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Evaluation of the Agronomic Traits of 80 Accessions of Proso Millet (*Panicum miliaceum* L.) under Mediterranean Pedoclimatic Conditions

Alessandro Calamai [1], Alberto Masoni [1,2,*], Lorenzo Marini [1], Matteo Dell'acqua [3], Paola Ganugi [1], Sameh Boukail [3], Stefano Benedettelli [1] and Enrico Palchetti [1]

[1] DAGRI, Department of Agriculture, Food, Environment and Forestry, University of Florence, Piazzale delle Cascine 18, 50144 Firenze, Italy; alessandro.calamai@unifi.it (A.C.); lo.marini@unifi.it (L.M.); paola.ganugi@unifi.it (P.G.); stefano.benedettelli@unifi.it (S.B.); enrico.palchetti@unifi.it (E.P.)
[2] Department of Biology, University of Florence, 50019 Florence, Italy
[3] Institute of Life Sciences, Scuola Superiore Sant'Anna, 56127 Pisa, Italy; matteo.dellacqua@santannapisa.it (M.D.); sameh.boukail@santannapisa.it (S.B.)
* Correspondence: alberto.masoni@unifi.it

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Abstract: The continuous increase in the world population and the associated food demands in the wake of climate change are pushing for the development and cultivation of climate-resilient crops that are able to efficiently use natural resources. Proso millet (*Panicum miliaceum* L.) might be a promising candidate crop thanks to its heat stress resistance and its limited water demand. To date, one of the most important strategies to increase grain yield and to improve other agronomic important traits is through an efficient breeding program based on a wide genetic variability of parental germplasm. In this study, we evaluated the agronomical traits of a world collection of 80 *P. miliaceum* accessions. The entire collection was evaluated over a 2 year field experiment under Mediterranean pedoclimatic conditions, which exhibited a wide range of variability for plant height (25–111 cm), grain yield (842–3125 kg ha^{-1}), total dry biomass (2767–10,627 kg ha^{-1}), harvest index (HI; 0.25–0.35), Growing Degree Days (GDDs; 581–899), and days to maturity (80–111 d). A non-parametric multivariate analysis of variance (Np-MANOVA) analysis indicated that GDDs to flowering, grain yield, total dry biomass, days to maturity, plant height, and seed yield per plant were useful parameters to differentiate the germplasm accessions. High heritability (>0.60) was observed in both years for plant height, leaf number, basal tiller, seed yield per plant, 100-seed weight, GDDs to flowering, and days to maturity. Grain yield, total dry biomass, and HI reported moderate heritability (0.30–0.60). The findings reported in the present study may provide valuable information that could support researchers in breeding programs to develop high grain-yielding accessions.

Keywords: proso millet; *Panicum miliaceum* L.; climate-resilient crops; breeding; germplasm

1. Introduction

By 2050, the world's population is expected to have increased rapidly, from about 7 billion to 9.2 billion people, boosting the global food demand by up to 60% [1,2]. Currently, cereal crop consumption supports approximately 50% of the total calorie intake of the world and is largely supplied by wheat, rice, and maize [3–5]. At the same time, climate change is accelerating land degradation and desertification, and extreme climatic events are lowering yields [6–8]. Global warming may reduce arable land due to the expansion of dryland regions by around 10% by the end of the 21st century, increasing global food shortages and even famine, especially in developing countries where populations are already affected by malnutrition [9–11]. In view of the current and future scenarios, scientists suggest that an efficient strategy could be to replace high water-demanding cereal crops with drought-adopted ones, focusing on climate-resilient crops to ensure high productive and nutritional value by efficiently utilizing natural resources [12–14].

Among the C_4 Panicoids (subfamily: *panicoideae*), proso millet (*Panicum miliaceum* L.) is known to possess morpho-physiological traits, conferring tolerance to abiotic stresses and greater adaptability than major grain cereal crops under different environmental conditions [15,16]. Based on the panicle morphology and shape, proso millet can be divided into five races: *miliaceum*, *patentissimum*, *contractum*, *compactum*, and *ovatum* [17,18]. *Panicum miliaceum* is one of the first domesticated crops in the world, and it was cultivated before the diffusion of rice, maize, and wheat [19,20]. Ten thousand years ago, it appeared as a staple food in the semiarid regions of East Asia (e.g., China, Korea, Japan, Russia, and India), and later spread throughout the entire Eurasian region [21,22]. Nowadays, millet grains still represent an important cereal food as a source of energy and protein for millions of people living in arid and semiarid areas in emerging countries, while millet biomass represents an interesting source of forage in some Asian countries, such as India [23,24]. In the Western world, *P. miliaceum* is considered a minor cereal due to its poor economic importance, and thus, it is usually used as feed or fodder for farm animals [19,21].

