

Original Article

Biomass characteristics in Mediterranean populations of *Piptatherum miliaceum* - A native perennial grass species for bioenergy

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Abstract

Native Mediterranean perennial grasses may represent a suitable feedstock for bioenergy production, offering high yields combined with environmental and economic advantages. Nonetheless, their potential is little investigated so far. This research aimed at evaluating bioenergy traits in Mediterranean germplasm of the perennial species *Piptatherum miliaceum* (L.) Coss., smilo grass, grown under rainfed conditions in Sardinia (Italy). Ten autochthonous populations were investigated for maturity, biometric traits, biomass production and quality in a spaced-plant field experiment; ANOVA and linear correlations were performed.

Smilo grass populations differed for flowering date, biometric traits, yield and biomass quality traits. Aboveground dry matter ranged from 651 to 1136 g plant⁻¹. The high productive populations out-yielded the test species (tall fescue). Cellulose content was approximately 40% in tillers and leaves. Heating value ranged from 16.3 to 18.2 MJ kg⁻¹ in leaves and tillers, respectively. The ultimate analysis of biomass revealed relatively high contents of chlorine and sulphur. A significant correlation was found between dry matter yield and number of tillers per plant. The general outcome of our experiment evidenced interesting traits for bioenergy production in smilo grass with favorable combinations of biomass yield and lignocellulosic contents. Additional research is necessary for investigating long-term performances of smilo grass crops and the effect of management on biomass yields and their chemical composition.

Keywords: Smilo grass, biomass, yield, lignocellulosic contents, ultimate analysis, bioenergy.

Abbreviations:

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CEL: (Cellulose Leaf); **CEP:** (Cellulose Panicle); **CET:** (Cellulose Tiller); **DMY:** (Dry Matter Yield); **DoY:** (Days of Years); **EL:** (Hemicellulose Leaf); **ET:** (Hemicellulose Tiller); **FLL:** (Flag Leaf length); **LF:** (Lignin Leaf); **LP:** (Lignin Panicle); **LT:** (Lignin Tiller); **ND:** (Nodes); **NT:** (Number of Tiller); **SBF:** (Stage Beginning Flowering); **SFF:** (Stage Full Flowering); **PL:** (Panicle length); **SLF:** (Stage Late Flowering); **TL:** (Tiller length); **To:** (Total length);

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1. Introduction

Several authors warned that bioenergy crops may substitute food crops in fertile lands for bioenergy production; moreover, the extension of previously unmanaged grassland areas may decrease as a response to energy policy guidelines and legislation in Europe and United States (Zegada-Lizarazu et al., 2010; Gelfand et al., 2011; Gelfand et al., 2013; Miyake et al., 2012; Popp et al., 2014). As an alternative option, the exploitation of grasslands (Heinsoo et al., 2010) and marginal lands (Zhuang et al., 2011), the use of agricultural residues (Muth et al., 2013), the cultivation of wild inedible species (Moshi et al., 2014) or sustainable lignocellulosic crops are being evaluated (Palmer et al., 2014) for the second generation bioenergy production. In Europe, the research and industrial efforts on biomass feedstock mainly focused on lignocellulosic energy crops, such as miscanthus (*Miscanthus* spp.), switchgrass (*Panicum virgatum* L.), and woody species (Venturi et al., 1999; Lewandowski et al., 1999; Kahle et al., 2001; Clifton-Brown et al., 2001; Angelini et al., 2009; Robbins et al., 2012).

Unfortunately, in Mediterranean environments, the sustainable cultivation of lignocellulosic non-food crops relies on a poor assortment of dedicated species and varieties (Mantineo et al., 2009; Ledda et al., 2013; Scordia et al., 2014), due to the scarcity of plants able to face summer drought under rainfed regime. In this framework, some perennial grasses may represent suitable potential bioenergy feedstock, as they show a drought avoidance or tolerance strategy (Volaire, 2009) associated to high water use efficiency (Lelièvre et al., 2011). Perennial grasses have several economic and environmental advantages (Palmer et al., 2014) such as low input requirements, adequate productivity, ability to grow on marginal lands, ecological benefits for soil and wildlife

