frontiers in Agronomy

TYPE Original Research
PUBLISHED 22 April 2026
DOI 10.3389/fagro.2026.1786084

OPEN ACCESS

EDITED BY
Desouza Blaise,
Central Institute for Cotton Research (ICAR), India

REVIEWED BY
Raja R.,
Central Institute for Cotton Research (ICAR), India
Belisario Dominguez-Mancera,
Universidad Veracruzana, Mexico

*CORRESPONDENCE
Davide Farruggia
davide.farruggia@unipa.it

RECEIVED 12 January 2026
REVISED 30 March 2026
ACCEPTED 31 March 2026
PUBLISHED 22 April 2026

CITATION
Dinolfo L, Prestigiacomo S, Lala N, Albano A, Farruggia D and Di Miceli G (2026) Agronomic and morphological characterization of *Medicago intertexta* L. for sustainable forage production in Mediterranean environments. *Front. Agron.* 8:1786084. doi: 10.3389/fagro.2026.1786084

Agronomic and morphological characterization of *Medicago intertexta* L. for sustainable forage production in Mediterranean environments

Lucia Dinolfo[1], Simona Prestigiacomo[1], Nicoletta Lala[1], Alessandro Albano[2], Davide Farruggia[1*] and Giuseppe Di Miceli[1]

[1]Department of Agricultural, Food and Forestry Sciences, Università degli Studi di Palermo, Palermo, Italy, [2]Department of Economics, Business and Statistics, University of Palermo, Palermo, Italy

Medicago intertexta L., a self-reseeding annual legume native to the Mediterranean Basin, shows high potential for sustainable forage production in semi-arid environments. This study investigated its presence in Sicilian forage–livestock systems, characterized the morphological variability of a local ecotype ("Monreale"), and evaluated seed yield under two plant densities across two growing seasons. Field surveys confirmed its natural abundance and agronomic value, as farmers recognized its persistence, palatability, and self-regeneration ability. Morphological characterization revealed notable intraspecific diversity, revealing distinct phenotypic groups with different agronomic performance and adaptive traits. Higher seeding density enhanced total seed yield per unit area but reduced individual plant productivity, while climatic variability between years significantly affected performance. The species' strong hardseededness ensures regeneration under adverse conditions, confirming its resilience to Mediterranean climatic stress. Overall, *M. intertexta* represents a valuable resource for developing low-input and climate-adapted forage systems. Further research should focus on optimizing seeding rates and exploiting morphological variability to select improved cultivars.

KEYWORDS
***Medicago intertexta*, Mediterranean environment, morphological variability, plant density, seed yield, sustainable forage**

1 Introduction

Forage production systems are increasingly concerned with the implications of global climate change. Shifts in bioclimatic conditions are affecting the production, availability, and quality of animal feed (Mbarki et al., 2020); therefore, species choice should prioritize low-input, resilient, and sustainable systems capable of withstanding adverse conditions. Self-reseeding annual legumes - particularly species in the genera *Medicago* and *Trifolium* - can support such systems when, after the initial sowing, stands are managed to provide forage and to produce sufficient seed for re-establishment in the following season (Bartholomew, 2014). In addition, hard-seed dormancy - a common survival strategy in Mediterranean annual legumes - confers multiple ecological advantages and is an important component of fitness in many natural systems (Norman et al., 1998; Revell et al., 2012). The soil seed bank functions as a vital reserve, especially in adverse winter growing seasons or after major disturbances when parent plants fail to set seed, thereby ensuring pasture regeneration (Taylor, 2005). These traits are fundamental in ley-farming systems, where legumes replace weedy fallows and contribute to livestock production (Bennett and Cocks, 1999).

With respect to annual self-reseeding *Medicago* species - widely adapted to diverse soils in arid Mediterranean environments - these legumes can enhance livestock production, nutrition, and soil fertility by fixing nitrogen, maintaining organic matter, and improving soil structure (Mbarki et al., 2020; Müller et al., 2022). In Mediterranean forage systems, diet quality influences intake and milk production in small ruminants; for example, goats grazing high-quality legumes show higher intake and milk yield (Bonanno et al., 2007). Among these species, *Medicago intertexta* (L.) Mill. (Calvary medick; *Fabaceae*) remains relatively underexplored agronomically. Morphologically, it is a stout, procumbent to ascending annual, 20–50 cm tall, with yellow flowers; it bears distinctive spiny, coiled pods on long, slender peduncles, and leaflets often marked by dark spots (Kernick, 1978; Pignatti et al., 2017; Bartolucci et al., 2018). Compared with other *Medicago* species, published information is limited primarily to genetics, geographic distribution, ecological preferences, and tolerance to abiotic stress. From a genetic and botanical perspective, *M. ciliaris* and *M. intertexta* are closely related and have been variously classified, although recent studies document morphological and phenological differences between them (Laouar et al., 2001; Laouar and Abdelguerfi, 1999; Cherifi, 2016). Genetic analyses of natural population from Tunisia show the two taxa to be distinct, with discriminating traits such as flowers per inflorescence and pod dimensions (Cherifi, 2016). Chromosome counts of 2n = 16 in *M. intertexta* are consistent with previous reports (Sadeghian and Hejazi, 2014; Fernandes and Queirós, 1978). While *M. ciliaris* is predominantly autogamous, *M. intertexta* is mainly autogamous but exhibits some allogamy and greater variability; it is closely allied to *M. ciliaris* and can hybridize with *M. muricoleptis* within section *Intertextae* (Lesins et al., 1971; Small et al., 1999; Abdelkefi et al., 2001).

Ecologically and biogeographically, *M. intertexta* is reported as native to south-eastern Europe (Greece, Italy), north Africa (northern Algeria, Morocco, Tunisia), and south-western Europe (Portugal, southern Spain), with introductions in France, New South Wales, Uruguay, Victoria, and Western Australia, including the Canary Islands (Spain) within Macaronesia (Tutin, 1968; Smythies, 1984–1986; Zoghlami and Hassen, 1999; Abdelguerfi-Laouar et al., 2003; Thanopoulos, 2007; Laouar and Abdelguerfi, 2014; Lepschi and Monro, 2014). The species shows intrinsic tolerance to high salinity and water stress, exceptional adaptability to hydromorphic (waterlogged) as well as arid soils, and broad elevational adaptation from lowland sites up to ~1,000 m a.s.l (Zahran et al., 2007; Laouar and Abdelguerfi, 2014; El-Shafey and Al-Sherif, 2020). Despite these favorable traits and its wide natural distribution, adoption in agricultural systems remains limited, largely due to insufficient agronomic evidence on management and, in particular, difficulties in seed production - as a self-reseeding species, morphological features such as pod architecture, dispersal traits, and hard seed can make commercial seed harvest and processing challenging. This study therefore aims to generate agronomic insight into *M. intertexta* under semi-arid Mediterranean conditions, focusing on: (i) a preliminary survey of the presence and role of *M. intertexta* in Sicilian forage systems and the collection of representative germplasm; (ii) morphological characterization of a *M. intertexta* ecotype; and (iii) evaluation of seed-yield dynamics in response to contrasting plant densities. Hypotheses. (H1) *M. intertexta* plays a meaningful role in Mediterranean forage systems. (H2) There is interspecific variation relevant to selection and genetic improvement. (H3) Increasing plant density per unit area in the field increases stand-level seed production. Overall, the work seeks to clarify the species' potential for sustainable forage production and to address key knowledge gaps that currently limit its practical deployment in semi-arid agricultural systems.

2 Materials and methods

2.1 An investigation into the presence and the role of *Medicago intertexta*

A preliminary assessment of the presence and distribution of *M. intertexta* was conducted through consultations of the database and scientific literature. Given the significant interest in this species and its ability to thrive in natural environments, this study aimed to investigate its presence in Sicilian farms and assess its role within local cropping systems. To this end, a survey was conducted across 20 forage-livestock farms. In particular, a structured questionnaire was administered to farmers, in order to obtain the following information: general characteristics about the farm and their forage resource management; the presence and the role of self-reseeding annual species. During the 2020–2021 growing season, on farms where the species was present, self-reseeding capacity was investigated as plant density (plants m^{-2}). Prior to the first autumn rain, and with farmer cooperation, three homogeneous areas per farm were selected and fenced (2 m × 4 m) to exclude grazing and allow monitoring of emergence. Seedling numbers were recorded within three delineated sub-areas per enclosure, each measuring 900 cm^2 (30 × 30 cm). At physiological maturity in June, seed samples were collected from the participating farms.