Compared to the other cereals, proso millet may represent a valuable crop, especially in Mediterranean areas, for its nitrogen use efficiency (NUE)—which is 1.5–4 times greater than that of C_3 cereals—its high leaf area index (LAI; 6.7), and its high radiation use efficiency (RUE; 2.5–4 g MJ^{-1}), which are comparable with that of maize growth under optimal conditions [22,25]. To produce 1 g of biomass, it requires about half the water that is needed to produce an equal amount of maize or wheat biomass [8,26]. *Panicum miliaceum* has recently received increasing interest due to its nutraceutical traits: grains are characterized by a high protein content (12.5%) and are generally rich in essential amino acids (e.g., methionine and cysteine), with the exception of lysine and threonine [12,20]. Human foods containing millet are promoted for their low glycemic index and their high fiber content, as well as for being gluten-free [27,28]. All of the above makes millet a potential candidate to source stress tolerance and nutritional traits for next-generation cereal breeding programs [15].

The objective of this study was to conduct an overall morphological characterization and a preliminary evaluation of the agronomical performances of a world collection of 80 proso millet (*P. miliaceum*) accessions over a 2 year field experiment. The collection used to estimate the agronomic trait values had several geographical proveniences and showed a broad variation for a number of traits. The results represent a useful tool for designing and implementing breeding programs aiming at the production of new and improved varieties.

2. Materials and Methods

2.1. Field Experiments

From a germplasm collection of wild and domesticated proso millet accessions (nearly 600 accessions) sourced from the United States Department of Agriculture, Darby, PA, USA [29], we selected 80 accessions to use in this study. The selection was done according to the results (data not shown) of a preliminary agronomical field screening carried out in 2017. The selected accessions showed the best promising traits and interesting seed colors, and possessed a complete bank passport of information (origin, senders, etc.). Our collection featured cultivated materials with several different countries of origin: Central Asia (*n* = 1), South Asia (*n* = 7), Southeast Asia (*n* = 8), West Asia (*n* = 15), East Asia (*n* = 3), North America (*n* = 1), Central America (*n* = 8), South America (*n* = 2), North Africa (*n* = 4), East Africa (*n* = 1), Central Europe (*n* = 7), Western Europe (*n* = 6), Eastern Europe (*n* = 13), and Oceania (*n* = 4). A detailed list of the materials with the corresponding passport information is provided in Supplementary Table S1.

The field evaluation of the collection was conducted for two consecutive years (i.e., 2018 and 2019) at the experimental farm belonging to the Tuscan Regional Administration located in Cesa (AR), Italy (43°18′32.4′′ N; 11°49′35.1′′ E; 253 m a.s.l). At the farm, the climate is typically Mediterranean and characterized by an average yearly temperature of 13.9 °C, with a minimum average temperature of 5.8 °C in January and a maximum average temperature of 24.0 °C in July. Typically, the annual precipitation ranges from 685 to 711 mm distributed across 89 rainy days (i.e., with rainfall above 1 mm). The meteorological data during the growing experimental period were recorded at the local weather station (Table 1). The experimental soil was characterized by a clay texture (25.4% sand; 30.1% silt; 44.5% clay), a pH of 7.1, low electrical conductivity (EC; 0.154 mS cm^{-1}), high cation exchange capacity (CEC; 27.46 meq 100 g^{-1}), and an organic matter content of 1.66% [27] (Table 2).

Table 1. Meteorological data for the experimental site during agronomic seasons 2018 and 2019.

Month	**Mean Temperature (°C)**		**Mean T Max (°C)**		**Mean T Min (°C)**		**Total Rainfall (mm)**		**Mean Relative Humidity (%)**	
Year	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
January	6.7	2.5	12.0	8.0	1.5	−2.0	27.8	38.8	87.2	83.5
February	3.9	6.8	8.2	14.4	−0.4	0.0	93.8	39.6	81.3	68.7
March	7.8	9.8	12.8	17.2	2.9	2.1	127.2	5,2	81.7	64.7
April	14.6	11.9	21.6	18.5	6.9	5.2	66.4	98.0	70.6	73.9
May *	17.6	13.5	23.6	18.9	11.6	8.2	140.6	128.6	77.2	80.1
June *	20.4	22.3	27.7	30.7	13.1	13.8	14.0	2.2	65.3	61.5
July *	23.7	24.0	32.0	32.2	15.4	15.8	22.6	202.6	61.3	62.7
August *	23.9	24.4	32.1	32.5	15.1	16.2	23.0	45.4	62.7	66.3
September	20.2	19.4	28.2	26.7	12.8	12.1	18.6	34.2	66.9	72.9
October	15.9	15.2	22.3	22.2	9.8	9.3	59.2	59.2	74.1	84.4
November	10.2	11.0	14.3	15.2	6.6	7.1	102.4	207.0	89.0	91.0
December	5.1	7.1	10.2	12.4	0.4	2.3	63.8	90.0	90.8	86.9
Mean [a]	**21.4**	**21.1**	**28.8**	**28.6**	**13.8**	**13.5**	**50.1**	**94.7**	**66.6**	**67.7**
Total Rain [b]	-	-	-	-	-	-	**200.2**	**378.8**	-	-
Total Rain [c]	-	-	-	-	-	-	**759.4**	**950.8**	-	-

[a] The monthly mean for the whole growth period, from planting to maturation; [b] The cumulative rainfall during grain development; [c] The cumulative year rainfall; * Months in which the crop was in the field.

