

Table 8 shows the mean and the standard deviation of the five clusters produced by K-means clustering, based on the first two principal components of the PCA. Means are expressed as unstandardized values, whereas the F ratio and P values are computed using standardized values. Cluster I includes the Sardinian population RG00, characterised by the highest values of SL, TL and FL, the highest COV and the earliest SE.

Table 7. Linear correlations (n = 11) between 16 morphological, agronomical and physiological traits[A] recorded for the 11 accessions under study and some environmental characteristics of collection site. Results refer to data recorded during the second year of the trial (2008)

	SL	TL	FL	SPTN	CT	SPTL	GL	LAI	DMY	SY	CH	COV	SE	Temperature	Rainfall
TL	0.893***														
FL	0.749**	0.872***													
SPTN	0.656*	0.814**	0.886***												
CT	0.276 n.s.	0.315 n.s.	0.394 n.s.	0.027 n.s.											
SPTL	0.471 n.s.	0.186 n.s.	–0.174 n.s.	–0.157 n.s.	–0.121 n.s.										
GL	0.324 n.s.	0.004 n.s.	–0.226 n.s.	–0.418 n.s.	0.201 n.s.	0.849***									
LAI	0.263 n.s.	0.474 n.s.	0.307 n.s.	0.113 n.s.	0.247 n.s.	–0.13 n.s.	–0.003 n.s.								
DMY	0.465 n.s.	0.696*	0.698*	0.539 n.s.	0.609*	–0.118 n.s.	–0.006 n.s.	0.384 n.s.							
SY	0.453 n.s.	0.624*	0.375 n.s.	0.376 n.s.	0.312 n.s.	0.295 n.s.	0.219 ns.	0.248 n.s.	0.790**						
CH	0.709*	0.920**	0.892***	0.793**	0.367 n.s.	–0.110 n.s.	–0.173 n.s.	0.560 n.s.	0.834**	0.602 n.s.					
COV	0.582 n.s.	0.788**	0.824**	0.817**	0.083 n.s.	–0.238 n.s.	–0,340 n.s.	0.381 n.s.	0.667*	0.443 n.s.	0.895***				
SE	–867***	–0.847**	–0.752**	–0.574 n.s.	–0.367 n.s.	–0.220 n.s.	–0.1591 n.s.	–0.409 n.s.	–0.473 n.s.	–0.437 n.s.	≠0.716*	–0.600 n.s.			
Temperature	0.503 n.s.	0.573 n.s.	0.371 n.s.	0.178 n.s.	0.429 n.s.	0.212 n.s.	0.246 n.s.	0.720**	0.495 n.s.	0.421 n.s.	0.599 n.s.	0.249 n.s.	–0.524		
Rainfall	–0.717*	–0.839**	–0.652*	–0.496 n.s.	–0.101 n.s.	–0.353 n.s.	–0.280 n.s.	–0.398 n.s.	–0.246 n.s.	–0.169 n.s.	–0.595 n.s.	–0.485 n.s.	–0.846***	–0.565 n.s.	
Altitude	–0.588 n.s.	–0.432 n.s.	–0.377 n.s.	–0.273 n.s.	–0.359 n.s.	–0.110 n.s.	–0.0467 n.s.	–0.761**	–0.497 n.s.	–0.399 n.s.	–0.633*	–0.399 n.s.	–0.939***	0.414 n.s.	

[A] SL: spike length. TL: tiller length. FL: flag leaf length. SPTN: number of spikelets spike⁻¹, CT: canopy temperature. SPTL: spikelet length. GL: glume length. LAI: leaf area index. DMY: dry matter yield. SY: seed yield. CH: canopy height. COV: plot coverage at the spring of the third year. SE: spike emergence. * Significant for $p \leq 0.05$. ** Significant for $p \leq 0.01$. *** Significant for $p \leq 0.001$. ns: not significant.