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habitats. Nonetheless, the native germplasm of Mediterranean perennial grasses is still little investigated for forage purposes (Annicchiarico et al., 2013), and nearly unexplored for bioenergy. *Piptatherum miliaceum* (L.) Coss. (Synonyms: *Oryzopsis miliacea* (L.) Asch. Et Schweinf, *Oloptum thomasi* (L.) Röser & Hamasha, *Achnatherum miliaceum* (L.) P. Beav., common name smilo grass, is a perennial species of Mediterranean basin origin, belonging to the *Graminaceae* family. It is naturalized in California, Western Nevada, South America, Australia, and New Zealand. Smilo grass is a hemicryptophyte (Pignatti, 1982), pollinated by wind (Proctor et al. 1996). According to Vogel et al. (1986), smilo grass has a C3 photosynthetic pathway. A wide range of ethnobotanical utilizations is reported for this species in Mediterranean basin countries. Baldoni (1981) described smilo grass as a plant supplying high amounts of palatable forage. Other authors stated that the species is grazed by livestock (Camarda, 1990; Celik, 1998) and can be considered as an important component in natural pastures (Kayiouli, 2006). Yet, smilo grass is mentioned as introduced range species for cultivation in desert regions but also for rangeland restoration and soil erosion control (Le Houérou, 2000). Other uses refer to its ability to remove and accumulate Pb and Zn from contaminated soils in mining polluted areas (Garcia et al., 2004; Conesa et al., 2009; Gonzalez-Fernandez et al., 2011) and to its medicinal proprieties (Falchi, 1981; Lentini and Venza, 2007, Cabello and Castro, 2012).

The native smilo grass grows in harsh environments (rocky soils, rocky slopes in direct sunlight, shallow soils, and roadsides) where nutrients availability is scarce. Moreover, smilo grass exhibited drought tolerant traits (Abd El-Rahman and El-monayeri, 1968), as its leaves dry up during summer, remaining in a semi-dormant state, and sprout again when sufficient rain has fallen. The substantial amounts of unpalatable dry matter available at the end of the growth season (Bullitta, 1993) suggest the potential use of this species for bioenergy.

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In the frame of a general activity aimed at the exploitation of Mediterranean herbaceous plant germplasm for multiple uses, our specific aims were:

- (i) to evaluate variability in cycle duration, biometric traits, biomass production and quality biomass characteristics in autochthonous smilo grass populations;
- (ii) to identify smilo grass populations suitable for bioenergy purposes under rainfed Mediterranean conditions;

2. Materials and methods

2.1 Experimental site and meteorological pattern during the trial

The field experiment was carried out at the experimental station of CNR (Leccari, Sassari, 40° 45' 12" N, 8° 25' 17" E; 27 m a.s.l.), in Sardinia (Italy), from 2012 to 2014. The climate of the area is typically Mediterranean with mild winter, characterized by a long-term average annual rainfall of 554 mm prevalingly distributed in autumn and winter months, and a mean annual air temperature of 16.2 °C. The soil at the experimental site has been classified as a Eutric, Calcaric and Mollic Fluvisol according to FAO classification (2006); soil is sandy-clay-loam, alkaline with a scarce average nitrogen content (0.96‰) and adequate phosphorous and organic matter content.

Daily maximum and minimum air temperatures and rainfall events were monitored in the experimental site by a weather station, and a data logger recorded meteorological data.

In the first annual growing season (2013) of smilo grass, total rainfall reached 718 mm, concentrated in winter and spring (Fig. 1). In the subsequent growing season (2014) was 626 mm with a better seasonal distribution. Total rainfall exceeded climatic means by 29.6 and 19.6% for the

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two growing seasons, respectively. Mean temperatures never dropped below the average monthly value of 7 °C (February 2013) and did not differ from long-term values, except in late spring 2012.

2.2 Plant material, experimental design and plot management

Since 2010, a collection of native grass plants for bioenergy potential evaluation has been carried out by the Istituto per il Sistema Produzione Animale in Ambiente Mediterraneo, Consiglio Nazionale delle Ricerche (CNR-ISPAAAM), in Sardinia. Seeds of *P. miliaceum* were collected from ten native populations, in sites differing for micro-environmental conditions and distributed all over the island (Table 1). After collection, seeds were sown in plastic pots and seedlings grown under a cold polycarbonate-covered tunnel for about 16 weeks. In April 2012, plants were transplanted to a spaced-plant field nursery, arranged in a completely randomized design with three replicates at the experimental field of CNR ISPAAAM in Sassari. Each replicated plot held 8 plants, spaced 0.5 x 0.5 m in row and between rows, for a total of 24 plants per populations. Plants of *Festuca arundinacea* Schreb. (tall fescue) cultivar Flecha were also transplanted and utilized as test in the nursery field. In fact, Flecha was found to be a benchmark, in a previous 4-year multisite experiment, for high yields and persistence over years (Pecetti et al., 2010). Moreover, tall fescue has the same photosynthetic pathway and propagation method (by seed). No fertilizers and irrigation were applied and weed control was performed by mechanical means. An emergency irrigation (50 mm) was provided just after transplanting to help root development.