Table 2. Physical and chemical properties of the soil used in the experiment.

Properties	**Value**	**Unit**
Sand	25.4	%
Silt	30.1	%
Clay	44.5	%
Total organic matter	1.66	%
Total nitrogen	0.12	%
Available phosphorous	11	ppm
pH	7.1	
Electrical conductivity (EC)	0.154	mS cm^{-1}
Cation exchange capacity (CEC)	27.46	meq 100 g^{-1}
Exchangeable Ca	21.25	ppm
Exchangeable Mg	5.17	ppm
Exchangeable Na	0.58	ppm
Exchangeable K	0.46	ppm

During both years, the experimental layout was of a randomized complete block design with two replications for each accession (total of 160 plots). The planting density was 55 plants m^{-2}, with a distance between rows of 60 cm and 3 cm between the plants in each row. The applied planting density was adopted based on recent studies [30] in order to optimize and standardize the experiment using an average density used in open field. Each experimental plot measured 2.4 m in length and 3 m in width (7.2 m^2), and was sown, as classically happen for spring crops in that area, at the beginning of May (4 May 2018 and 9 May 2019). Harvesting was performed on 25 August 2018 and on 30 August 2019. The precedent crop in both years was bread wheat (*Triticum aestivum*). The seedbed was prepared using a disc harrow (20–25 cm depth) in winter, followed by a spike-tooth harrow (6–8 cm depth) before seeding. Fertilization was applied at sowing time using 150 kg ha^{-1} of NPK (10-10-10), the adoption of a limited supply of fertilizers is motivated by the desire to minimize the influence of this factor on the experimental device since the aim was precisely to verify the vegetative-productive behavior of the accessions in standard conditions.

The following crop management was performed using manual weeding and without the use of irrigation and pesticides, in fact weeds generally represent a big problem for the cultivation of millet but in our case, given the experimental nature of the cultivation and the limited degree of infestation, we proceeded to perform two manual weeding. Regarding irrigation, since the purpose of the experiment was to determine the feasibility of the cultivation of millet in semi-arid areas and with reduced water consumption, together with the satisfactory rainfall recorded in the two years of activity, it was considered important do not administer water by irrigation. Finally, both thanks to the careful agronomic management of the field trial, and thanks to a favorable climatic course during the two years of experimentation, no parasitic attacks such as to require phytosanitary treatments have been carried out.

2.2. Data Collection

Harvesting was performed when the seeds were fully matured according to the phenological BBCH (Biologische Bundesanstalt, Bundessortenamt, und Chemische Industrie) scale [31]. At maturity, the plants were classified in different *P. miliaceum* races according to their panicle morphology and shape [17,18]. Three randomly selected plants for each plot were harvested and used to measure the vegetative traits, such as plant height, leaf number, basal tiller number, and grain yield per plant. Plant height was measured as the distance from the ground level to the end of panicle. For each experimental plot, a 0.50 m^2 area was harvested by hand, oven-dried at 55 °C for 48 h, and then used to estimate the productive traits (i.e., total dry biomass, grain yield, and 100-seed weight). Total dry biomass and grain yield were subsequently converted into kilograms per hectare (kg ha^{-1}). The harvest index (HI) was calculated as the ratio between grain yield and total dry biomass of the whole sampled plants. This parameter indicates the partitioning of photosynthesis products to the harvestable portion of the crop [32]. Days to maturity were calculated from the emergence stage to the harvest cultivation stage, while cumulative Growing Degree Days (GDDs) were recorded from the emergence until at least 50% of the plants in a plot had reached the flowering phenological stage or beyond, using the following formula [33]:

$$\mathrm{GDD} = \sum_{i=1}^{n} \mathrm{DTT} \quad (1)$$

$$\mathrm{DTT} = [(T_{max} + T_{min})/2] - T_{base} \quad (2)$$

where DTT is the daily thermal time recorded, T_{min} is the minimum daily temperature (°C), T_{max} is the maximum daily temperature (°C), T_{base} is the base air row temperature set equal to 10 °C for millets and sorghum, and i = 1,2,3,4 ... n are the days for which cumulative GDDs is calculated [34,35].

In addition, considering the importance of GDDs in proso millet growth and productivity, the accessions were subdivided into the following precocity classes: early, medium, and late maturity [32,36,37].