2.2 Trial 1 - morphological characterization of collected germplasm

The trial was conducted during a single growing season 2021/22 at the experimental farm "Sparacia" (Department of Agricultural, Food and Forest Sciences, University of Palermo, Cammarata, Agrigento, Italy, 37°38'09.0"N 13°45'46.6"E, 415 m above sea level), located in a semi-arid, hilly area in western Sicily, approximately 100 km south of Palermo. In the site, both climatic patterns and soil conditions are typical of the Mediterranean dry environments.

The soil is classified as a Vertic Haploxerept (Soil Survey Staff, 2014) and has a clayey texture (62% clay, 33% silt, 5% sand).At the experimental site, soils are typically subalkaline and characterized by relatively high available phosphorus levels (about 76 mg kg^{-1}), with total nitrogen of 1 g kg^{-1} and organic matter content of 7 g kg^{-1}. In the field, where wheat was the preceding crop, the seedbed was prepared using plowing followed by complementary operations. The seeding was carried out by hand in December 2021 using three germinable seeds sown per planting spot spaced 0.50 m within rows, and the rows were spaced at 1 m. The soil was left unfertilized. Weed control and thinning operations were conducted manually through two interventions, at the emergence of the third true leaf and at 60 days after sowing. The field consisted of 50 individual plants.

The plants were characterized based on the criteria/descriptors for Annual Medicago established by the International Board for Plant Genetic Resources (IPGRI, 1991).

Plants were harvested in June 2022 and the following parameters were recorded: plant weight (fresh and dry biomass), plant height, number of stems (measured 3 cm above the stem base), number of racemes per plant and for each raceme the number of pods, weight of 1000 seeds. In addition, for up to 50 pods per plant (or fewer if the total number of pods per plant was less than 50), the following data were collected: the number of seeds per pod, weight of seeds, and pod dimensions (diameter A and diameter B, Figure 1).

From the data collected the following production factors were calculated: total biomass (DM kg ha^{-1}), number of pods per plant, number of seeds per plant, seed weight per plant, seed yield (kg ha^{-1}), harvest Index (HI).

2.3 Trial 2 - seed yield at different plant densities

The trial was conducted during two growing seasons 2021/22 and 2022/23 at the experimental farm “Sparacia”, in the same field as Trial 1. For both years in the field, where wheat was the preceding crop, the seedbed was prepared using plowing followed by complementary operations. The soil was left unfertilized. The seeding was carried out by hand in December 2021 and 2022 using an initial seed rates of 60 and 320 germinable seeds per square meter. Thinning operations were performed at two points during the crop cycle: once at the emergence of the third true leaf and again 60 days after sowing. These interventions aimed to achieve final plant densities of 20 (T1) and 160 (T2) plants per square meter. Each plot (6 × 6 m) consisted of six rows, each 6 m long, spaced 1 m apart. Weeds were hand-weeded twice: at the three-true-leaf stage and 60 days after sowing. At physiological maturity, in the field within a 2 m^2 sampling area, undisturbed plant height was measured, and the total plant biomass was collected. In the laboratory, the following parameters were recorded: biomass (fresh and dry weight) and the number of pods, seed yield, and weight of 1000 seeds. A sample of fresh biomass was dried at 65 °C using a convection oven until a constant weight was reached and then weighed. From the data collected, the following production factors were calculated: total biomass (DM kg ha^{-1}), number of pods per plant, number of seeds per plant, seed weight per plant, and harvest index (HI). The field experiment was arranged in a completely randomized block design with three replicates. Furthermore, in the laboratory, a germination test was conducted to determine how plant density might influence seed germination, including the percentage of soft and hard seeds. For each treatment (T20, T160), a sample of 100 seeds was placed on moist filter paper in a Petri dish, labeled, and incubated in a germinator for 24 days at 20 °C. Each sample was replicated four times.

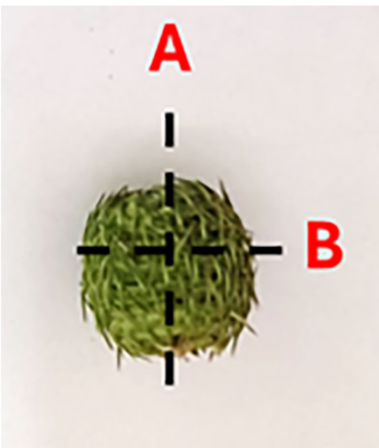

FIGURE 1
Measurement of pod dimensions in *Medicago intertexta*: **(A)** longitudinal diameter; **(B)** transverse diameter.

2.4 Plant characteristics

2.4.1 Correlation

Relationships among morphological traits and agronomic performance were assessed using Pearson correlation coefficients *(r)*, to identify plant characteristics most closely associated with productivity (e.g., biomass and pod production), highlighting key traits as indicators of performance and potential targets for selection or management.

2.4.2 Cluster analysis

A hierarchical clustering approach was applied to identify groups of plants with similar morphological and productive characteristics. In the first stage, plants were grouped based on morphological and yield-related traits, allowing the identification of plant types differing in growth and productivity. Variables were standardized to ensure comparability, and clustering was performed using Euclidean distance. The optimal number of clusters was determined through dendrogram interpretation and silhouette analysis (Rousseeuw, 1987). Principal Component Analysis (PCA) was used to support the interpretation of clusters by summarizing the main sources of variability among traits (Jolliffe, 2002). In the second stage, clustering was refined by considering pod-level variability (number of seeds and pod size). Since each plant produced multiple pods, a distribution-based approach (Wasserstein distance) was used to account for variability and consistency in reproductive traits (Kantorovich, 1939), enabling the identification of subgroups differing not only in productivity but also in reproductive patterns.

2.4.3 Analysis of variance with nested factors

An analysis of variance (ANOVA) with nested factors was performed to evaluate the effects of year, plant density, and their interaction on the variables of interest (Snedecor and Cochran, 1989). Replication was treated as nested within each year × density combination to reflect the experimental design. This approach allowed the assessment of how environmental conditions (year) and management factors (density) influence plant growth and seed production, with the interaction term providing insight into whether plant responses to density were stable or dependent on climatic variability, with implications for management under Mediterranean conditions.

3 Results

A fine-scale reading of seasonal weather is crucial, because the timing—not only the totals—of temperature and rainfall strongly influences emergence, growth, flowering, and seed set in semi-arid Mediterranean systems. Thermo-pluviometric trends during the 2021/2022 and 2022/2023 growing seasons are reported in Figure 2.

Both seasons display a typical semi-arid Mediterranean pattern: temperatures decline from autumn to a winter minimum (Jan–Feb) and rise steadily through spring, closely tracking the polyannual mean. Rainfall is concentrated in autumn–winter, but its timing and amount differ between years. In 2021/2022, several intense events in October–December produced rapid increases in cumulative rainfall, approaching/exceeding the long-term average by late winter. In 2022/2023, rainfall was more irregular and generally lower, with a slower cumulative rise and a marked deficit relative to the polyannual mean from winter into spring. In both years, a pronounced dry spell begins in late spring and extends through summer. The weather data collected from the Agrometeorology Service Network of the Sicilian Region (SIAS, 2025).

3.1 An investigation into the presence of *Medicago intertexta*

The assessment of *M. intertexta* via literature review confirmed its widespread distribution in Sicily—northeast (Sciandrello et al., 2014), northwest (Caldarella et al., 2009), northern areas (Domina et al., 2019; Cambria and Tavilla, 2020), and the Sicilian hinterland (Gianguzzi et al., 2011)—within a region noted for diverse flora, climate, and soils. The on-farm survey in Sicilian forage–livestock systems confirmed the species' occurrence within forage stands. Three farms with high natural prevalence of *M. intertexta* were identified:

- farm 1, Monreale (PA; 37°58′ N, 13°07′ E);
- farm 2, Prizzi (PA; 37°46′ N, 13°24′ E);
- farm 3, Vicari (PA; 37°49′ N, 13°31′ E).