2.3 Data collection

Plants were cut the first time in July 2012 to stimulate a synchronous regrowth in all plants and plots. Phenological observations were carried out during the subsequent growing seasons. Plant

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phenology was monitored weekly to define the beginning, evolution and duration of the following stages: tillers elongation, heading, flowering, seed setting and ripening. The beginning of each stage was expressed as the day of the year (DoY) from January 1st.

The variability of biometric parameters in smilo grass populations was determined at the end of heading stage in 2013. We measured the length of reproductive tillers (from the first visible node from soil to the insertion of panicle), the length of panicles, the number of nodes per reproductive tiller, the length and width of the flag leaf on thirty tillers per population (ten tillers per plot).

Aboveground plant dry matter yield (DMY) was estimated once a year in July 2013 and 2014, for the first and second growing seasons, respectively. DMY was determined on an individual plant basis, as average of dry weight of 18 plants per populations (6 plants per plot).

The number of total tillers per plant was determined at harvest in both years, by counting tillers in two plants per plot (6 plants per population). Finally, biomass partitioning among tillers, leaves and panicles was estimated for both years on 150 reproductive tillers per accessions (50 tillers per plot). Fresh tillers were separated into components and each component was weighted and then oven-dried at 60 °C up to constant weight and dry weight and dry weight rate (%) calculated. An amount of DM of each component was accurately ground and stored for biomass quality analysis.

2.4 Biomass quality analysis

Neutral and acid detergent fibers (NDF and ADF) and acid detergent lignin (ADL) were determined for both years by using the procedure of Van Soest et al. (1991). The cellulose content was calculated as ADF minus ADL, hemicellulose as the difference between NDF and ADF, and lignin as the ADL value.

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Dry milled subsamples of each biomass component and species were sent to a chemical laboratory (Laboratorio di Ricerca e ANalisi, LARIAN, Pomezia, Rome) for the determination of their calorific value, the carbon, hydrogen, nitrogen, sulphur and chlorine contents and the proximate analysis (ash, volatile matter and fixed carbon). Analyses of samples were carried out in duplicate, according to the Technical Specification UNI CEN/TS (2005), and to the American Society for Testing and Materials standardized procedures (ASTM, 2004).

2.5 Statistical analysis

The variability in biometric, biomass production, biomass quality and phenological traits, was investigated through a two-way fixed model of analysis of variance (ANOVA) with “Accession” (A) and “Block” (B) as fixed effects. For the traits observed for two years, the interaction Accession \times Year (A \times Y) was investigated to assess the stability of traits, by implementing a mixed model ANOVA with “Block” (B) and “Accession” (A) as fixed effects, and “Year” (Y) as a random effect, assuming that effect of climatic conditions during each year were randomly uncontrolled. Traits not showing significant interaction A \times Y were computed in the statistical analyses as average of the two years.

Fisher's least significant difference (*LSD*) method was used for comparing means in the ANOVA.

All ANOVAs were performed using Statgraphics Centurion XVI (StatPoint, Inc., USA).

Linear correlation coefficients were used to determine the relationship within morphological traits, and between them and qualitative and phenological data.

3. Results and discussion

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Among the traits observed for two years, only the percentages of biomass partitioning for tillers, leaves and panicles showed significant interaction A x Y. Thus, these data are reported separately for the two years 2013 and 2014.