2.3. Statistical Analysis

The differences in the agronomic performances among accessions were assessed using a general linear mixed model (GLMM), fitted using R software v3.6 with R/lme4 package [38,39], and considering years and accessions as the random factors and the assigned morphological race as the fixed factor. A post-hoc Tukey test for multiple comparisons among morphological races was carried out using the same software. The correlation between all of the collected variables was assessed using a Pearson correlation model implemented in the R/corrplot package [40]. A principal component analysis (PCA) on the vegetative and production traits was carried out considering the overall mean values of both years using the R/factoextra [40], in order to estimate the relative importance of each trait in capturing data variation and the importance of the morphological race, origin, and GDDs to flowering parameters as possible factors structuring the germplasm collection. In addition, a non-parametric multivariate analysis of variance (np-MANOVA) was performed to test the differences among the clusters previously hypothesized by using R/vegan [38]. Finally, the broad-sense heritability ($h^2{}_b$) was estimated for each trait in each experimental year according to Equation (3) and using the software META-R v6.04 (CIMMYT Research Data & Software Repository Network) [41], and classified as low (<0.30), moderate (0.30–0.60), or high (>0.60) according to Vetriventhan and Upadhyaya [42].

The broad-sense heritability of a given trait at an individual environment is calculated as:

$$h^2{}_b = \sigma^2{}_g/\sigma^2{}_g + (\sigma^2{}_e/\text{nreps}) \quad (3)$$

where $\sigma^2{}_g$ and $\sigma^2{}_e$ are the genotype and error variance components, respectively, and nreps is the number of replicates.

3. Results

3.1. Variability for Agronomic Traits and Heritability

The 80 accessions of proso millet could be classified into four races: *miliaceum* (52.5%), *contractum* (22.5%), *patentissimum* (17.5%), and *compactum* (7.5%). No accession in the collection was identified as being from the *ovatum* race. The outcomes of the GLMM model indicate that the genotypes differed significantly ($p < 0.01$) for plant height, grain yield, total dry biomass, HI, GDDs to flowering, and days to maturity among the single years (Table 3). All proso millet accessions showed a wide range of variability for all of the measured traits, especially for plant height (25–104 cm in 2018 and 33–111 cm in 2019), grain yield (842–2982 kg ha^{-1} in 2018 and 891–3125 kg ha^{-1} in 2019), total dry biomass (2889–9664 kg ha^{-1} in 2018 and 2767–10,627 kg ha^{-1} in 2019), HI (0.25–0.33 in 2018 and 0.27–0.35 in 2019), GDDs to flowering (581–891 in 2018 and 592–899 in 2019), and days to maturity (80–109 days in 2018 and 83–111 days in 2019) for both years. Overall, the accessions showed significantly ($p < 0.01$) lower mean values in 2018 compared to 2019 for plant height (67.48 cm and 69.82 cm, respectively), grain yield (1708 kg ha^{-1} and 1832 kg ha^{-1}, respectively), total dry biomass (6001 kg ha^{-1} and 6279 kg ha^{-1}, respectively), HI (0.28 and 0.30, respectively), GDDs to flowering (740.8 and 743.3, respectively), and days to maturity (97.8 days and 98.8 days, respectively) (Table 3).

Plant height, leaf number, seed yield per plant, grain yield, total dry biomass, GDDs to flowering, and days to maturity were found to significantly differ between races ($p < 0.01$ for all parameters) (Table 4). The *compactum* race was characterized by a short plant (53.6 cm) and the lowest leaf number (5.99 on average), and produced the lowest grain yield and total dry biomass (1428 kg ha^{-1} and 4902 kg ha^{-1}, respectively). Plants of the *compactum* type required less GDDs than the other races to reach the flowering stage (679 GDDs) and were characterized by early maturity (86 days). On the contrary, the accessions of *contractum* and *miliaceum* produced the highest average grain yield (1900 kg ha^{-1} and 1860 kg ha^{-1}, respectively), total dry biomass (6506 kg ha^{-1} and 6432 kg ha^{-1}, respectively), and plant height (75.1 cm and 71.5 cm, respectively), but differed for leaf number (7.11 and 6.56, respectively), seed yield per plant (10.28 g and 8.94 g, respectively), GDDs to flowering (772 and 732, respectively), and days to maturity (98 days and 95 days, respectively). The race *patentissimum* exhibited intermediate values for plant height (69 cm), grain yield (1790 kg ha^{-1}), total dry biomass (6291 kg ha^{-1}), and days to maturity (92 d), yet did not differ significantly from the race *miliaceum* for leaf number, seed yield per plant, and GDDs to flowering.

Table 3. Mean, range, and broad-sense heritability (h^2_b) of the agronomic traits evaluated in the two-year field experiment.