Management differed among farms but converged on late-spring utilization at maturity: farm 1 practiced late-season cattle grazing after early maturation; Farms 2–3 produced hay, leaving stands undisturbed until mid-May, with *M. intertexta* contributing ~30% of the forage. Farmers also reported the species to be resilient,

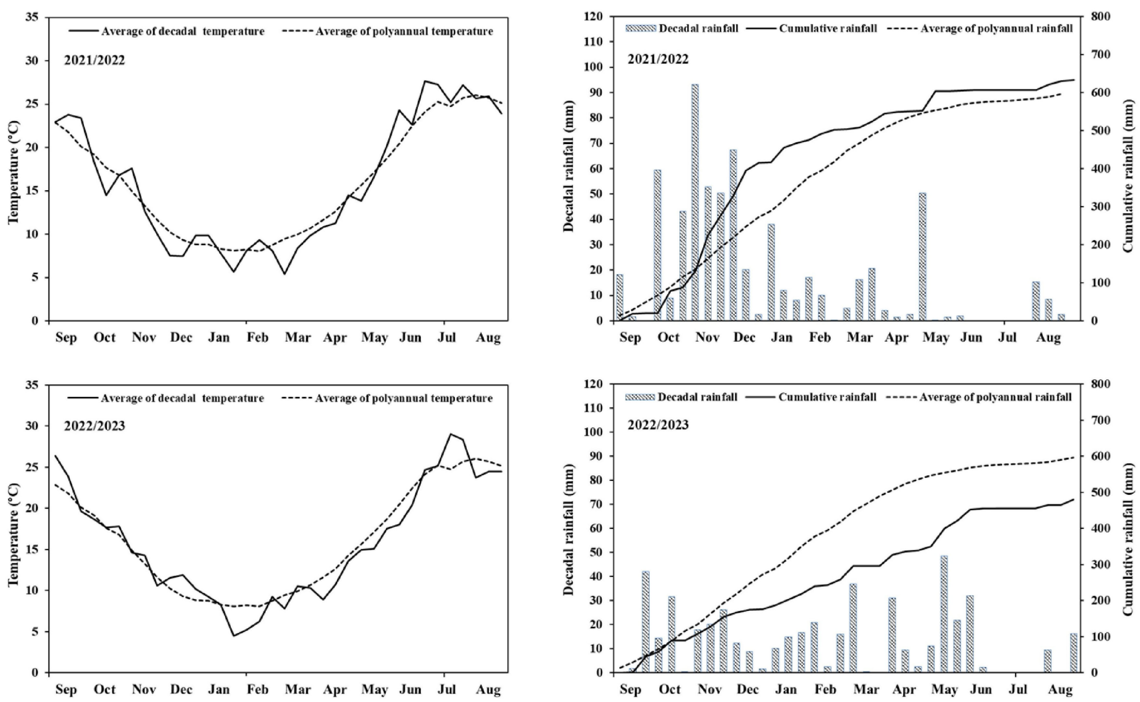

FIGURE 2
Thermo-pluviometric trends during the 2021/2022 and 2022/2023 growing seasons. The graphs show the average decadal temperature (°C) and rainfall (mm) compared with polyannual average.

palatable, productive, and self-regenerating across rotations, with consistent year-to-year re-establishment. Seedling monitoring across the three farms revealed two occurrence patterns: dense (150–160 plants m^{-2}) and sporadic (10–30 plants m^{-2}). Seed samples were collected in May 2021; one ecotype (Monreale) provided sufficient material for subsequent field trials, including an evaluation of seed production at two sowing densities.

3.2 Trial 1 - morphological characterization of collected germplasm

To examine how morphological traits relate to agronomic performance, pairwise associations among measured variables were quantified. Pearson correlation coefficients were calculated to identify trait combinations most closely linked with biomass accumulation and seed yield, providing candidate proxies for selection and management. The correlation matrix (Figure 3) among 17 morphological and agronomic traits in *Medicago intertexta*, shows strong positive correlations ($r > 0.80$) were found between total biomass and: total pods per plant ($r = 0.88$), racemes with two pods ($r = 0.84$), total racemes per plant ($r = 0.92$), seeds per plant ($r = 0.88$), and seed weight per plant ($r = 0.84$). Seed yield correlated strongly with total pods per plant ($r = 0.90$), racemes with two pods ($r = 0.87$), racemes with three pods ($r = 0.82$), total racemes per plant ($r = 0.86$), and most strongly with seeds per plant ($r = 0.93$).

Based on 17 morphological descriptors measured on 50 plants of the Monreale ecotype, hierarchical clustering (Figure 4) separated the material into two unequal groups at a branch height of ~10, where branch height reflects dissimilarity (greater heights = stronger differentiation): Cluster 1 (left) is larger and contains several sub-clusters, whereas Cluster 2 (right) is smaller, shows fewer sub-clusters with mostly lower internal heights (<4), and a few individuals stand out as distinct within their groups (e.g., plants 35 and 39 in Cluster 1; plant 9 in Cluster 2).

Principal component analysis (PCA) confirmed the clustering, with the first two components (Dim1 = 52.7%, Dim2 = 13.3%; ~66.0% total) clearly separating two groups, Cluster 1 (blue) and Cluster 2 (red)—in the Dim1–Dim2 space (Figure 5).

To compare seed productivity between groups, a boxplot summarizes seed yield by cluster (Figure 6). Cluster 2 exhibited higher median yield (800 kg ha^{-1}) compared to Cluster 1 (400 kg ha^{-1}), along with greater variability. Box boundaries represent the interquartile range (IQR) with the median indicated by the central line; whiskers extend to 1.5×IQR; individual plants are shown as points; colors distinguish cluster membership.

A similar pattern is observed for biomass (Figure 7): higher median biomass in Cluster 2 (3500 kg ha^{-1}) than Cluster 1 (2000 kg ha^{-1}), again with greater variability in Cluster 2.

To assess potential within-group heterogeneity, follow-up analyses were conducted on each cluster. These analyses aimed to identify possible substructure and trait differentiation through hierarchical

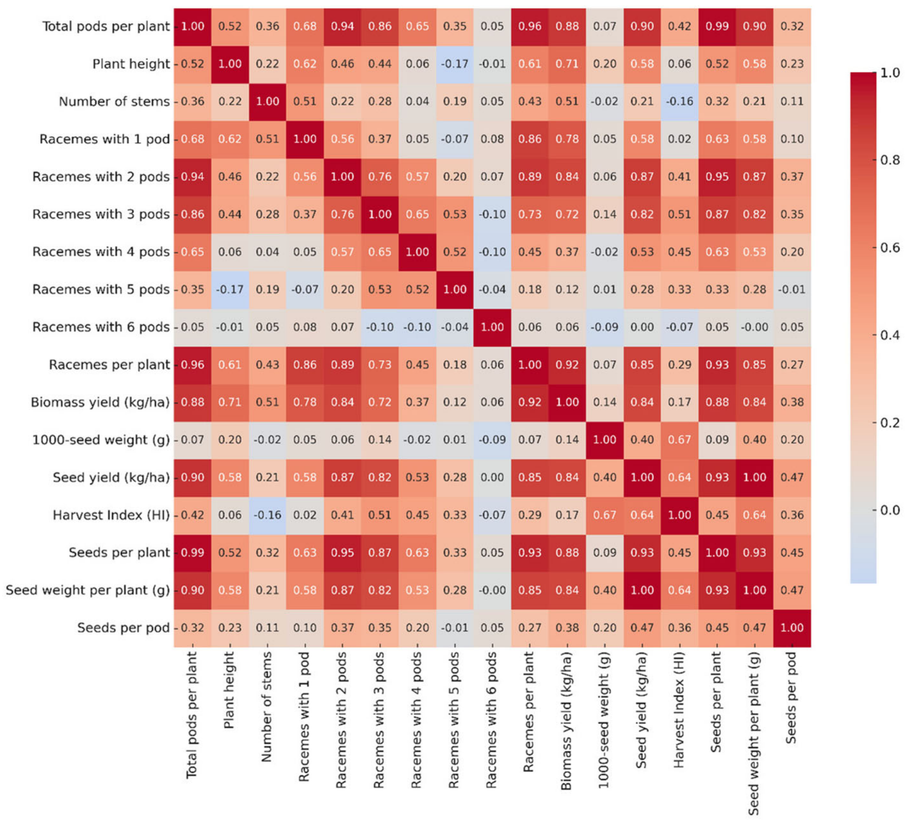

FIGURE 3
Correlation matrix among morphological and agronomic traits in *Medicago intertexta*. The color scale represents Pearson's r (–1 to +1).

