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Characterization of pastures legumes with potential for biomass production and for mediterranean pastures restoration

Carita, T[1]; Pereira, G[1,2]

[1]National Institute of Agricultural and Veterinary Research-Elvas, Ap 6, 7351-901 Elvas, Portugal

[2]GeoBiotec, NOVA School of Science and Technology, Caparica, Portugal

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Abstract

True extensive livestock systems - those based on the use of permanent pastures and grazable agricultural co-products, with low use of external production factors, and which promote ecosystem services, combat desertification and create economic conditions for the population to settle in the territory - are probably one of the most vital ecosystems in the world, particularly in the Mediterranean. These agrarian systems provide us high quality products, fodder, ecosystem services and harbour a great plant and animal biodiversity, both above and below the soil surface.

In some regions of the world, the area of permanent pasture has decreased and changes in land use continue to threaten its extent. In Portugal, many of these pastures (which occupy > 50% of the utilised agricultural area) need to be restored, preferably using nature itself, and thus increase agricultural productivity and soil health, as well as improving land management.

INIAV-Elvas (southern Portugal) has morphologically and phenologically characterised a collection of 66 ecotypes belonging to the genera *Ornithopus* and *Trifolium* genera. The aim is to evaluate ecotypes of native legume species of mainland Portugal which have the potential to successfully establish and develop under very limiting agro-ecological conditions. Descriptors developed by INIAV-Elvas, based on those developed by "Bioversity International" and UPOV-International Union for the Protection of New Varieties of Plants for species in the same group, were used to characterise this plant material. High winter growth and early flowering are important descriptors for the improvement of the Mediterranean agro-sylvo-pastoral sector. So, the ecotypes considered to have the best potential are: Ornitopus compressus 15692 e 15682; Ornithopus pinnatus 13563; *Trifolium cherleri* 15710; *Trifolium glomeratum* 15648 and *Trifolium lappaceum* 14174.

Introduction

Permanent pastures are a key feature of the European rural landscape, representing not only a significant agricultural resource but also supporting multiple non-provisioning ecosystem service. However, this vital

farming system is at risk of loss or degradation due to changes in agricultural land use and management practices (Elliot et al., 2024). Schils et al. (2022) emphasize that, despite apparent shifts in the dietary preferences of the European population, prioritizing the protection of permanent grasslands in Europe is essential to prevent further losses in area and to safeguard the provision of ecosystem services. In Portugal, 52% of agricultural land consisted of permanent pastures. Meadows and permanent pastures covered approximately 2 million hectares, 68% of which consisted of unimproved pastures, lacking interventions such as sowing, fertilizing, watering, or drainage (INE, 2019). Many of these pastures are either natural (characterized by low and irregular yields) or degraded. It is necessary to intervene in permanent pastures to enhance their productivity and quality, enabling increased stocking densities and reducing the need for supplemental feed.

When faced with the challenge of restoring poorly productive or degraded permanent pastures, sustainability must be a central consideration. One effective strategy to enhance pasture functionality involves improving its floristic composition. This can be achieved through appropriate grazing management and/or the introduction of seeds from species well adapted to the specific environmental conditions. The introduction of new plant genetics, particularly legumes offers numerous benefits, including increased biodiversity, improved grass yield and quality, reduced dependence on fertilisers, lower greenhouse gas emissions, and enhanced landscape aesthetics. The addition of legumes, in particular, plays a crucial role in improving soil fertility by enriching it with mineral elements (especially nitrogen) and organic matter, creating conditions that support the development of other high-quality species.

For many years, INIAV-Elvas has been developing programs focused on the conservation and improvement of various legume species suitable for grasslands. These programs aim to contribute to the economic and environmental sustainability of Mediterranean farming systems.

In this study, we aimed to evaluate ecotypes of native legume species from mainland Portugal that have the potential to successfully establish and develop under highly limiting agro-ecological conditions.

Methods

The Portuguese ecotypes used were collected in the wild, in uncultivated fields; the prospecting and germplasm collection missions followed the rules laid down in the 'Seed Collecting Manual for wild species' published by the 'European Native Seed Conservation Network' (ENSCONET 2009).

Caraterizaram-se um total de 66 ecótipos, pertencente aos géneros *Ornithopus* e *Trifolium*. As espécies consideradas foram: *Ornithopus compressus*-Ocom ((90% Hard seeds; Deep root system; Well adapted to light-textured, acidic soils), *Ornithopus pinnatus* (Opin), *Trifolium cherleri* (Tche), *Trifolium glomeratum* (Tglo) e *Trifolium lappaceum* (Tlap).