Trait	2018			2019		
	Mean	**Range**	**h^2_b**	**Mean**	**Range**	**h^2_b**
Plant height (cm)	67.48 b	25–104	0.85	69.82 a	33–111	0.86
Leaf number	6.70 a	3–11	0.82	6.52 a	3–10	0.87
Basal tiller	3.9 a	2–6	0.83	3.7 a	2–6	0.82
Seed yield per plant (g)	8.54 a	2.6–16.7	0.71	8.96 a	2.8–15.9	0.75
Grain yield (kg ha^{-1})	1708 b	842–2982	0.55	1832 a	891–3125	0.58
Total dry biomass (kg ha^{-1})	6001 b	2889–9664	0.53	6279 a	2767–10,627	0.58
Harvest index	0.28 b	0.25–0.33	0.58	0.30 a	0.27–0.35	0.59
100-seed weight (g)	0.56 a	0.35–0.71	0.73	0.54 a	0.32–0.71	0.77
GDDs to flowering	740.8 b	581–891	0.77	743.3 a	592–899	0.79
Days to maturity	97.8 b	80–109	0.73	98.8 a	83–111	0.75

Means followed by the same letters in the same row are not significant at $p > 0.05$, while means followed by different letters in the same row are significant at $p < 0.05$. GDDs, growing degree days.

In general, the estimates of broad-sense heritability were found to be moderate–high for all of the agronomic traits evaluated, ranging from 0.53 for total dry biomass to 0.85 for plant height in 2018 and from 0.58 for grain yield and total dry biomass to 0.87 for leaf number in 2019 (Table 3). High heritability (>0.60) was observed in both years for plant height (0.85–0.86), leaf number (0.82–0.87), basal tiller (0.82–0.83), seed yield per plant (0.71–0.75), 100-seed weight (0.73–0.77), GDDs to flowering (0.77–0.79), and days to maturity (0.73–0.75). The millet collection exhibited moderate heritability for grain yield (0.55–0.58), total dry biomass (0.53–0.58) and HI (0.58–0.59).

3.2. Principal Component Analysis and the Relationship between Traits

The first three principal components (PCs) computed for the agronomic traits explained about 71% of the total variation among the traits evaluated (Figure 1 and Table 5). GDDs to flowering, grain yield, total dry biomass, days to maturity, and plant height were the most important variables, and contributed largely to the first principal component (PC1; 0.623, 0.582, 0.533, 0.449, and 0.425, respectively), explaining 33% of the total variation. The second component (PC2) accounted for 25% of the total variation and differentiated the accessions by seed yield per plant and days to maturity (0.536 and 0.374, respectively). The third component (PC3) explained an additional 13% of the total variation and was attributed 100-seed weight for positive loadings (0.513) and basal tillers for negative loadings (−0.783). In the np-MANOVA results, the origin and race membership did not seem to represent good clustering factors ($p > 0.05$ for both), but three major groups were identified according to the cumulative GDDs to flowering groups ($p < 0.05$). The group in the top area of the PCA graph (Figure 1), named A, contained the early-flowering proso millet accessions (590–690 GDDs); the lower group, named C, comprised the late-flowering accessions (790–890 GDDs); and the central group, named B, contained the medium-flowering accessions (690–790 GDDs).

Table 4. Mean and range of the agronomic traits evaluated in proso millet races based on the mean data of the two years.

Trait	Mean				Range			
	compactum	***contractum***	***miliaceum***	***patentissimum***	***compactum***	***contractum***	***miliaceum***	***patentissimum***
Plant height (cm)	53.6 c	75.14 a	71.52 ab	68.8 b	34–62	53–97	38–100	58–90
Leaf number	5.99 c	7.11 a	6.56 b	6.18 b	4–7	5–10	4–9	4–9
Basal tiller	3.6 a	3.9 a	3.9 a	3.9 a	3–5	3–5	3–5	4–5
Seed yield per plant (g)	6.64 c	10.28 a	8.94 b	8.22 b	3.7–12.1	6.1–19.1	4.6–14.1	3.7–13.8
Grain yield (kg ha^{-1})	1428 b	1900 a	1860 a	1790 ab	911–2045	1209–2984	994–2893	1004–2743
Total dry biomass (kg ha^{-1})	4902 c	6506 a	6432 a	6291 b	2909–7550	3960–9775	3422–9477	3334–9230
Harvest index	0.28 a	0.30 a	0.30 a	0.29 a	0.25–0.31	0.26–0.34	0.26–0.35	0.26–0.33
100-seed weight (g)	0.54 a	0.55 a	0.55 a	0.54 a	0.36–0.69	0.45–0.69	0.46–0.68	0.43–0.67
GDDs to flowering	679 c	772 a	732 b	721 b	590–760	609–890	606–886	602–881
Days to maturity	86 b	98 a	95 b	92 ab	83–91	92–111	90–108	88–105

Means followed by the same letters in the same row are not significant at $p > 0.05$, while means followed by different letters in the same row are significant at $p < 0.05$.