3.1 Phenological stages

In both 2013 and 2014, after the cut at the beginning of July, plants remained in a semi-dormant state during summer. The emission of new tillers from the crown started in the first days of September, after late-summer rains and when temperatures began to drop. Tillers elongation in smilo grass continued during winter months, ending at the beginning of heading stage (113-126 DoY). Significant differences were found among smilo grass populations for the start of their phenological stages only for heading and full flowering date (Table 2). Full flowering began between 151 in PM09 and 164 DoY in PM07, with most populations exhibiting the full flowering stage between 154 and 158 DoY, and the seed setting between 171 (in most populations) and 174 DoY in PM04. Foliar senescence started immediately after seed setting and was complete at seed ripening. Nonetheless, tillers remained green and were still green at harvest time. Smilo grass showed a later cycle than tall fescue Flecha. Specifically, the beginning of the heading stage delayed about 40 days respect to tall fescue. Nonetheless, the whole cycle in smilo grass ended 25 days after seed setting in tall fescue, indicating shorter time spans for the reproductive stages in smilo grass, presumably due to the higher temperatures during the end of its growing seasons.

It has been reported that the starting and duration of phenological stages in bioenergy species is correlated to DMY. In switchgrass, Hopkins et al. (1995) found that early maturing genotypes showed positive correlations with higher DMYs. Pedroso et al. (2011) confirmed the same outcome with the same species. Clifton-Brown et al. (2001) studied the performances of 15 miscanthus

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genotypes in different European sites, and found that higher DMYs were also associated with late flowering time or no flowering and that these genotypes kept a higher amount of green photosynthetic area, continuing to accumulate dry matter. In our experiment, no direct correlations were found between flowering time and DMY (Table 6). However, late-flowering populations showed a positive correlation with flag leaf length ($r = 0.667$, $p < 0.05$) and a delayed foliar senescence that can be related to a wider growth period and potential higher biomass accumulation, as in other biomass species (Jones et al., 2014).

Later flowering was positively correlated with cellulose content in reproductive organs ($r = 0.770$, $p < 0.05$), whereas it was negatively correlated with hemicellulose content in tillers ($r = -0.679$, $p < 0.05$). Both cellulose and hemicellulose contents are important for the quality of biomass and their evolution in relation to phenology has to be further investigated.

3.2 Biometric parameters

Smilo grass populations differed for morphological traits (Table 3). Tiller length (TL) ranged between 107 and 124 cm (mean 115.9). Panicle length (PL) ranged from 39 to 44 cm (mean 41.3) and tiller total length (To) from 146 and 164 cm (population mean 157). No correlations were observed between panicle and tiller lengths and other traits (Table 6), except for the flag leaf length ($r = 0.681$ and $r = 0.686$ respectively, $p < 0.05$). The flag leaf width ranged between 0.6 and 0.8 cm (mean 0.69). The mean number of nodes per tiller ranged from 6.6 to 7.6 (mean 7.2). This trait was found to be negatively correlated with the ADL content in tillers ($r = -0.793$, $p < 0.05$), indicating a better biomass quality for energy purposes in plants having a higher amount of young tissues, as it could be expected.

3.3 Tiller number, plant aboveground biomass yield and its partitioning

The number of tiller per plant ranged from 214 to 338 in PM03 and PM05, respectively (Table 4). The populations PM05 and PM06 showed a superior number of tiller per plant compared to PM03 and Flecha tall fescue. Tiller number per plant in smilo grass was positively correlated ($r = 0.692$, $p < 0.05$) with aboveground plant dry matter yield (Table 6). For switchgrass, tiller mass has been reported to be a better predictor of biomass yield than tiller density (Fike et al., 2006).

Percentage of DM at harvest (data not shown) reached 65.9 in tall fescue Flecha, whereas it was 62.5% as average of the smilo grass populations under study.

Aboveground biomass ranged from 652 to 1138 g plant⁻¹ in tall fescue and PM05, respectively (Table 4). The high yielding populations PM05 and PM06 significantly differ from several of the remaining populations, out-yielding tall fescue Flecha for about 60%. In our environment, the performances of smilo grass compared to Flecha tall fescue are very interesting. In fact, the highly winter-active Mediterranean-type fescue Flecha proved to be drought tolerant and high yielding in a nearby site (Pecetti et al., 2010). Due to the very limited information on smilo grass, direct comparisons with other smilo grass genotypes are currently not possible, to our knowledge. However, the abovementioned performances in high yielding populations of smilo grass slightly exceeded the peak values for biomass yield from individual plants of both upland and lowland switchgrass cultivars grown in New Jersey (Cortese and Bonos, 2013).