Table 8. Mean and standard deviation (± SD) of 13 morphological and agronomic traits (unstandardised data) in each of the five clusters (I to V) formed by K-means clustering. Differentiation between clusters is assessed by an F statistic (F-ratio) and associated *p* value, computed from standardized mean values of accessions (RG00-RG18)

Traits	I RG00	II Rg 01, RG11, RG15, RG16, RG18	III RG02, RG09	IV RG17	V RG06, RG07	F-ratio*	*p*
		(mean ± SD)	(mean ± SD)		(mean ± SD)		
TL (cm)	61.0	48.3 ± 7.7	48.9 ± 4.4	34.1	34.4 ± 4.6	12.85	0.000
SL (cm)	39.0	25.0 ± 3.9	27.4 ± 3.2	23.0	19.6 ± 3.6	13.22	0.000
FL (cm)	17.2	13.4 ± 1.6	13.5 ± 1.8	6.8	11.3 ± 1.2	19.98	0.000
SPTN	28.8	26.3 ± 2.5	28.9 ± 1.7	20.6	24.8 ± 2.9	9.05	0.000
SPTL (cm)	1.1	0.9 ± 0.1	1.0 ± 0.1	1.2	0.9 ± 0.2	5.40	0.003
GL (cm)	0.7	0.6 ± 0.1	0.6 ± 0.1	0.8	0.6 ± 0.1	3.78	0.016
CT (°C)	32.3	33.1 ± 1.8	31.0 ± 2.2	31.3	32.9 ± 2.1	1.60	0.206
LAI	4.7	5.1 ± 0.9	4.2 ± 0.4	4.5	3.7 ± 1.4	2.39	0.094
DMY (t ha^{-1})	2.6	2.7 ± 0.9	1.9 ± 1.2	0.9	1.2 ± 0.6	4.16	0.010
SY (g m^{-2})	30.2	31.6 ± 1.6	23.8 ± 1.4	19.4	8.3 ± 0.6	2.88	0.044
CH (cm)	38.5	31.5 ± 4.1	28.5 ± 3.5	7.6	14.3 ± 2.9	41.92	0.000
COV (%)	82.1	72.3 ± 8.4	72.5 ± 19.4	40.5	61.0 ± 12.4	5.19	0.004
SE (days)	195	202.5 ± 2.3	204.0 ± 2.2	206	204.8 ± 5.1	8.59	0.000

* F-ratios are calculated from analyses of variance for each variable and the *p* value indicates the probability that the F-ratios are not significant. TL: tiller length. SL: spike length. FL: flag leaf length. SPTN: number of spikelets spike^{-1}. SPTL: spikelet length. GL: glume length. CT: canopy temperature. LAI: leaf area index. DMY: dry matter yield. SY: seed yield. CH: canopy height. COV: plot coverage at the spring of the third year. SE: spike emergence.

The second cluster groups five accessions with high DMY and SY. Cluster III includes two accessions showing intermediate traits. Accession in Cluster IV (RG17) exhibited low values of morphological traits, but had long glumes and spikelets and prostrate habit. Cluster V grouped two accessions characterised by low values of SL and SY. No significant differences beween the five clusters were found in terms of LAI and CT.

Discussion

The characterization of persistent perennial ryegrass populations in the studied 3-year field trial was performed under fluctuating weather conditions. In fact, the first two years can be considered as common years for the experimental site, but with milder temperatures than average during autumn and winter in 2006-2007 and an irregular distribution of spring rains in 2008. The meteorological trend of the 3rd year is closer to the definition of sub-humid climate of Barbero & Quezel (1982). Furthermore, two out of the three experimental years were characterised by an annual precipitation about 200 mm lower than the normal values for the collection sites of accessions. Such contrasting inter-annual conditions gave us the additional opportunity to study the plastic adaptation of the eleven populations to fluctuations of weather conditions, a character that has been associated both with the productivity and the persistence of perennials (Porqueddu & González, 2006; Lelièvre *et al.*, 2008; Molle *et al.*, 2008; Pecetti *et al.*, 2011).

Chapman *et al.* (2011) indicated that very few studies have so far explicitly surveyed the expression of plant traits held by perennial ryegrass cultivars over the years. They reported, as examples, results from other authors about the effect of genotype × environment (G × E) interaction for water soluble carbohydrates (Parsons *et al.,* 2004) and for maturing date (NZPBRA, 2010) and concluded that the existence of G × E interactions affecting other specific traits in perennial ryegrass cannot be ruled out. The results of this work highlight that, taking into account our specific experimental conditions and the observed 11 accessions, TL, FL and SPTN were the morphological traits correlated with persistence and also that some accessions have benefited from the additive effect of the A × Y interaction, which was considered in our study in the same way as G × E interaction. Among all the other observed traits, CH seemed to be the most important for defining highly persistent materials; it was also positively and significantly correlated with