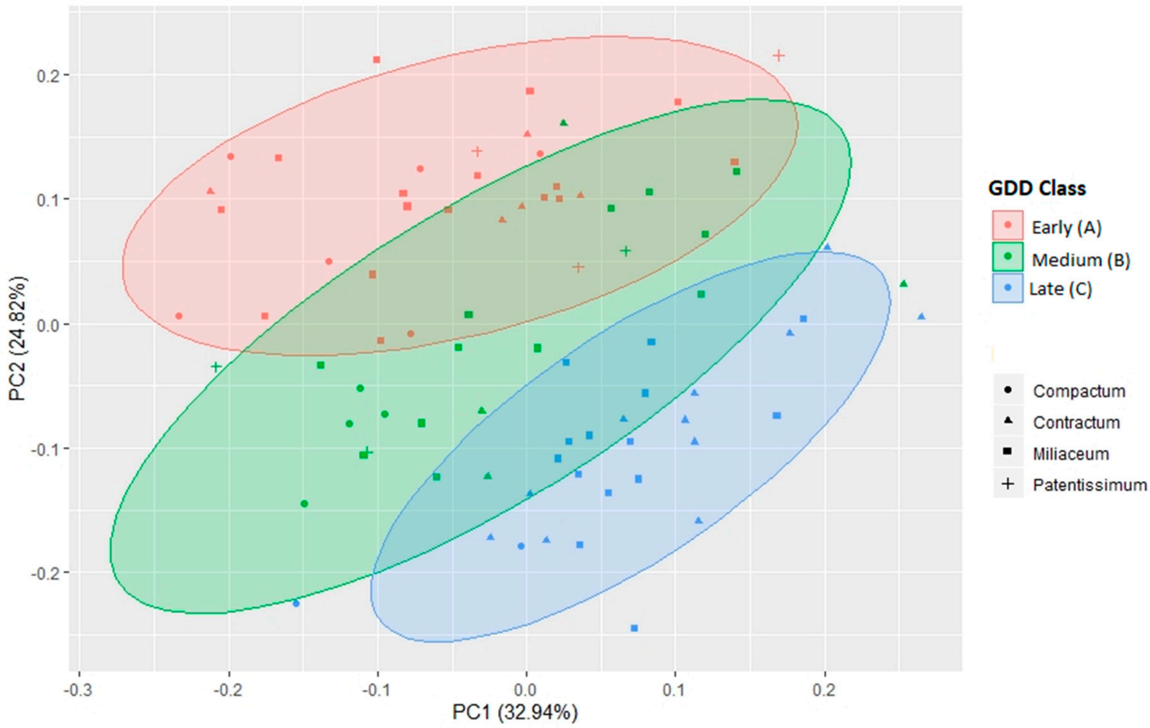

Figure 1. Principal component graph of vegetative and productive traits based on the first two components.

Table 5. Principal component analysis factor loadings (PC1, PC2, and PC3) among the agronomic traits in proso millet based on the mean data of the two years.

Trait	PC1	PC2	PC3
Plant height (cm)	0.425	−0.074	−0.264
Leaf number	0.351	0.207	−0.234
Basal tiller	−0.079	0.112	−0.783
Seed yield per plant (g)	0.309	0.536	−0.034
Grain yield (kg ha^{-1})	0.582	0.198	0.097
Total dry biomass (kg ha^{-1})	0.533	0.234	0.136
100-seed weight (g)	−0.053	0.213	0.513
GDDs to flowering	0.623	0.189	0.097
Days to maturity	0.449	0.374	0.023
Standard deviation	1.9217	1.4947	1.0778
Proportion of variance	32.941	24.823	12.947
Cumulative proportion	32.941	57.764	70.711

Colors represent the three different GDD clusters while point form the race of each accession. Group A = Early flowering accessions (560–690 GDD); Group B = Medium flowering accessions (690–790 GDD); Group C = Late flowering accessions (790–890 GDD).

The correlation coefficients between the agronomic traits are reported in Table 6. Grain yield showed a high correlation (r = 0.688) with total dry biomass, and both reported intermediate correlations with plant height (r = 0.445 and r = 0.436, respectively), seed yield per plant (r = 0.538 and r = 0.521, respectively), GDDs to flowering (r = 0.680 and r = 0.594, respectively), and days to maturity (r = 0.655 and r = 0.638, respectively), and low correlations with leaf number (r = 0.288 and r = 0.287, respectively). GDDs to flowering had a positive significant correlation with days to maturity (r = 0.741), and the same positive pattern was also observed between plant height and leaf number, GDDs to flowering, days to maturity, and seed yield plant (r = 0.547, r = 0.300, r = 0.309, and r = 0.244, respectively).

Table 6. Correlation coefficients among the agronomic traits in proso millet based on mean data of two years.