A substantial variability for biomass partitioning was found across all populations. This trait was influenced by environmental conditions of the year, which induced a different allocation of biomass among aboveground plants components. In 2013, no smilo grass population exceeded the tillers biomass rate found in tall fescue (53.3%), whereas in 2014, only a population showed a higher biomass accumulation in tillers than tall fescue. In 2014, biomass accumulation in tillers was higher

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in all populations and in the test species than in 2013 (63.5 vs 50%), and three populations exceeded 65% of biomass accumulated in tillers. The lowest percentage of tillers weight was 48% in 2013 and 59% in 2014.

As leaves are regarded, both in 2013 and in 2014 tall fescue showed the highest amount on DM accumulated in leaves (about 34%), an outcome to be expected from a perennial grass selected for forage production. In smilo grass, the mean rate of leaves was 26.5% in 2013 (range 24-29.4) and 15% in 2014 (range 12.3-17.8).

Both in 2013 and 2014, the contribution of reproductive organs to the biomass in smilo grass ranged from 23 to 31% in 2013 (mean 27.3) and from 16 to 27% in 2014 (mean 21.7) and markedly differed compared to tall fescue.

3.4 Lignocellulosic contents of biomass

Concerning the lignocellulosic contents of the smilo grass populations the results, reveal that there were differences in their composition dependent on plant components (Table 5). In tillers, the cellulose content ranged from 35.7 in tall fescue to 40.6% in PM10, respectively. All smilo grass populations had a higher cellulose content compared to Flecha tall fescue, and among them PM10 and PM02 significantly differed from PM08. The hemicellulose content ranged from 22.6 in tall fescue to 29.6% in PM03. All smilo grass populations had a higher hemicellulose content compared to Flecha tall fescue and among them PM01, PM03, and PM07 significantly differed from PM04 and PM10. The populations PM05 and PM10 showed a lignin content higher than PM01, PM02, PM06, PM09 and tall fescue. The cellulose content of tillers was positively correlated ($r = 0.668$ $p < 0.05$) with flowering date, whereas the lignin content was negatively correlated ($r = -0.793$, $p < 0.05$) with the number of nodes per tiller (Table 6).

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In leaves, the cellulose content ranged from 32.3 in PM09 to 44.1% in tall fescue, with the former showing the lowest significant value among all populations. The hemicellulose content ranged from 21.9 in tall fescue to 27.7% in PM09, respectively, whereas the lignin content never exceeded 10%. The hemicellulose content of leaves was negatively correlated ($r = -0.690$, $p < 0.05$) with cellulose content of reproductive organs (Table 6).

Reproductive organs showed a higher cellulose content in tall fescue (41%) compared to smilo grass populations where it ranged from 28.3 to 35.7%. The hemicellulose content ranged from 29.5 to 35%, whereas it was significantly lower in tall fescue (19.2%). The lignin content varied from 9.1 to 13.8% and did not significantly differ from Flecha tall fescue, except for PM04. The cellulose content of reproductive organs was positively correlated ($r = 0.770$, $p < 0.05$) with full flowering date, whereas was negatively correlated ($r = -0.913$, $p < 0.001$) with the cellulose content in tillers (Table 6). Therefore, several populations showed variability in lignocellulosic contents of different plant components. On the other hand, the total lignocellulosic contents of aboveground biomass in smilo grass were also affected by the different contributions of tillers, leaves and reproductive organs, respectively.

The overall content of cellulose, hemicellulose and lignin in smilo grass populations and their variations are quite similar to those reported for several cultivars of switchgrass (NREL, 2004; David and Ragauskas, 2010). High concentrations of cellulose and hemicellulose and low concentrations of lignin are desirable for high bioethanol conversion (Lemus et al., 2002). On the contrary, lignin content is important for combustion. Therefore, among the high yielding populations of smilo grass, at a very similar content of cellulose in tillers, PM06 could be regarded as promising germplasm for bioethanol production because also presented a lower content of lignin (Table 5).