Each ecotype was characterised for 2 consecutive years. To characterise this plant material (10 plants/access/year), several descriptors developed by INIAV-Elvas were used, based on those of Bioversity International and UPOV (International Union for the Protection of New Varieties of Plants) for species in the same group: Winter vigour; Spring vigour; No. of days to flowering (Flo-I); No. of days to end of flowering (Flo-F); Duration of flowering (Durflor); Plant size; No. of internodes; Longest stem including flower head; Leaf colour; Length; No. of internodes; Stem pubescence; Length of longest stem including flower head; Stem thickness; Stem anthocyanin colouration; Leaf colour; Length (normally developed centre leaflet immediately below terminal flower; Frequency of plants with coloured leaflet markings; Centre leaflet length; Centre leaflet width; Flower colour; Seed colour; Seed weight 1000 seeds and seed yield.

The data were analysed by numerical taxonomy techniques, using the NTSYS-pc (Numerical Taxonomy and Multivariate System) programme.

Results

A wide range of variation was registered among the ecotypes of the five annual herbaceous legume species, particularly in flowering period and cycle duration.

O. compressus is the earliest species, requiring approximately 152 days from transplanting to the start of flowering, whereas *T. lappaceum* is the latest, taking an average of 170 days. Regarding flowering duration, *O. pinnatus* exhibited the longest average flowering period (42 days) but showed the weakest vegetative vigour. The species characterised exhibited significant differences in their cycle duration. For example, the average cycle duration of *O. pinnatus* is 50 days, while that of *T. lappaceum* is only 19 days (Table 1). Additionally, there is substantial intraspecific variability.

Discussion and Implications

The species evaluated in this study exhibit the characteristics of pioneer species, meaning they are plants capable of growing in inhospitable environments with unfavorable conditions, being the first to colonize areas where many other plants cannot survive. When analysing the results, it is important to consider that the long-term goal is to identify and select species and ecotypes with the ability to adapt to challenging soil and climatic conditions (e.g., light-textured, shallow soils with acidic pH, and a Mediterranean climate characterized by increasingly shorter and warmer springs).

The ecotypes identified as having the greatest genetic potential were: Ocom_15692, Ocom_15682, Opin_13563, Tche_15710, Tglo_15648, and Tlap_14174. These ecotypes demonstrated the most stable performance over two years of characterization and exhibited reproductive and vegetative cycles suitable for Mediterranean dryland farming, including high winter vegetative vigor and an early growth cycle.

INIAV should continue to develop programs for the conservation and characterization of plant genetic resources in consistent way, given the extensive collection of forage accessions that have been preserved and which hold significant potential. This approach will enable the identification of new solutions capable of addressing the needs of diverse national agricultural systems in the face of constant environmental changes.

Table 1 - Limits of variation and coefficient of variation for some of the descriptors evaluated

	Opin				***Ocom***			
	Min	Max	Mean	CV(%)	Min	Max	Mean	CV(%)
Winter vigour*	9	3	4	13,4	5	3	6,19	27,7
Spring vigour*	8	2	7	17,8	9	5	4,55	34,8
Number of days to flowering (no. of days)	142	168	156	5,9	134	165	152	6,2
Duration of flowering (no. of days)	17	50	42	22,4	25	61	31,61	37,7
Cycle duration (no. of days)	24	60	60	22,9	42	80	40,64	31,7

*1: Very vigorous to 9: Very little vigorous

Table 1 - Limits of variation and coefficient of variation for some of the descriptors evaluated
(continuation)

	Tglo				*Tche*				*Tlap*			
	Min	Max	Mean	CV(%)	Min	Max	Mean	CV(%)	Min	Max	Mean	CV(%)
Winter vigour*	7	3	5,01	15,7	7	3	4,52	20,1	9	1	5,09	44,8
Spring vigour	8	3	5,99	21,7	7	3	5,27	22,5	7	2	3,89	38,6
Number of days to flowering (no. of days)	152	183	167	4,6	146	176	161	5,5	150	181	170	5,4
Duration of flowering (no. of days)	13	44	26,01	29,5	42	47	34,86	20,6	12	28	19,25	30,1
Cycle duration (no. of days)	26	58	41,55	18,9	37	63	51,45	16,3	24	46	35,05	17,2

*1: Very vigorous to 9: Very little vigorous

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