Trait	Plant Height (cm)	Leaf Number	Basal Tiller	Seed Yield Per Plant (g)	Grain Yield (kg ha^{-1})	Total Dry Biomass (kg ha^{-1})	Harvest Index	Days to Maturity	100-Seed Weight	GDDs to Flowering
Plant height (cm)	1	-	-	-	-	-	-	-	-	-
Leaf number	0.547 **	1	-	-	-	-	-	-	-	-
Basal tiller	0.062	−0.007	1	-	-	-	-	-	-	-
Seed yield per plant (g)	0.244 *	0.167	0.094	1	-	-	-	-	-	-
Grain yield (kg ha^{-1})	0.445 **	0.288 **	0.225 *	0.538 **	1	-	-	-	-	-
Total dry biomass (kg ha^{-1})	0.436 **	0.287 **	0.485 *	0.521 **	0.688 **	1	-	-	-	-
Harvest Index	−0.086	−0.115	−0.121	−0.095	−0.118	−0.182	1	-	-	-
Days to maturity	0.309 **	0.259 *	−0.176	0.100	0.655 **	0.638 **	0.125	1	-	-
100-seed weight (g)	0.147	0.155	0.223	0.143	0.052	0.073	0.027	0.206	1	-
GDDs to flowering	0.300 **	0.255 *	0.175	−0.108	0.680 **	0.594 **	0.125	0.741 **	−0.206	1

* Significant at $p < 0.05$; ** Significant at $p < 0.01$.

4. Discussion

In this study, we evaluated the agronomic performances of 80 different proso millet accessions in two consecutive cultivation years (2018 and 2019) under Mediterranean pedoclimatic conditions. The characterization of the agronomic traits of germplasms is considered an important step to select genotypes adaptable to different environments and with desirable productive traits to be used in future breeding programs for the constitution of new improved varieties [43].

Overall, the field experiment of 2019 showed better agronomic traits values than that of 2018. This could be attributed to the drought stress that occurred during the ear emergence stage, which negatively affected plant growth [14] in the first experimental year (i.e., 2018). The morphological and productive variability of the *P. miliaceum* accessions and races assessed during the 2 year field experiment was comparable to that of other studies on millet germplasm evaluation [16,33,42,44]. For example, plant height ranges recorded were found to be within the range from 39 to 173 cm reported by Salini [45], also consistent with the other small millets species which exhibited similar values, such as pearl milled (62–160 cm) and barnyard millet (79–156 cm), with the only exception being kodo millet, which exhibits shorter plants (34–101 cm) [42–44,46] Moreover, our data confirmed that the *compactum* race was characterized by short and low grain yielding plants compared to the *contractum*, *miliaceum*, and *patentissimum* races [16,45] suggesting that is unlikely that all accessions of the *compactum* race evaluated are suitable for mechanical harvesting due to grain losses during cutting operations [47].

In general, worldwide cultivation of proso millet has declined in the last decade, particularly because of its low grain yield (average world yield of 890 kg ha^{-1}) compared to the major cereal crops [2,20]. Although the response of the grain yield parameter does not depend only by the genotype chosen, but also on the rainfall, temperatures, and agricultural techniques applied [26,32], the present results showed that high-yielding accessions could reach 3125 kg ha^{-1} of cereal grain. This is in agreement with previous studies that reported that the use of a promising genotype leads to an average grain yield of 2016 kg ha^{-1} in the United States and up to 2600 kg ha^{-1} in India [16,48]. In addition, the correlation results indicated that the performances of the accessions were positively correlated with plant height and leaf number, probably because these traits are linked to intercepting more light, thereby increasing photosynthesis efficiency [48,49], it is however important to keep in mind that some pearl millet genotypes have been breed to limit height in very dry area to avoid lodging. Indeed, millets are known to accumulate more dry matter compared to wheat, maize, and sorghum, producing biomass useful for providing forage and biogas production [32,50,51]. In fact, this parameter can reach 13,961 kg ha^{-1} under dryland conditions and 14,407 kg ha^{-1} when irrigated in a short period of time [52].

Crops characterized by low grain yield and high total biomass weight generally result in low HI ratios [32,53]. Among these, *P. miliaceum* and other millets (i.e., *Pennisetum glaucum*, *Setaria italica*, and *Echinochloa frumentacea*) have been reported to have lower HI ratios (i.e., 0.25–0.35, 0.20–0.30, 0.30–0.35, and 0.36–0.41, respectively) compared to major cereal crops (0.40–0.60) such as wheat, rice, maize, and sorghum [32,54–56]. The rise in HI ratio also results in an increase in grain yield, and thus might be an important goal in breeding programs. This could be achieved by reducing tillering, as occurs in maize and sorghum, because intra-plant competition for assimilates between tillers and seeds is considered a major cause of low grain yield [49,53]. The low effect of basal tiller on grain yield was previously noted in barnyard and proso millet, suggesting that this trait is less relevant and that its improvement could be of low priority [44,45].