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3.5 Heating value, proximate and ultimate analysis of biomass

In tillers, the calorific value (Table 7) reached on average 18.2 MJ kg⁻¹ and it was higher than in leaves and reproductive organs. The proximate analysis also showed differences among plant components. In particular, the ash contents of leaves was about three times as higher than in tillers. According to Cassida et al. (2005), ash content of switchgrass is primarily affected by environmental factors and could be reduced in delayed harvests, due to the translocation of mineral towards roots. The ultimate analysis indicated for smilo grass biomass typical values of C, H, O and N of herbaceous fuels (Oberberger et al., 2006) and values are quite similar to those reported for switchgrass (David and Ragauskas, 2010). However, our first information on the ultimate analysis of smilo grass biomass also revealed substantial higher contents of sulphur and chlorine, compared to switchgrass. Similar high chlorine contents were already found in the same area for *Cynara cardunculus* var. *atilis* (cardo) from Ledda et al. (2013), who also indicated operational measures required for processing its biomass via combustion.

To our knowledge, this is the first evaluation of bioenergy traits for Mediterranean smilo grass populations and, therefore, cannot be considered an exhaustive study. However, our research indicated several important bioenergy traits in smilo grass germplasm under evaluation. Moreover, additional positive features of smilo grass need to be taken into account. In fact, such a perennial grass species has a native status, can be propagated by seed, proved to be very persistent under drought and tolerant to insects and diseases, and can be easily integrated into existing Mediterranean farming systems (Author personal comm.). Finally, the possibility to use smilo grass as a dual purpose (bioenergy but also forage) crop, as indicated for other forage species (Sanderson et al., 2004), may make it more acceptable to farmers than the cultivation of rhizomatous species. In

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addition, establishment and harvest of smilo grass can be performed with farm machinery, whereas the management of rhizomatous species require expensive extra-farm equipment.

4. Conclusions

Results indicate interesting features for bioenergy in the investigated smilo grass populations grown under rainfed Mediterranean conditions. Some populations exhibited a positive combination of high biomass yields and favorable lignocellulosic contents. The ultimate analysis of biomass showed a similar composition among genotypes with a good calorific value but with relatively high content of some elements. Additional research is necessary for investigating long-term performances of smilo grass populations in multi-location studies and for elucidating the effects of management on biomass yields and their chemical composition. Overall, the results encourage studies on little investigated native Mediterranean perennial grass, whose potential for bioenergy is still unexplored.

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Table 1. List of collection and surveying sites of smilo grass germplasm in Sardinia (Italy).

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Populations	FAO Soil Classification	Altitude (m a.s.l.)	Latitude	Longitude	Annual rainfall (mm)	Use of soil
PM01	Eutric, Dystric and Lithic Leptosols	29	40°51'	8°14'	500	Roadside
PM02	Eutric, Lithic Leptosols	353	40° 23'	8°41'	880	Pasture
PM03	Eutric, Calcaric Mollic Fluvisols	15	39°57'	8°40'	620	Roadside
PM04	Eutric, Lithic Leptosols	98	39°33'	8°56'	560	Uncultivated
PM05	Eutric, Calcaric Mollic Fluvisols	26	39°11'	9°08'	430	Fallow
PM06	Eutric, Dystric and Lithic Leptosols	28	39°08'	9°32'	560	Grazed
PM07	Haplic Nitisols	35	39°22'	8°58'	490	Roadside
PM08	Eutric, Calcaric Mollic Fluvisols	12	41°14'	9°11'	514	Fallow
PM09	Eutric, Lithic Leptosols	56	40°44'	8°25'	590	Cultivated
PM10	Rock Outcrop	117	39°23'	9°03'	530	Grazed

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Table 2.

Main phenological stages in ten smilo grass populations (days of the year from January 1st)^a.

Populations	Beginning of heading		End of heading		Full flowering		Seed setting		Leaf senescence	
Flecha	82.7	a	103.8	a	123.5	a	150.0	a	135.0	a
PM01	125.8	b	139.6	bc	158.5	bcd	173.3	b	172.0	b
PM02	124.5	b	138.2	bc	161.1	cd	171.7	b	176.0	b
PM03	126.5	b	138.9	bc	155.4	bcd	174.2	b	174.7	b
PM04	114.1	b	141.2	bc	158.2	bcd	172.8	b	174.7	b
PM05	120.2	b	138.9	bc	152.9	bc	171.5	b	177.3	b
PM06	120.6	b	136.8	b	154.2	bcd	171.5	b	172.0	b
PM07	113.2	b	145.9	c	164.6	d	171.5	b	176.0	b
PM08	116.6	b	136.1	b	157.4	bcd	171.7	b	172.0	b
PM09	124.5	b	138.9	bc	151.3	b	171.5	b	174.7	b
PM10	124.5	b	138.9	bc	156.8	bcd	173.1	b	177.3	b

^aAs average of 2013-2014. Means followed by the same letters within a column are not statistically different ($p \leq 0.05$).