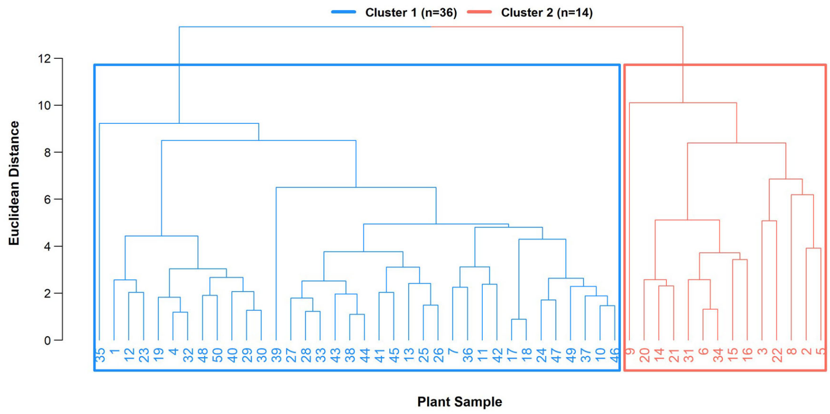

FIGURE 4
Dendrogram showing hierarchical clustering of 50 plants from the Monreale ecotype based on 17 morphological descriptors.

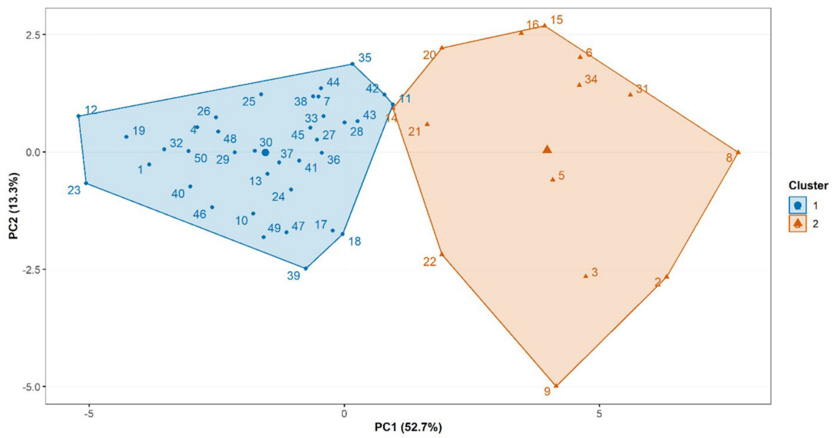

FIGURE 5
Principal component analysis (PCA) of phenotypic traits.

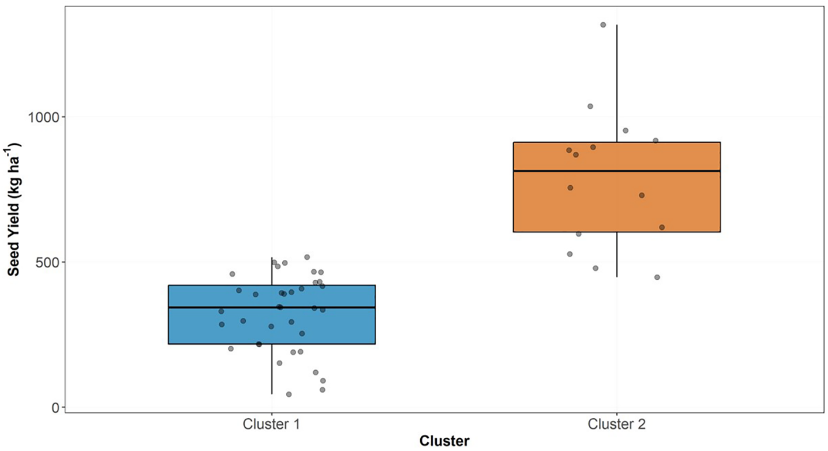

FIGURE 6
Boxplot of seed yield (kg ha^{-1}) by cluster.

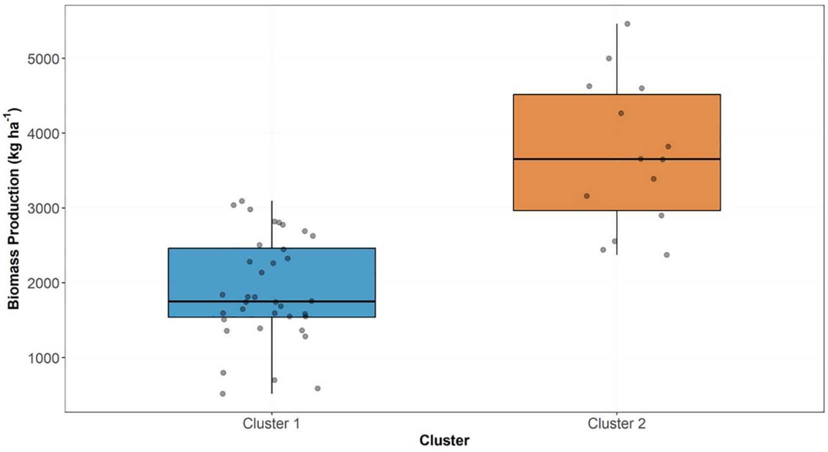

FIGURE 7
Boxplot biomass production (kg ha^{-1}) by cluster.

clustering and comparative distributional assessments of key morphological traits (long diameter, short diameter, and seed number). Regarding cluster 2 (n = 14), the follow-up results (Figure 8) point to minimal internal substructure: the dendrogram offers little evidence of coherent subgroups, while the boxplots show broad overlap in trait values across the putative partitions, suggesting limited justification for further subdivision.

Unlike Cluster 2, the two sub-clusters here (Figure 9) show meaningful differences, one group exhibits higher medians and broader spread for several traits, revealing substantial internal heterogeneity with potential relevance for selection.

3.3 Trial 2 - seed yield at different plant densities

Presented below are the main outcomes from the density trial on *M. intertexta* (ecotype Monreale) conducted across two growing seasons. Across two plant densities and two years (Table 1), plant height was significantly affected by density and year ($p < 0.001$), with a significant density × year interaction ($p < 0.01$). The highest mean height occurred in T20 (2023) at 63 cm. In both years, higher density was associated with shorter plants.

Mean total biomass did not differ between years, and the density × year interaction was not significant; the density effect was significant ($p < 0.01$), with the highest value in T160 (4967 kg DM ha^{-1}). A consistent trend toward greater biomass per unit area at higher density was observed. The harvest index (seed yield/total biomass per unit area) showed no significant effects of treatment or year.

Seed yield differed significantly between sowing densities (T20 vs. T160) and between years ($p < 0.01$), while the density × year interaction was not significant. The higher density T160 produced greater overall seed yield than T20. The results show as T160 increased total yield and 1000-seed weight (16.3 g vs. 13.2 g, $p < 0.001$) but reduced pods per plant and seeds per plant, the latter being markedly lower (31 vs. 290, $p < 0.001$). T20 showed the opposite pattern (more pods and seeds per plant but lower total yield) (Table 2).

Across seasons, plant density consistently affected seed germinability, altering the proportions of hard (dormant) and soft (germinable) seeds (and, consequently, total germinability), as well as the share of dead seeds (Figure 10). In 2022, T160 showed a higher hard-seed fraction and lower soft-seed fraction than T20 (hard: 76 vs 65%; soft: 16 vs 20%), while dead seeds were lower in T160 (8 vs 15%). In 2023, the same density pattern held for hard and soft seeds (58 vs 53% and 38 vs 45%, respectively), but dead seeds were minimal and similar (3 vs 2%). Consequently, total germinability = hard+soft differed by density in both years but in opposite directions (2022: T160 > T20; 2023: T160 < T20), in line with a significant Year × Density interaction [hard: marginal (≈0.050); soft: n.s. (0.4595); dead and total germinability: significant (0.00341)].

4 Discussion

Evidence on the presence and role of *M. intertexta* in Sicilian forage systems highlights the combined effect of management practices and species traits in sustaining field persistence and spread. Observed practices, such as delayed grazing and haymaking at maturity, align with traits that favor persistence, including early maturity, staggered flowering, slender peduncles, spiny pods generally avoided by livestock, a high proportion of hardseededness, and early attainment of physiological seed maturity (Small and Jomphe, 1989; Kemp, 1989). Farmer reports of consistent self-regeneration align with classic mechanisms in annual medics, particularly weather-resistant pods and hardseededness, which allow natural re-establishment even when winter rainfall is insufficient for seed set (Crawford and Nankivell, 1989). These features are also congruent with ley-farming systems that rely on self-regenerating annual legumes rotated with annual crops (Edwards et al., 2019). Finally, the contrasting on-farm density patterns observed here provide a clear rationale for focusing subsequent experiments on plant-density effects.