other traits (TL, FL, SPTN, DMY). Also, the correlation matrix showed a positive correlation between SY and DMY, not in agreement with results of previous studies on Mediterranean perennial grasses (Falcinelli *et al.*, 1988). This characteristic is relevant for the potential interest that the persistent accessions could have for the seed industry (Sampoux *et al.*, 2011). However, the small plot dimension could limit the generalisation of DMY and SY results obtained in our experiment. Earliness was nearly significantly correlated with COV and the earliest flowering accession RG00 maintained the highest COV in the last year. The same trend was reported for Australian perennial ryegrass varieties by Waller & Sale (2001), who stated that early flowering is important, when the growing season is short, to avoid the deleterious effects of moisture stress on plant survival with the onset of the dry season. The same relationship between persistence and flowering time was also observed for other perennial grasses such as tall fescue (Piano *et al.*, 2005; Pecetti *et al.*, 2007) and cocksfoot (Volaire & Lelièvre, 1997; Shaimi *et al.,* 2009). Canopy temperature at complete flowering was not correlated with persistence and spike emergence date. This could mean that the earliest and most persistent accessions do not necessarily maintain high water potential and do not have higher dehydration avoidance (sensu Norton & Volaire, 2012) than late and less persistent accessions. These results partially disagree with Abberton *et al.* (2008), who linked the desiccation delay and the maintenance of high relative water content with early flowering, considering such traits as key targets for the selection of drought avoidant genotypes suitable for European dry environments. These contrasting results could be explained by the relative small number of accessions and by their limited area of origin.

Investigating the associations between phenotypic traits and environmental parameters at sites of origin of accessions, we can observe that accessions collected at sites with the lowest rainfall showed the earliest spike emergence and the highest vigour (as described by TL, FL and SL) in our experimental conditions. Accessions collected in the coldest and most elevated sites showed the lowest leafiness (LAI) at autumn regrowth. However, the environmental features of collection sites did not correlate to persistence (COV) in the third year.

In our experiment, the persistence of accessions was assessed by a measurement of soil coverage (COV), in the last year of the experiment (February 2009). Soil coverage (COV) depends on organ (tiller and leaf) size and on number of organs (which depends on number of plants and of tillers per plant). Differences in COV between the five clusters were evident (from 40 to 82%). The two accessions from Cluster V (RG06 and RG17) had significantly lower COV than the best one (RG00). Accessions in Cluster V and, even more RG17 (Cluster IV) had much lower canopy height (CH) than most of the other accessions. Meanwhile, RG06 and RG17 were late flowering accessions (SE) whereas RG00 was the earliest one. It is clear that the weak soil coverage of RG06, RG07 and RG17 at the end of the experiment is at least partly due to a poor plant size, already noticeable in the second year through the CH measurement. Assessing the persistence of accessions from an agronomic point of view (*i.e.,* assessing the ability of stands to survive and produce satisfactorily three years after sowing), makes it possible to identify promising populations for forage or alternative uses in Mediterranean rainfed conditions. The Sardinian accession RG00, included in Cluster I, has the required features of a persistent perennial ryegrass to be used for conventional forage use. Accessions from Cluster II also seem suitable for forage production, with mean DMY as good as RG00 and with low A × Y interaction (Fig. 2) that achieves quite predictable performances. On the other hand, accessions of Cluster V, with acceptable soil coverage in the third year and small plant size, are convenient as mixture components for alternative multifunctional uses, including erosion control, recovery of degraded areas and firebreak management, that require dense, low productive and persistent cover crops. Further research is advisable on these interesting accessions to better study their agronomic performances (forage and seed production) on larger plots, observing the effects of fertilization and cutting regimes on their productivity levels at farm scale.

As conclusions, the variability observed in Corsican and Sardinian populations for morphological and productive traits and for adaptation to fluctuating weather conditions allowed the identification of natural populations of perennial ryegrass with contrasting persistence. Early flowering, vigorous and productive accessions identified in this study represent a valuable germplasm to breed improved perennial ryegrass varieties for forage/hay production. The short sized and less productive accessions could be used in multifunctional mixtures, for soil coverage purposes. Such contrasting accessions represent valuable resources to

be exploited into breeding programs based on Mediterranean germplasm, in order to meet the critical need of perennial ryegrass varieties able to survive the hot and dry summer in rainfed conditions in a context of climate change and multipurpose exploitation of the species.