Table 3.

Main morphological traits for ten smilo grass populations during 2013.

Populations	To cm		TL cm		PL cm		ND no.		FLW cm		FLL cm	
PM01	145.8	a	106.7	a	39.1	ab	7.3	cde	0.73	d	25.1	a
PM02	160.7	cd	116.5	bc	44.2	e	7.4	cde	0.71	cd	31.5	e
PM03	164.1	d	123.5	d	40.6	abc	7.5	de	0.81	e	31.8	e
PM04	157.1	bc	113.7	b	43.4	de	7.1	cd	0.70	cd	31.0	e
PM05	159.0	cd	119.2	cd	39.8	ab	7.1	bc	0.72	d	28.9	cd
PM06	162.0	cd	120.5	cd	41.5	cd	6.7	ab	0.59	a	26.7	abc
PM07	161.2	cd	118.3	bcd	42.9	cde	7.2	cde	0.69	cd	29.3	cde
PM08	146.1	a	107.5	a	38.6	a	6.6	a	0.74	d	26.3	a
PM09	163.0	d	119.8	cd	43.2	de	7.5	de	0.65	bc	26.6	ab
PM10	153.0	ab	113.2	b	39.8	ab	7.6	e	0.60	ab	27.2	abc

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TL: tiller length, PL: Panicle length, To: total length (TL+PL); ND: node number; FLW: flag leaf width; FLL: flag leaf length

Means followed by the same letters within a column are not statistically different ($p \leq 0.05$).

Table 4.

Tillers number, plant aboveground biomass and its partitioning into components of ten smilo grass populations.

Populations	Tillers plant ⁻¹ (no.) ^a		Dry matter plant ⁻¹ (g) ^a		Tillers (%)		Leaves (%)		Panicles (%)							
					2013	2014	2013	2014	2013	2014						
Flecha	203.8	a	652.0	a	53.3	e	62.4	ab	33.9	d	33.6	e	12.4	a	3.9	a
PM01	244.6	ab	768.9	ab	49.7	abcd	62.0	ab	28.3	bc	16.0	abcd	25.9	bcd	22.0	cdefg
PM02	263.7	abc	977.9	bcd	52.4	de	60.5	a	27.1	abc	13.2	abc	23.2	b	26.3	fg
PM03	214.0	a	766.1	ab	51.5	bede	70.7	c	26.7	abc	12.3	a	25.3	bcd	17.0	bc
PM04	286.3	abc	783.4	ab	50.0	abcd	67.6	bc	29.4	c	15.0	abcd	24.4	bc	17.3	bc
PM05	337.7	c	1137.7	d	50.5	abcde	59.0	a	24.3	a	14.1	abcd	29.0	bcd	26.9	g
PM06	334.5	bc	1089.5	cd	48.5	ab	62.0	ab	24.0	a	17.4	d	31.5	d	20.6	bcdef
PM07	275.6	abc	812.3	ab	48.7	abc	63.0	ab	25.8	abc	17.3	cd	29.7	bcd	19.7	bcd
PM08	284.7	abc	867.9	abc	48.1	a	60.8	a	25.8	ab	14.3	abcd	30.4	cd	24.9	defg
PM09	267.8	abc	813.3	ab	51.4	bede	68.0	bc	27.0	abc	17.8	d	25.2	bc	15.8	b
PM10	273.9	abc	748.4	ab	49.3	abcd	61.0	a	26.5	abc	12.9	ab	28.2	bcd	26.0	efg

^aAs average of 2013-2014. Means followed by the same letters within a column are not statistically different ($p \leq 0.05$).

Table 5.

Mean values^a of cellulose, hemicellulose and lignin contents in plant components of ten smilo grass populations (% DM).