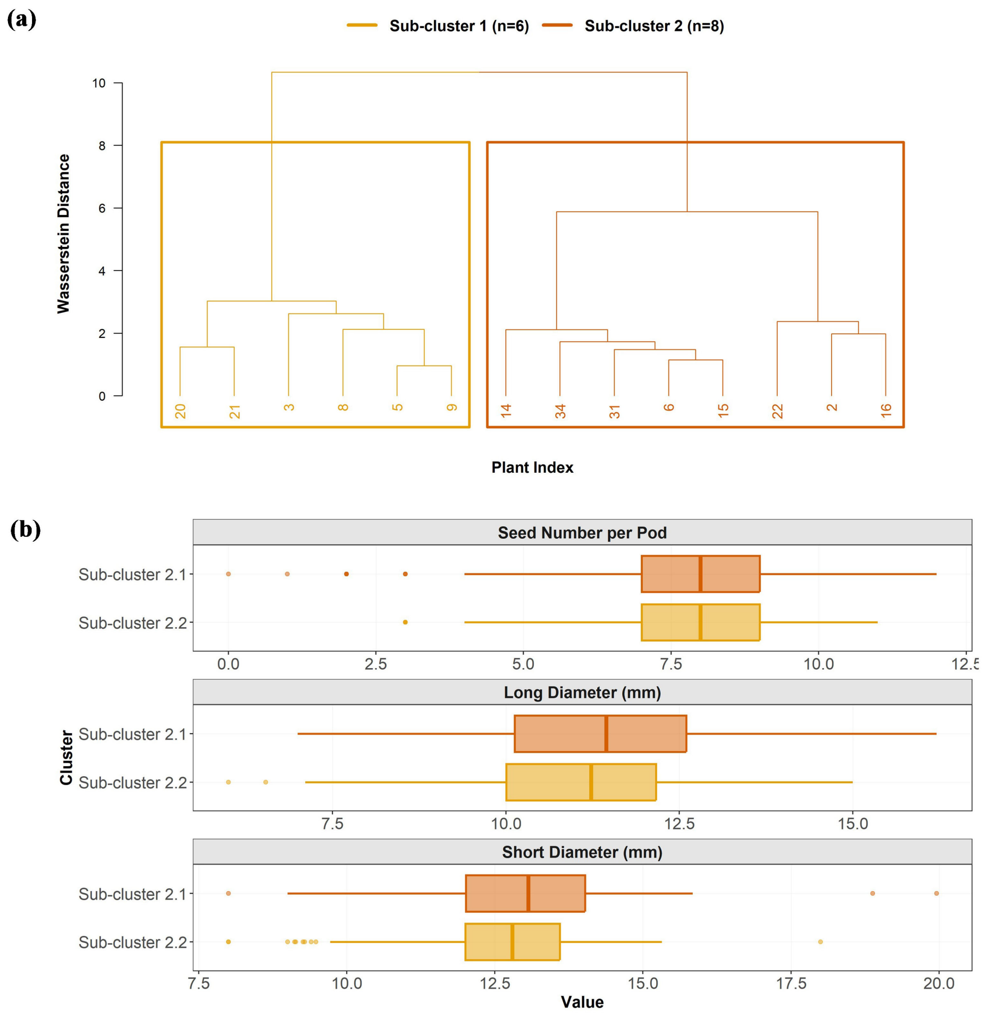

FIGURE 8
Follow-up of Cluster 2 (n = 14). **(a)** Hierarchical dendrogram (Wasserstein distance) resolving two tentative sub-clusters (n = 6; n = 8). **(b)** Boxplots of seed number per pod, long diameter, and short diameter by sub-cluster.

The morphological characterization of the ecotype Monreale of *M. intertexta* provides a basis for evaluating this genetic resource. The observed associations indicate that reproductive traits (raceme and pod numbers) are closely linked to both biomass and, especially, seed yield, confirming their role as key yield components (Wang et al., 2023). Although correlations with biomass and seed yield are broadly similar, those with seed yield tend to be slightly stronger, suggesting that some traits, such as three pods per raceme, can increase seed yield without necessarily affecting total biomass. Overall, the results support prioritizing improvements in yield components over strategies focused solely on vegetative growth. The observed variability is consistent with intra-accession diversity expected in annual *Medicago*, driven by partial outcrossing and micro-environmental effects, which generate differences in biomass, seed yield, and stress responses (Ellis et al., 1985; Van de Wouw et al., 1999). The dendrogram's asymmetric cluster sizes and the presence of distinct individuals (e.g., 35, 39, 6) point to heterogeneous phenotypes within the ecotype and potential selection candidates. The observed separation of plant groups highlights two components capturing about 66% of the variance, indicating that a compact set of traits effectively summarizes the major axes of phenotypic differentiation. Cluster 2 showed higher median biomass and seed yield but greater variability, suggesting high-performing yet less stable ideotypes. The overlap observed in the targeted analyses of Cluster 2 indicates that further sub-clustering is unlikely to add resolution, whereas the exploration of Cluster 1 shows comparable variability in seed number across its subgroups; given subgroup 2's generally lower performance, prioritizing subcluster 1 for germplasm advancement appears justified. Seed yield and quality are influenced by genotype, agronomic practices, and environmental conditions (Hampton, 1991; Dordas, 2006; Hakl et al., 2021); making plant density a key factor for optimizing biomass and seed production (Boelt et al., 2015; Thomas, 2016). Across two seasons, results highlight productive flexibility under contrasting conditions. The inverse density–height relationship reflects reduced competition at lower densities (greater access to light, nutrients, water), while biomass showed no significant density effect but tended to increase at higher density, suggesting a trade-off between stand-level production and individual plant growth (Postma et al., 2021). Practically, higher

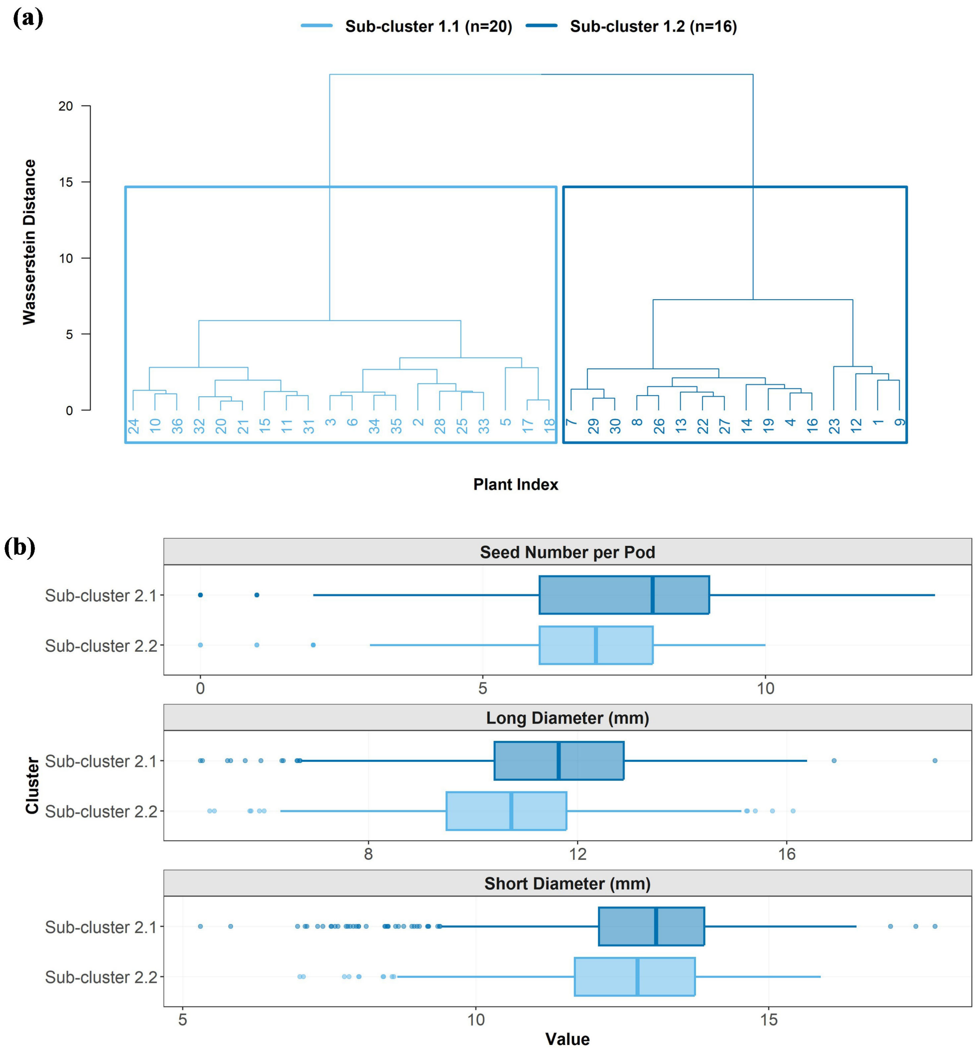

FIGURE 9
Follow-up of Cluster 1 (n = 36). **(a)** Hierarchical dendrogram (Wasserstein distance) resolving two sub-clusters (n = 20; n = 16). **(b)** Boxplots of seed number per pod, long diameter, and short diameter by sub-cluster.