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References

Abberton MT, Marshall AH, Humphreys MW, Macduff JH, Collins RP Marley CL, 2008. Genetic improvement of forage species to reduce the environmental impact of temperate livestock grazing systems. Adv Agron 98: 311-355.

Annicchiarico P, Pecetti L, Abdelguerfi A, Bouzerzour H, Kallida R, Porqueddu C, Simões NM, Volaire F, 2013. Optimal forage grass germplasm for drought-prone Mediterranean environments. Field Crops Res 148: 9-14.

Balfourier F, Charmet G, 1991. Relationships between agronomic characters and ecogeographical factors in a collection of French perennial ryegrass population. Agronomie 11: 645-657.

Balfourier F, Oliveira JA, Charmet G, Arbones E, 1997. Factorial regression analysis of genotype by environment interaction in ryegrass populations, using both isozyme and climatic data as covariates. Euphytica 98: 37-46.

Barbero M, Quezel P, 1982. Classifying mediterranean ecosystems in the mediterranean rim countries and in Southwestern U.S.A. Proc Symp Dynamics and management of Mediterranean – Type Ecosystems (Conrad C, Oechel WC, eds). Pacific Southwest Forest and Range Exp Stat, Berkeley, CA, USA. pp: 69-74.

Blum A, Mayer J, Gozlan G, 1982. Infrared thermal sensing of plant canopies as a screening technique for dehydration avoidance in wheat. Field Crops Res 5: 137-140.

Bransby DI, Matches AG, Krause GF, 1977. Disk meter for rapid estimation of herbage yield in grazing trials. Agron J 69: 393-396.

Chapman DF, Edwards GR, Nie ZN, 2011. Plant responses to climate and relationships with pasture persistence. Pasture persistence – Grassland Research and Practice Series 15: 99-108.

Charmet G, Balfourier F, Bion A, 1990. Agronomic evaluation of a collection of French perennial ryegrass populations: multivariate classification using genotype × environment interactions. Agronomie 10: 807-823.

Choo TM, 1984. Association between growth habit and persistence in red clover. Euphytica 33: 177-185.

Cunningham PJ, Graves WL, Chakroun M, Mezni MY, Saidi S, Bounejmate M, Porqueddu C, Reed KFM, 1997. Novel perennial forage germplasm from North Africa and Sardinia. Aust Plant Introd Rev 27: 13-46.

Douglas JT, Crawford CE, 1994. An evaluation of the drop-disc technique for measurements of herbage production in ryegrass for silage. Grass Forage Sci 49: 252-255.

Elazreg H, Chtourou-Ghorbe N, Ghariani S, Chakroun M, Trifi-Farah N, 2011. Studying genetic diversity of the Tunisian *Lolium perenne* and *Festuca arundinacea* with AFLP markers. J Food Agric Environ 9: 409-415.

Falcinelli M, Veronesi F, Lorenzetti S, 1988. Evaluation of an Italian germplasm collection of *Lolium perenne* L. through a multivariate approach. Proc Eucarpia Fodder Crops Section Meeting, Lusignan (France), Sept 22-24. pp: 22-24.

Franca A, Porqueddu C, Roggero PP, Sulas L, 1995. Adattamento e produttività di alcune graminacee da prato e da pascolo in ambiente semi-arido mediterraneo. Rivista di Agronomia 29(2): 171-177.

Franca A, Loi A, Davies WD, 1998. Selection of annual ryegrass for adaptation to semi-arid conditions. Eur J Agr 9: 71-78.

Franca A, Porqueddu C, Dettori D, 2007. Evaluating new grass-legume mixtures for pasture improvement in a semi-arid environment. In: Permanent and temporary grassland: plant, environment and economy (De Vliegher A, Carlier L, eds). Grassland Sci Eur 12: 43-46.

Fulkerson WJ, Neal JS, Clark CF, Horadagoda A, Nandra KS, Barchia I, 2007. Nutritive value of forage species grown in the warm temperate climate of Australia for dairy cows: grasses and legumes. Livest Sci 107: 253-264.

Gauch HG, 2007. MATMODEL vers. 3.9: Open source software for AMMI and related analyses. Crop and Soil Sciences. Cornell University, Ithaca, NY, USA.

Gauch HG, Furnas RE, 1991. Statistical analysis of yield trials with MATMODEL. Agron J 83: 916-920.