Millets have a relatively short maturation time (3–4 months), which makes it a desirable crop for sustainable rain-fed agriculture [26]. Generally, among millet groups, proso requires less GDDs than foxtail and pearl millet to reach the flowering stage [57–59]. However, the entire *P. miliaceum* germplasm tested covered a very large range of precocity, as previously recorded by Vetriventhan and Upadhyaya [16] based on the ICRISAT (International Crops Research Institute for the Semi-Arid Tropics, Patancheru, India) germplasm, by Sanon et al. [33] based on the local millet varieties cultivated in West Africa, and by Salini et al. [45] based on 364 accessions tested at the Department of Millets, Centre for

Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatroe, India. From the results of the non-parametric multivariate analysis of our data, it was possible to identify three classes of precocity based on cumulative GDDs to flowering. The late-flowering millet accessions required high cumulative GDDs and resulted in a better yield production compared to the early and medium ones, due to the longest growing cycle, which increased the grain yield and biomass accumulation [43,44,60]. On the contrary, Eric et al. [61] reported the lowest grain yield using late maturity accessions, because the plants flowered under limited moisture conditions when the amount of rainfall was less than at the beginning of the season. These findings suggest that breeding programs should try to develop new and improved varieties within the different precocity classes suitable for different environmental conditions. Moreover, our data do not show any evidence of accession clusterization according to their geographical origin, in contrast to what was found by Rajput and Santra [62]. In fact, considering our data, the accessions share similar trait combinations independently by their origin or their race. This could be due to the short history of breeding this crop or the heterogeneous group selected in each area [63]. Therefore, the passport information obtained from the germplasm bank could contain inaccurate attributions concerning the taxonomy identification made by the seed donors.

At the same time, the heritability estimates of the single traits suggest that the collection studied could be appropriate for effective selection [64]. In fact, according to several authors, plant height, leaf number, basal tiller, seed yield plant, 100-seed weight, GDDs to flowering, and days to maturity in millets underline a high genetic component ($h^2{}_b > 0.7$) and represent good traits to be selected in a breeding program [42,43,45]. On the contrary, the selection of traits with moderate heritability, such as grain yield, total dry biomass, and HI, would be difficult to set up. This could be achieved by indirect selection using the high heritability traits characterized by a high positive correlation with the trait to be improved [44,45,61].

Finally we should state that despite the promising yields and the positive traits that may confer resilience to environmental stress, *P. miliaceum* remains a minor cereal primarily cultivated in semi-arid, low input dryland agriculture regions of Africa and southeast Asia, as a subsistence crop for local consumption, with very limited quantities recorded in international trade of West Countries [19]. Commercial millet production is risky for Western farmers, because the absence of large market outlets means that fluctuations in output cause significant price fluctuations [21]. Many specific agronomic constraints affect its cultivation (poor soil fertility, low and erratic rainfall, high temperature after sowing, loss of grain to birds, pest and weed management) limiting its spread and forcing to develop new technologies for crop and resource management, in order to promote its production on a large scale, including breeding of new varieties [65]. Moreover, only limited experience has been acquired on millet breeding in developed countries, compared what has been done for wheat and maize [66]. Among the different types of millet only pearl millet, and to a small extent finger millet, has so far been researched at the international level. Hybrid grain cultivars have been developed for pearl millet in India and the United States, but perform best in areas where rainfall is reliable [67]. In drier areas with limited and fluctuating rainfall, where it is difficult for breeders to identify dual-purpose grain/stover modern varieties, open-pollinated varieties or composite cross population (CCP) that give stable grain and straw yields and suit the prevailing rainfall pattern, should be developed and adopted [11,68].

5. Conclusions

Despite the decline in *P. miliaceum* cultivation due to its low grain yield, this crop has generated great interest in recent years as a promising sustainable candidate for the Tuscanian semi-arid zones as a renewal crops in a sustainable rotational crop program, which could contribute to crop diversification, as well as diet and use of land. Its planting time, indeed, fits well in rotation with winter annual crops such as winter wheat or warm-season broadleaf crops such as sunflower or sugar beet. In this study, the evaluation of the agronomical performances of 80 accessions over a 2 year field experiment showed that the entire collection under evaluation exhibited a wide range of variability for plant height, grain yield, total dry biomass, HI, GDDs to flowering, and days to maturity. Overall, the I.Pm. 673 (ID: Ames 11678; India) and GR 665 (ID: PI 517019; Morocco) accessions showed the highest grain yield, while GR 658 (ID: PI 517017; Morocco) and Index Seminum #568 (ID: PI 649371; Germany) reported a greater total dry biomass. All of this information could be utilized in future breeding programs for the development of new and improved genotypes adaptable to different environments and with desirable productive traits.

Supplementary Materials: The following are available online at http://www.mdpi.com/2077-0472/10/12/578/s1, Table S1: List of materials evaluated during the two experimental years. NA: not available

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