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Challenges and innovations for grasslands resilience

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Intraspecific variability at early establishment and winter survival of some minor forage species

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Abstract

Efforts continue to diversify grasslands with minor forage species to meet the current and future challenges. From a cultivation and breeding perspective, the key question is how much intraspecific variation exists in desired traits, as this determines the potential for future breeding. This study compared six dicot forage species (*Achillea millefolium* (*Achmil*), *Carum carvi* (*Carcar*), *Cichorium intybus* (*Cicint*), *Lotus corniculatus* (*Lotcor*), *Medicago lupulina* (*Medlup*), *and Plantago lanceolata* (*Plalan*)) to investigate intraspecific variation in early establishment (dry matter yield and weed mass) and winter survival. Four to seven cultivars and wildtypes (hereafter accessions) of each species were grown in a single-site field experiment arranged in a randomized block design with three replicates. Data were collected from the first harvest after sowing and the following winter. All plant species showed potential for successful early establishment and winter survival with significant differences among species. Although there was considerable intraspecific variation within species, this variation was statistically significant only for some species. The findings reveal the potential of minor species to be considered in future grassland farming and point out the possibility of improving germplasm through plant breeding.

Keywords: accessions, early establishment, minor forage species, winter survival

Introduction

Grasslands face multiple stressors, including drought, reduced species diversity, and particularly harsh winter conditions, which negatively impact crop physiology and productivity (Andrews, 1987). Enhancing both intraspecific and interspecific diversity has been proposed as a strategy to improve resilience and performance under these stresses (Isselstein & Komainda, 2021). The integration of underutilized species into cultivated grasslands represents a promising approach, yet these species have received limited breeding attention. Legumes and forbs, in particular, offer potential benefits for climate adaptation, forage quality, ruminant health, and nitrogen use efficiency due to their functional traits and secondary metabolites (Hamacher *et al.*, 2021). Since successful establishment and early growth are critical for integrating new germplasms, this study aims to evaluate early establishment and winter survival in selected underutilized species to identify intraspecific variability to better assess suitability for inclusion in grasslands mixtures.

Materials and methods

A field trial testing the effects of cultivars and wildtypes (hereafter accessions) of minor dicot forage species, (i.e., *Achillea millefolium* (*Achmil*), *Carum carvi* (*Carcar*), *Cichorium intybus* (*Cicint*), *Lotus corniculatus* (*Lotcor*), *Medicago lupulina* (*Medlup*) *and Plantago lanceolata* (*Plalan*)) was established in May 2023 in Göttingen (Central Germany) on a Luvisol soil with a silty loam texture in a randomized complete block design with three replications and a plot size of 4.8 m^2. The number of accessions

Table 1. Estimated forage dry matter yield, weed mass, and median winter survival in the early establishment of *Achillea millefolium* (*Achmil*), *Carum carvi* (*Carcar*), *Cichorium intybus* (*Cicint*), *Lotus corniculatus* (*Lotcor*), *Medicago lupulina* (*Medlup*) and *Plantago lanceolata* (*Plalan*).

Species	n[1]	Dry matter yield (g m^{-2})			Weed mass (g m^{-2})			Winter survival		
		Mean	Range[2]	Pop	Mean	Range	Pop	Median	Range	Pop
Achmil	4	47.8 b	131.6	0.06	77.4 a	102.4	0.41	8.0 a	5	0.13
Cicint	4	130.7 a	201.5	0.56	49.6 a	113.9	0.4	7.0 ab	5	0.12
Carcar	4	50.3 b	177.6	0.43	79.0 a	166.9	0.22	6.5 ab	8	0.13
Lotcor	7	36.3 b	77	0.48	97.8 a	210.5	0.05	4.0 c	5	0.14
Medlup	4	77.1 b	164.7	0.002	69.3 a	105.2	0.13	7.0 ab	8	0.07
Plalan	7	56.6 b	139	0.009	88.5 a	173.3	0.05	5.0 b	7	0.02

The Pop columns provide *P*-values for the main effect of accessions per species as obtained from analyses of intraspecific variability. Different lowercase letters indicate significant differences among the species ($P \leqslant 0.05$).

[1] Number of accessions per species.

[2] Distance between minimum and maximum values per target variable (range).

differed among species because of variable access in the market (*Lotcor* and *Plalan*: 7 and all other species 4 accessions). After seeding, two cleaning cuts against annual weeds were conducted. The early establishment of plants was assessed using dry matter yield and weed mass in autumn to define their competitive strength against weeds. For this, plots were harvested once in September during the establishment year 2023 using a plot harvester (Model HD 1500 Wintersteiger). Subsamples were dried (60°C, 48 h) to determine the dry forage yield. Winter hardiness was visually assessed in the spring of 2024 (first winter after establishment) using a rating scale from 1 (no plants survived) to 9 (all plants survived). Calcium ammonium nitrate (30 kg N ha^{-1}) was applied to each non-leguminous plot after seeding. Statistical analyses were performed with R studio package 'nlme' (Pinheiro *et al.*, 2025) using linear mixed effects modelling for the accumulated forage yield and weed mass. The main effect of species was treated as a fixed and the block was used as a random effect. Tukey's HSD test was used post hoc to compare species means for forage yield and weed mass. The intraspecific variability was calculated as distance between minimum and maximum values per target variable (Range). A Kruskal–Wallis comparison of medians was applied for winter survival in the package 'agricolae' (De Mendiburu, 2023). The main effect of accessions was tested for each species separately with the block as random effect to determine the intraspecific variability per species for forage yield and weed mass. Kruskal–Wallis test was performed to evaluate accessions effects on winter survival.

Results and discussion

The average temperature and rainfall during the growing season (April-October) were 14.8°C and 438 mm, respectively, which was 3% warmer and 12% wetter than the long-term values (average temperature and precipitation were 14.4°C and 385 mm, respectively from 2013 to 2022). During winter 2023/2024 (December-February), the average temperature was 4.6°C which was warmer than the long-term value (3.0°C for 2013–2022). Temperatures remained below 0°C for 19 days in the considered winter period (average −2.8°C). The main effect of species was significant for all target variables ($P \leqslant 0.001$), except weed mass (Table 1).

All investigated species were successfully established and survived the first winter. However, clear differences among species were observed, with *Cicint* showing the highest forage yield ($P \leqslant 0.001$), whereas differences in weed mass (an indicator of competitive strength) were not statistically significant among species. Notably, with 28% *Cicint* had the lowest proportion of weeds, which was considerably lower compared to the other species. All species showed a huge variation among the accessions within species

but significant effects were found only for *Medlup* (forage yield), *Lotcor* (weed mass), and *Plalan* (forage yield and weed mass) (Table 1). Regarding winter survival, *Lotcor* showed significantly lower survival rates than other species. The values of winter survival were generally above five, indicating moderate survival. *Lolium perenne*, the most important forage grass in Germany, has a winter survival scale of, on average, 4–5 according to the official species variety list (Bundessortenamt, 2024). Interestingly, although there was a wide range of variation among accessions within the species, these effects were not statistically significant for all species. This indicates that the observed variation was not fully explained by the investigated factors, suggesting that the emergence and establishment were also influenced by additional variables that were not identified in this experiment. Such patterns are typical of wildflower species, which often exhibit delayed and variable germination rates.

Conclusion

All species except *Lotus corniculatus* showed moderate to high winter survival rates under the given conditions. However, it is important to note that the results of this study were based on a single site and the first year only. Therefore, there is a need to take a closer look at the variation among accessions within species and consider a broader range of traits, including long-term performance and nutritive value, ideally through studies extending over additional seasons and at multiple locations.

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