density may maximize stand biomass, with implications for nutritional management in dairy ewes (Bonanno et al., 2019). Despite management-driven variability reported elsewhere, HI was stable across treatments and years (Hay, 1995). Regarding seed production, *M. intertexta* is in line with other annual *Medicago* species. In Mediterranean environments, species such as *Medicago truncatula* and *Medicago polymorpha* can produce up to 500–1000 kg ha^{-1} of seed under favorable conditions, although production is highly variable depending on environmental conditions and management (Cocks, 1988; Spanu and Peddis, 2022). Seed yield is a composite trait determined by pods and seeds per plant, number of inflorescences, and mean seed weight (Yassin, 1973; Laouar and Abdelguerfi, 1999). In this study, plant density influenced these components, showing a trade-off between per-plant performance and stand-level productivity. Higher plant density (T160) increased seed yield per hectare, but reduced seed mass per plant, mainly due to fewer seeds rather than smaller seed size, as indicated by higher 1,000-seed weight at high density. These results suggest that increased density intensifies resource competition, particularly for light, reducing individual plant performance while enhancing yield at the stand level (Ghiasy-Oskoee et al., 2019; Postma et al., 2021). Seasonal conditions further modulated these responses. In this warm semi-arid Mediterranean environment, rainfall timing strongly influenced density effects: the 2021/2022 season was characterized by well-distributed autumn and winter rainfall followed by a clear spring dry-down, whereas 2022/2023 experienced later and more irregular rainfall. These differences explain the observed year effects and the Year × Density interaction in seed yield. Overall, higher plant density increased yield across years, although rainfall timing remained a key factor under semi-arid Mediterranean conditions. The shift toward a higher hard-seed fraction and a lower soft-seed fraction at higher density (T160) suggests a maternal-environment effect, mediated by canopy structure (light attenuation, resource competition), on seed-coat development and dormancy, responses frequently reported in self-reseeding annual legumes (Nichols et al., 2012; Teixeira et al., 2022). Year effects and the Year × Density interaction for dead seeds and total germinability

TABLE 1 Two-year field experiment on Medicago intertexta (2021/22–2022/23).

Plant density	Plant height (cm)				Total biomass (kg DM ha^{-1})				Seed yield (kg ha^{-1})				Hi (%)			
	2022	2023	Mean	P	2022	2023	Mean	P	2022	2023	Mean	P	2022	2023	Mean	P
T20	59	63	61	***	4260	4450	4355	**	723	779	751	**	0.17	0.18	0.18	ns
T160	34	44	39		4967	5285	5126		773	832	803		0.16	0.16	0.16	
Mean	46	54			4613	4867			748	807			0.16	0.17		
P	***			**	ns			ns	**			ns	ns			ns

Trait means by plant density (T20, T160) and year are reported for plant height (cm), total biomass (kg DM ha^{-1}), seed yield (kg ha^{-1}), harvest index. The table also summarizes ANOVA results for Density, Year, and D×Y.
**p < 0.01; ***p < 0.001; ns, not significant.

TABLE 2 Seed-production components of *Medicago intertexta* over two seasons (2021/22–2022/23) by plant density (T20, T160): pods plant^{-1}, seeds pod^{-1}, seeds plant^{-1}, and 1,000-seed weight (g).

Plant density	Pods plant^{-1} (n)				Seeds pod^{-1} (n)				Seeds plant^{-1} (n)				1000-seed weight (g)			
	2022	2023	Mean	P	2022	2023	Mean	P	2022	2023	Mean	P	2022	2023	Mean	P
T20	37.8	38.7	38.3	ns	8.7	6.5	7.6	***	328	253	290	***	11.0	14.4	13.2	***
T160	5.3	5.4	5.4		6.4	5.2	5.8		34	29	31		14.3	18.2	16.3	
Mean	22	22			7.5	5.9			181	141			12.6	16.8		
P	***			ns	***			**	***			***	***			ns

For each trait, columns report values for 2022 and 2023 and the across-year Mean; The table also summarizes ANOVA results for Density, Year, and D×Y.
**p < 0.01; ***p < 0.001; ns, not significant.

10.3389/fagro.2026.1786084

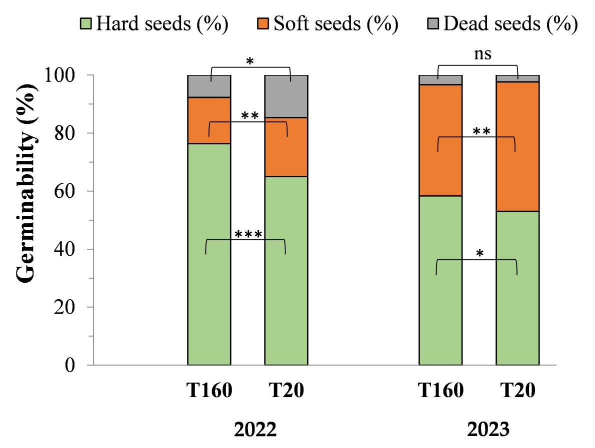

FIGURE 10
Germinability by sowing density (T160, T20) in 2022 and 2023. Asterisks mark the within-year contrast T160 vs T20 for each fraction (Hard/Soft/Dead): p < 0.05 (*), p < 0.01 (**), p < 0.001 (***); ns, not significant.

emphasize rainfall timing over totals: in 2021/22, earlier, concentrated autumn–winter precipitation and a sharper dry-down align with more hard seed and a higher dead-seed share (especially at low density), whereas in 2022/23 late spring rain pulses and smoother thermal rise likely sustained embryo viability, reduced mortality, increased the soft-seed fraction, and slightly elevated total germinability under T20 (Porqueddu et al., 1996; Martínez-Berdeja et al., 2023). Accordingly, higher density favors seed bank formation (more hard seeds) and support pasture persistence, while lower density favors immediate establishment (more soft seeds); optimizing density should balance short-term recruitment and long-term self-regeneration (Nichols et al., 2012; Martínez-Berdeja et al., 2023).

5 Conclusions

This study highlights the agronomic relevance of *Medicago intertexta*, a self-reseeding annual legume with considerable potential for sustainable forage systems in semi-arid Mediterranean environments. Its widespread presence in Sicilian farms and recognized persistence and self-regeneration confirm its adaptability and value within ley farming systems. The observed morphological diversity within the Monreale ecotype indicates substantial genetic variability that could be exploited for future selection and breeding efforts. Results indicate that optimizing plant density is essential to balance stand-level productivity and long-term pasture persistence, while hard seed dormancy plays a key role in maintaining the soil seed bank under variable climatic conditions. Further research should optimize harvesting and management practices to enhance seed production and system efficiency, refine seeding density, and evaluate factors affecting yield and forage quality, including crude protein content, as well as breeding for climate adaptation. Overall, *M. intertexta* emerges as a promising candidate for developing resilient and sustainable Mediterranean pastures.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

LD: Formal analysis, Data curation, Writing – review & editing, Conceptualization, Methodology, Software, Investigation, Writing – original draft. SP: Software, Writing – original draft, Writing – review & editing, Investigation, Formal analysis, Data curation. NL: Writing – review & editing, Investigation, Software, Data curation, Formal analysis. AA: Software, Writing – review & editing, Writing – original draft, Formal analysis, Data curation, Methodology. DF: Data curation, Writing – review & editing, Validation, Formal analysis, Visualization, Investigation, Software. GD: Project administration, Funding acquisition, Writing – original draft, Resources, Validation, Visualization, Conceptualization, Supervision, Writing – review & editing, Methodology.