Gauch HG, Piepho HP, Annicchiarico P, 2008. Statistical analysis of yield trials by AMMI and GGE: further considerations. Crop Sci 48(3): 866-889.

Harris CA, Clark SG, Reed KFM, Nie ZN, Smith KF, 2008. Improvement of tall fescue and cocksfoot for marginal environments in southern Australia. Aust J Exp Agr 47: 436-448.

Hawkins DM, 1982. Topics in applied multivariate analysis. Cambridge University Press, Cambridge, UK.

Lelièvre F, Volaire F, 2009. Current and potential development of perennial grasses in rainfed Mediterranean farming systems. Crop Sci 49: 2371-2378.

Lelièvre F, Norton MR, Volaire F, 2008. Perennial grasses in rainfed Mediterranean farming systems. In: Sustainable Mediterranean grasslands and their multi-functions (Porqueddu C, Tavares de Sousa MM, eds). CIHEAM Zaragoza. Opt Méditerr A 79: 137-146.

Lelièvre F, Sala S, Volaire F, 2010. Climate change at the temperate-Mediterranean interface in Southern France and impacts on grasslands production. In: The contributions of grasslands to the conservation of Mediterranean biodiversity (Porqueddu C, Ríos S, eds). CIHEAM Zaragoza. Opt Méditerr A 92: 187-192.

Lopes MS, Reynolds MP, 2010. Partitioning of assimilates to deeper roots is associated with cooler canopies and increased yield under drought in wheat. Funct Plant Biol 37(2): 147-156.

Manly BFJ, 1994. Multivariate statistical methods: a primer. Chapman & Hall, London. 215 pp.

Masson P, Gintzburger G, 2000. Les légumineuses fourragères dans les systèmes de production méditerranéens: utilisations alternatives. In: Legumes for Mediterranean forage crops, pastures and alternative uses (Sulas L, ed). CIHEAM Zaragoza. Opt Méditerr A 45: 395-406.

Migliore J, Baumel A, Juin M, Diadema K, Hugot L, Verlaque R, Médail F, 2011. Genetic diversity and structure of a Mediterranean endemic plant in Corsica (*Mercurialis corsica*, Euphorbiaceae). Popul Ecol 53: 573-586.

Ministère de l'Agriculture et de la Pêche, Inventaire Forestier National, 2006. Les indicateurs de gestion durable des forêts françaises, Édition 2005, DGFAR, Paris.

Molle G, Decandia M, Cabiddu A, Landau SY, Cannas A, 2008. An update on the nutrition of dairy sheep grazing Mediterranean pastures. Small Rumin Res 77: 93-112.

Nie Z, 2011. Use of perennial grass in grazing systems of Southern Australia to adapt to a changing climate. In: Climate change – Research and technology for adaptation and mitigation (Blanco J, Kheradman H, eds). InTech. pp: 431-452.

Nie Z, Chapman DF, Tharmaraj J, Clements R, 2004. Effects of pasture species mixture, management, and environment on the productivity and persistence of dairy pastures in south-west Victoria. 1. Herbage accumulation and seasonal growth pattern. Aust J Agr Res 55(6): 625-636.

Norton MR, Volaire F, 2012. Selection of pasture and forage species adapted to changing environmental conditions in Mediterranean climates. In: New approaches for grassland research in a context of climate and socio-economic changes (Acar Z, López-Francos A, Porqueddu C, eds). CIHEAM Zaragoza. Opt Méditerr A 102: 119-127.

NZPBRA, 2010. National forage variety trials. Data summaries: perennial ryegrass 1991-2009. New Zeal Plant Breeding Res Assoc, Christchurch, New Zealand.

Parsons AJ, Rasmussen S, Xue H, Newman JA, Anderson CB, Cosgrove GP, 2004. Some high sugar grasses don't like it hot. Proc of the New Zealand Grassland Association 66: 265-272.

Pecetti L, Romani M, Carroni AM, Annicchiarico P, Piano E, 2007. The effect of endophyte infection on persistence of tall fescue (*Festuca arundinacea* Schreb.) populations in two climatically contrasting Italian locations. Aust J Agric Res 58: 893-899.

Pecetti L, Annicchiarico P, Abdelguerfi A, Kallida R, Mefti M, Porqueddu C, Simões NM, Volaire F, Lelièvre F, 2011. Response of Mediterranean tall fescue cultivars to contrasting agricultural environments and implications for selection. J Agron Crop Sci 197(1): 12-20.