Populations	Tiller			Leaves			Reproductive organs										
	cellulose	hemicellulose	lignin	cellulose	hemicellulose	lignin	cellulose	hemicellulose	lignin								
Flecha	35.7	a	22.6	a	10.5	a	44.1	d	21.9	a	5.8	a	41.0	e	19.2	a	8.8
PM01	38.7	bc	29.3	d	11.0	ab	38.7	b	27.2	cd	8.2	b	29.9	ab	34.8	e	11.2
PM02	40.5	c	28.1	bcd	11.2	ab	41.7	bcd	22.1	ab	8.4	b	32.1	abcd	30.7	bc	10.4

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PM03	38.1 abc	29.6 d	12.8 bc	42.0 bcd	26.9 cd	7.1 ab	31.7 abcd	32.5 bcde	11.0
PM04	40.1 bc	26.8 b	11.7 abc	40.4 bc	25.7 cd	8.4 b	28.3 a	33.6 de	13.8
PM05	38.8 bc	28.1 bcd	13.1 c	41.4 bcd	25.5 cd	8.6 b	33.9 cd	34.4 de	9.1
PM06	39.7 bc	28.7 cd	10.7 ab	40.5 bc	24.6 abc	9.0 b	32.4 bcd	32.0 bcd	11.9
PM07	38.5 bc	29.3 d	11.7 abc	39.0 bc	24.8 bc	8.8 b	29.4 ab	34.7 de	12.8
PM08	37.9 ab	28.2 bcd	11.8 abc	38.6 b	27.6 d	7.1 ab	30.1 ab	35.0 e	9.5
PM09	39.6 bc	27.8 bcd	10.2 a	32.3 a	27.7 d	9.9 c	35.7 d	29.5 b	10.1
PM10	40.6 c	27.3 bc	13.2 c	39.5 bc	25.8 cd	7.9 ab	30.5 abc	33.4 cde	12.3

^aAs average of 2013-2014. Means followed by the same letters within a column are not statistically different ($p \leq 0.05$).

Table 6.

Linear correlation (r values) between 14 morphological and qualitative traits recorded for ten Sardinian native populations of smilo grass.

	To	FLL	ND	DMY	NT	HL	LF	CEL	LP	CEP	HT	LT	CET	SBF
FLL	0.757*													
ND	n.s.	n.s.												
DMY	n.s.	n.s.	n.s.											
NT	n.s.	n.s.	n.s.	0.692*										
EL	n.s.	n.s.	n.s.	n.s.	n.s.									
LF	n.s.	0.723*	n.s.	n.s.	n.s.	n.s.								
CEL	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.							
LP	n.s.	n.s.	n.s.	n.s.	0.752*	n.s.	n.s.	n.s.						
CEP	n.s.	n.s.	n.s.	n.s.	n.s.	-0.690*	n.s.	n.s.	n.s.					
ET	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	-0.913***				
LT	n.s.	n.s.	0.793*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.			
CET	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.		
SBF	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.784*	-0.696*	n.s.	n.s.	n.s.	0.668*	
SFF	n.s.	0.664*	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	0.770*	-0.679*	n.s.	n.s.	n.s.

To: Tiller total length; **ND:** number of nodes; **DMY:** plant aboveground dry matter; **NT:** number of tillers; **HL:** leaf hemicellulose; **CEL:** leaf cellulose; **LF:** leaf lignin; **LP:** panicle lignin; **CEP:** panicle cellulose; **HT:** tiller hemicellulose; **LT:** tiller lignin; **CET:** tiller cellulose; **SBF:** beginning of flowering; **SFF:** full flowering.. *Significant for $p \leq 0.05$, ***Significant for $p \leq 0.001$, n.s. = not significant.

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Table 7.

Means and standard deviation (SD) for heating value, proximate and ultimate analysis in plant components of native smilo grass populations (% of dry weight).

Plant components	Heating value (MJ kg ⁻¹)	Volatile matter (%)	Fixed carbon (%)	Ash content (%)	C (%)	H (%)	N (%)	S (%)	Cl (%)	O (%)
Leaves										
mean	16.3	75.7	13.8	11.5	48.5	6.2	1.7	1.0	1.2	43.6
SD	0.3	5.9	6.7	3.5	0.6	0.9	0.4	0.3	0.3	1.6
Tillers										
mean	18.2	70.0	11.0	4.6	49.0	5.5	1.3	1.2	1.7	44.2
SD	0.9	4.7	0.8	1.1	1.6	0.7	0.3	0.5	0.2	1.8
Reproductive Organs										
mean	16.7	69.8	13.3	8.3	48.5	5.9	1.7	1.3	1.4	43.9
SD	0.7	6.4	4.0	2.5	1.3	0.9	0.7	0.5	0.4	1.7

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