Funding

The author(s) declared that financial support was received for this work and/or its publication. This research was funded by “Ministero dell’agricoltura, della sovranità alimentare e delle foreste”, Italy, “SFEFA” Project (grant number: B79C21000370001) and by Assessorato Regionale dell’Agricoltura, dello Sviluppo Rurale e della Pesca Mediterranea, Regione Sicilia (Misura 10, Sottomisura 10.2, Operazione 10.2a, PSR Sicilia 2014–2020), regional project “COSA—Conservazione e caratterizzazione di Accessioni Siciliane di Specie Agrarie erbacee” (grant number: G31J20000120009).

Conflict of interest

The author(s) declared that this work was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The author GD declared that they were an editorial board member of Frontiers at the time of submission. This had no impact on the peer review process and the final decision.

Generative AI statement

The author(s) declared that generative AI was not used in the creation of this manuscript.

Any alternative text (alt text) provided alongside figures in this article has been generated by Frontiers with the support of artificial intelligence and reasonable efforts have been made to ensure accuracy, including review by the authors wherever possible. If you identify any issues, please contact us.

Publisher's note

All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations, or those of the publisher, the editors and the reviewers. Any product that may be evaluated in this article, or claim that may be made by its manufacturer, is not guaranteed or endorsed by the publisher.

References

Abdelguerfi-Laouar, M., Abdelguerfi, A., Bouznad, Z., and Guittonneau, G. G. (2003). Autoécologie et distribution du complexe d'espèces Medicago ciliaris–M. intertexta en Algérie. *Acta Botanica Gallica* 150, 253–265. doi: 10.1080/12538078.2003.10515995

Abdelkefi, A., Boussaid, M., and Marrakchi, M. (2001). "Complexe d'espèces et régime de reproduction dans la section Intertextae: Medicago ciliaris, Medicago intertexta," in *Quality in Lucerne and Medics for Animal Production*. Ed. J. Lloveras (CIHEAM, Zaragoza), 107–110.

Bartholomew, P. W. (2014). Self-seeding warm-season legumes for low-input forage production in the southern Great Plains of the USA. *Agr. Sci.* 5, 1112–1118. doi: 10.4236/as.2014.512121

Bartolucci, F., Peruzzi, L., Galasso, G., Albano, A., Alessandrini, A. N. M. G., Ardenghi, N. M. G., et al. (2018). An updated checklist of the vascular flora native to Italy. *Plant Biosyst. - Int. J. Dealing Aspects Plant Biosyst.* 152, 556–592. doi: 10.1080/11263504.2017.1419996

Bennett, S. J., and Cocks, P. S. (1999). *Genetic Resources of Mediterranean Pasture and Forage Legumes* (Dordrecht: Springer Netherlands), pp. 9–19.

Boelt, B., Julier, B., Karagić, D., and Hampton, J. (2015). Legume seed production meeting market requirements and economic impacts. *Crit. Rev. Plant Sci.* 34, 412–427. doi: 10.1080/07352689.2014.898477

Bonanno, A., Di Grigoli, A., Stringi, L., Di Miceli, G., Giambalvo, D., Tornambè, G., et al. (2007). Intake and milk production of goats grazing sulla forage under different stocking rates. *Ital. J. Anim. Sci.* 6, 605–607. doi: 10.4081/ijas.2007.1s.605

Bonanno, A., Di Grigoli, A., Vitale, F., Di Miceli, G., Todaro, M., Alabiso, M., et al. (2019). Effects of diets supplemented with medicinal mushroom myceliated grains on some production, health, and oxidation traits of dairy ewes. *Int. J. Med. Mushrooms* 21, 89–103. doi: 10.1615/IntJMedMushrooms.2018029327

Caldarella, O., Gianguzzi, L., Romano, S., and Fici, S. (2009). The vascular flora of Nature Reserve "Pizzo Cane, Pizzo Trigna and Grotta Mazzamuto" (NW Sicily). *Webbia* 64, 101–151. doi: 10.1080/00837792.2009.10670854

Cambria, S., and Tavilla, G. (2020). Check-list of the vascular flora of the "Bosco di Gibilmanna", a Special Area of Conservation (S.A.C.) in northern Sicily (Italy). *Biodiversity J.* 11, 369–382. doi: 10.31396/biodiv.jour.2020.11.2.369.382

Cherifi, K. (2016). Evidence of natural hybridization and introgression between Medicago ciliaris and Medicago intertexta. *Int J Environ Agric Res* 29–35. doi: 10.22192/ijarbs.2016.03.09.012

Cocks, P. S. (1988). Seed production and seed survival under grazing of annual medics (Medicago spp.) in north Syria. *J. Agric. Sci.* 110, 455–463. doi: 10.1017/s0021859600082009

Crawford, E. J., and Nankivell, B. G. (1989). Effect of rotation and cultivation systems on establishment and persistence of annual medics. *Aust. J. Exp. Agric.* 29, 183–188. doi: 10.1071/EA9890183

Domina, G., Di Gristina, E., Scafidi, F., Calvo, R., Venturella, G., and Gargano, M. L. (2019). The urban vascular flora of Palermo (Sicily, Italy). *Plant Bios.* 154 (5), 627–634. doi: 10.1080/11263504.2019.1651787

Dordas, C. (2006). Foliar boron application improves seed set, seed yield, and seed quality of alfalfa. *Agr. J.* 98, 907–913. doi: 10.2134/agronj2005.0353

Edwards, T., Howieson, J., Nutt, B., Yates, R., O'Hara, G., and Van Wyk, B. E. (2019). A ley-farming system for marginal lands based upon a self-regenerating perennial pasture legume. *Agron. Sustain. Dev.* 39, 13. doi: 10.1007/s13593-019-0558-2

Ellis, R. H., Hong, T. D., and Roberts, E. H. (1985). *Handbook of Seed Technology for Genebanks. Volume II: Compendium of Specific Germination Information and Test Recommendations* (Rome, Italy: IBPGR).

El-Shafey, N. M., and Al-Sherif, E. (2020). Wild medics from different original habitats can be used as forage legumes in salt-affected soil. *J. Clean. WAS (JCleanWAS)* 4, 47–55. doi: 10.26480/jcleanwas.02.2020.47.55

Fernandes, A., and Queirós, M. (1978). Contribution à la connaissance cytotaxinomique des Spermatophyta du Portugal. IV: Leguminosae (Suppl. 3).

Ghiasy-Oskoee, M., AghaAlikhani, M., Mokhtassi-Bidgoli, A., Sefidkon, F., and Ayyari, M. (2019). Seed and biomass yield responses of blessed thistle to nitrogen and density. *Agr. J.* 111, 601–611. doi: 10.2134/agronj2018.05.0323

Gianguzzi, L., D'Amico, A., Caldarella, O., and Romano, S. (2011). La flora vascolare delle Rocche di Entella (entroterra della Sicilia occidentale). *Nat. Sicil* s.4, 35, 363–403.

Hakl, J., Pisarčik, M., Fuksa, P., and Šantrůček, J. (2021). Potential of lucerne sowing rate to influence root development and its implications for field stand productivity. *Grass Forage Sci.* 76, 378–389. doi: 10.1111/gfs.12546

Hampton, J. G. (1991). Herbage seed lot vigour: Do problems start with seed production? *J. Appl. Seed Prod.* 9, 87–93.

Hay, R. K. M. (1995). Harvest index: A review of its use in plant breeding and crop physiology. *Ann. Appl. Biol.* 126, 197–216. doi: 10.1111/j.1744-7348.1995.tb05015.x

IPGRI (1991). *Descriptors for annual Medicago* (Rome: International Plant Genetic Resources Institute).

Jolliffe, I. T. (2002). *Principal Component Analysis. 2nd ed* (New York. USA: Springer).

Kantorovich, L. V. (1939). Mathematical methods of organizing and planning production. *Manage. Sci.* 6, 366–422. doi: 10.1287/mnsc.6.4.366

Kemp, P. R. (1989). "Seed banks and vegetation processes in deserts," in *Ecology of Soil Seed Banks*. Eds. M. A. Leck, V. T. Parker and R. L. Simpson (Academic Press, San Diego), 257–281.

Kernick, M. D. (1978). Indigenous and semi-arid forage plants of north Africa, the Near and Middle East. *Ecological management of arid and semi-arid rangelands in Africa and Near and Middle East* (Rome, Italy: FAO), Vol. 4, 519–689.