Piano E, Annicchiarico P, 1995. Persistence of ladino white clover ecotypes and its relationship with other agronomic traits. Grass Forage Sci 50: 195-198.

Piano E, Bertoli FB, Romani M, Tava A, Riccioni L, Valvassori M, Carroni AM, Pecetti L, 2005. Specificity of host-endophyte association in tall fescue populations from Sardinia, Italy. Crop Sci 45: 1456-1463.

Pignatti S, 1982. La flora d'Italia. Edagricole, Bologna.

Pontes LS, Carrère P, Andueza D, Louault F, Soussana JF, 2007. Seasonal productivity and nutritive value of temperate grasses found in semi-natural pastures in Europe: responses to cutting frequency and N supply. Grass Forage Sci 62: 485-496.

Porqueddu C, Sulas L, 1998. Mediterranean grassland systems. In: Ecological aspects of grassland management (Nagy G, Peto K, eds). Grassland Sci Eur 3: 335-345.

Porqueddu C, González F, 2006. Role and potential of annual pasture legumes in Mediterranean farming systems. Pastos XXXVI(2): 125-142.

Porqueddu C, Maltoni S, 2006. Experiences and perspectives of restoration with herbaceous species in Italy. In: Soil-Bioengineering: ecological restoration with native plant and seed material (Krautzer B, Hacher E, eds). Proc Int Conf HBLFA Raumberg-Gumpenstein (Austria), 5-9 September 2006. pp: 219-226.

Reed KFM, Cunningham PJ, Barrie JT, Chin JF, 1987. Productivity and persistence of cultivars and Algerian introductions of perennial ryegrass (*Lolium perenne* L.) in Victoria. Aust J Exp Agric 27: 267-274.

Reed KFM, Cunningham PJ, Byron A, Anderson MW, 1999. Perennial grasses of Mediterranean origin offer advantages for central western Victorian sheep pasture. Aust J Exp Agric 39: 275-284.

Sampoux JP, Bandouin P, Bayle B, Beguier V, Bourdon P, Chosson JF, Deneufbourg F, Galbrun C, Ghesquiere M, Noel D *et al.,* 2011. Breeding perennial ryegrasses for forage uses: an experimental assessment of trait changes in diploid perennial ryegrass (*Lolium perenne* L.) cultivars released in the last four decades. Field Crop Res 123: 117-129.

Sanna F, 2009. Le aree a pascolo in ambiente forestale mediterraneo: valutazione di metodologie di rilevamento pastorale e valorizzazione di specie native, per la gestione multiuso di aree forestali. Tesi di dottorato. Facoltà di Agraria. Università degli Studi di Sassari – XX ciclo. 197 pp.

Shaimi N, Kallida R, Volaire F, Al Faiz C, 2009. Summer dormancy in orchardgrass: evaluation and characterization through ecophysiological and genetic studies. Crop Sci 49: 2353-2358.

Schubiger FX, Lehmann J, Daccord R, Arrigo Y, Jeangros B, Scehovic J, 2001. Nutritive value of grassland plants. 5. Digestibility of organic matter. Revue Suisse Agric 33: 275-279.

Slack K, Fulkerson WJ, Scott JM, 2000. Regrowth of prairie grass (*Bromus willdenowii* Kunth) and perennial rye-

grass (*Lolium perenne* L.) in response to temperature and defoliation. Aust J Agr Res 51(5): 555-561.

Taylor NL, Quesenberry KH, 1996. Red clover science. Kluwer Acad Publ, Dordrecht, The Netherlands.

Volaire F, Lelièvre F, 1997. Production, persistence, and water-soluble carbohydrate accumulation in 21 contrasting populations of *Dactylis glomerata* L. subjected to severe drought in the south of France. Aust J Agric Res 48: 933-944.

Waller RA, Sale PWG, 2001. Persistence and productivity of perennial ryegrass in sheep pastures in South-Western victoria: a review. Aust J Exp Agric 41(1): 117-144.

Walter H, Lieth H, 1967. Klimadiagram-Weltatlas. VEB Gustav Fischer Verlag, Jena.

Warren WJ, 1963. Estimation of foliage denseness and foliage angle by inclined point quadrats. Aust J Bot 11: 95-105.