Laouar, M., and Abdelguerfi, A. (1999). Variabilité de la production de gousses et des graines chez quelques populations spontanées de Medicago intertexta. *Cahiers Options Méditerranéennes* 39, 111–117.

Laouar, M., and Abdelguerfi, A. (2014). Little effect of ecological factors and symbiotic specificity on the distribution of Medicago subsect. Intertextae (Urban) Heyn (Fabales, Fabaceae) in the Mediterranean Basin. *Biod. J.* 5, 481–498.

Laouar, M., Kies, N., Abdellaoui, K., Bennour, A., Bettahar, N., Kadi, S., et al. (2001). "Effet du stress hydrique sur le comportement physiologique de dix populations de Medicago intertexta," in *Quality in Lucerne and Medics for Animal Production*. Ed. J. Lloveras (CIHEAM, Zaragoza), 167–171.

Lepschi, B., and Monro, A. (2014). "Australian plant census (APC)," in *Council of Heads of Australian Herbaria* (Camberra, Australia: Council of Heads of Australian Herbaria). Available online at: http://www.anbg.gov.au/chah/apc/index.html (Accessed October 24, 2025).

Lesins, K., Singh, S. M., and Erac, A. (1971). Relationship of taxa in the genus Medicago as revealed by hybridization. V. Section Intertextae. *Can. J. Gen. Cyt.* 13, 335–346. doi: 10.1139/g71-052

Martínez-Berdeja, A., Tovar-Herrera, O. E., Naranjo, S., and Munguía-Rosas, M. A. (2023). Precipitation timing and soil substrate drive phenology and fitness of Arabidopsis thaliana in a Mediterranean environment. *Fun. Ecol.* 37, 2774–2787. doi: 10.1111/1365-2435.14391

Mbarki, S., Skalicky, M., Vachova, P., Hajihashemi, S., Jouini, L., Živčák, M., et al. (2020). Comparing salt tolerance at seedling and germination stages in local populations of Medicago ciliaris L. to Medicago intertexta L. and Medicago scutellata L. *Plants* 9, 526. doi: 10.3390/plants9040526

Müller, F., Masemola, L., Britz, E., Ngcobo, N., Modiba, S., Cyster, L., et al. (2022). Seed germination and early seedling growth responses to drought stress in annual Medicago L. and Trifolium L. forages. *Agronomy* 12, 2960. doi: 10.3390/agronomy12122960

Nichols, P. G. H., Revell, C. K., Humphries, A. W., Howie, J. H., Hall, E. J., Sandral, G. A., et al. (2012). Temperate pasture legumes in Australia — their history, current use and future prospects. *Crop Past. Sci.* 63, 691–725. doi: 10.1071/CP12194

Norman, H. C., Cocks, P. S., Smith, F. P., and Nutt, B. J. (1998). Reproductive strategies in Mediterranean annual clovers: Germination and hardseededness. *Aust. J. Agric. Res.* 49, 973–982. doi: 10.1071/A97105

Pignatti, S., Guarino, R., and La Rosa, M. (2017). *Flora d'Italia. 2nd ed. vol. 2* (Milano: New Business Media), 556–571.

Porqueddu, C., Bullitta, S., and Loi, A. (1996). Hardseededness and pattern of hard seed breakdown in Sardinian populations of Medicago polymorpha under field conditions. *J. Agric. Sci.* 126, 161–168. doi: 10.1017/S0021859600073093

Postma, J. A., Hecht, V. L., Hikosaka, K., Nord, E. A., Pons, T. L., and Poorter, H. (2021). Dividing the pie: A quantitative review on plant density responses. *Plant Cell Environ.* 44, 1072–1094. doi: 10.1111/pce.13968

Revell, C. K., Ewing, M. A., and Nutt, B. J. (2012). Breeding and farming system opportunities for pasture legumes facing increasing climate variability in the south-west of Western Australia. *Crop Past. Sci.* 63, 840–847. doi: 10.1071/CP12160

Rousseeuw, P. J. (1987). Silhouettes: A graphical aid to the interpretation and validation of cluster analysis. *J. Comput. Appl. Math.* 1, 53–65. doi: 10.1016/0377-0427(87)90125-7

Sadeghian, S., and Hejazi, S. H. (2014). Cytogenetic studies in some species of Medicago L. in Iran. *Eur. J. Biol.* 73, 21–30. doi: 10.3923/ajbs.2010.188.194

Sciandrello, S., D'Agostino, S., and Minissale, P. (2014). The vascular flora of the Taormina Region (Peloritani Mountains – northeast Sicily). *Webbia* 69, 301–324. doi: 10.1080/00837792.2014.966487

SIAS (Servizio Informativo Agrometeorologico Siciliano). (2025). SIAS — Servizio informativo agrometeorologico siciliano. Available online at: http://www.sias.regione.sicilia.it/ (Accessed October 24, 2025).

Small, E., and Jomphe, M. (1989). A synopsis of the genus Medicago (Leguminosae). *Can. J. Bot.* 67, 3260–3294. doi: 10.1139/b89-405

Small, E., Warwick, S. I., and Brookes, B. (1999). Allozyme variation in relation to morphology and taxonomy in Medicago sect. Spirocarpos subsect. Intertextae (Fabaceae). *Plant Syst. Evol.* 214, 29–47. doi: 10.1007/BF00985730

Smythies, B. E. (1984–1986). *Flora of Spain and the Balearic Islands: Checklist of Vascular Plants* Vol. 3 (Berlin-Dahlem, Germany: Englera), 1–882.

Snedecor, G. W., and Cochran, W. G. (1989). *Statistical Methods* (Ames, Iowa, USA: Iowa State University Press).

Soil Survey Staff (2014). *Keys to Soil Taxonomy* (Washington, DC: USDA–NRCS).

Spanu, E., and Peddis, A. (2022). "Enhancing native species seed supply to improve the resilience of Mediterranean pastures", in: *Grassland at the heart of circular and sustainable food systems* (Paris, France), 481–484.

Taylor, G. B. (2005). Hardseededness in Mediterranean annual pasture legumes in Australia: A review. *Aust. J. Agric. Res.* 56, 645–661. doi: 10.1071/AR04284

Teixeira, C. S., Hampton, J. G., and Moot, D. J. (2022). Time of sowing and cultivar effects on hardseededness and germination of subterranean clover seeds. *N. Z. J. Agric. Res.* 65, 309–333. doi: 10.1080/00288233.2020.1861030

Thanopoulos, R. (2007). The genus Medicago in Greece: 1. A review of species diversity, geographical distribution and ecological adaptation. *Flora Mediterr.* 17, 217–276.

Thomas, B. (Ed.) (2016). *Encyclopedia of Applied Plant Sciences* (Amsterdam, Netherlands: Academic Press).

Tutin, T. G., Heywood, V. H., Burges, N. A., Moore, D. M., Valentine, D. H., Walters, S. M., et al. (Ed.) (1968). *Flora Europaea* Vol. 2 (Cambridge, UK: Cambridge University Press).

Van de Wouw, M., Hanson, J., and Nokoe, S. (1999). Observation strategies for morphological characterisation of forages. *Genet. Resour. Crop Evol.* 46, 63–71. doi: 10.1023/A:1008627527822

Wang, X., Zhong, P., Yang, Z., Lai, Y., Li, S., Chai, H., et al. (2023). Effects of plant density on alfalfa (Medicago sativa L.) seed yield in western Heilongjiang areas. *Open Life Sci.* 18, 20220792. doi: 10.1515/biol-2022-0792

Yassin, T. E. (1973). Genotypic and phenotypic variances and correlations in field beans (Vicia faba L.). *J. Agr. Sci.* 81, 445–448. doi: 10.1017/S0021859600086494

Zahran, H. H., Marín-Manzano, M. C., Sánchez-Raya, A. J., Bedmar, E. J., Venema, K., and Rodríguez-Rosales, M. P. (2007). Effect of salt stress on the expression of NHX-type ion transporters in Medicago intertexta and Melilotus indicus plants. *Physiol. Plant* 131, 122–130. doi: 10.1111/j.1399-3054.2007.00940.x

Zoghlami, A., and Hassen, H. (1999). "Observations on the distribution and ecology of annual Medicago species in northern Tunisia", in: *Genetic Resources of Mediterranean Pasture and Forage Legumes* (Dordrecht: Springer Netherlands), 235–238.