Evaluation of new pasture legumes options in the Pampa-Patagonia transitional region of Argentina

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ABSTRACT

Biodiversity in forage legumes adapted to semiarid-arid conditions is scarce. In the Pampa-Patagonia transitional region of Argentina (PTRA), the annual rainfall is 440 mm with annual evapotranspiration of 1690 mm. Only *Vicia villosa* Roth shows agronomic adaptation to such an environment. Dry matter production of 19 selected legume species was compared to *V. villosa* (cv. Patagonia INTA) in the first year of establishment during 2015-to-2019. *Medicago truncatula* Gaertn. and *Trifolium subterraneum* L. (cv. Goulburn) showed high winter dry matter production, similar to *V. villosa*, but poor biomass production in spring. Spring dry matter contributed ≈ 80% of the total biomass per year. Preliminary results showed that *Onobrychis viciifolia* Scop. overcame the rest of the accessions evaluated, except for *V. villosa*. *O. viciifolia* could be considered a complementary species to *V. villosa*, a candidate for initiating a process of breeding as a new pasture legume for semiarid-arid environments. This is the first record of the potential use as a pasture of *O. viciifolia* in the PTRA.

Keywords: semiarid-arid regions; vetch; sainfoin.

RESUMEN

La biodiversidad de leguminosas forrajeras adaptadas a condiciones semiáridas-áridas es escasa. En la región de transición Pampa-Patagonia de Argentina (PTRA), la precipitación anual es de 440 mm con una evapotranspiración anual de 1690 mm. Solo Vicia villosa Roth muestra adaptación agronómica a dicho ambiente. Se comparó la producción de materia seca de 19 especies de leguminosas seleccionadas con V. villosa (cv. Patagonia INTA) en el primer año de establecimiento durante 2015-2019. Medicago truncatula Gaertn. y Trifolium subterraneum L. (cv. Goulburn) mostraron una alta producción de materia seca invernal, similar a la de V. villosa, pero una pobre producción de biomasa en primavera. La materia seca de primavera aportó ≈ 80% de la biomasa total por año. Los resultados preliminares mostraron que Onobrychis viciifolia Scop. superó al resto de las accesiones evaluadas, excepto a V. villosa. O. viciifolia podría considerarse una especie complementaria a V. villosa, candidata a iniciar un proceso de mejoramiento como nueva leguminosa de pastoreo para ambientes semiáridos-áridos. Este es el primer registro del uso potencial como forrajera de O. viciifolia en la PTRA.

***Palabras clave:** regiones semiáridas-áridas, vicia, esparceta.*

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INTRODUCTION

The semiarid-arid Pampa-Patagonia transitional region of Argentina (PTRA) encompasses nearly 18 million hectares. The predominant farming systems combine cereal cropping with cattle production. Annual precipitation (PP) varies between 370 to 590mm, with 67±13% of the precipitation distributed from autumn until spring, but high evapotranspiration (PET) especially during the summer months. During the spring-summer period, the aridity index (AI=PP/PET) is less than 0.2, corresponding to the arid climate type (UNEP 1993). The mean annual temperature is about 14.8ºC with an average minimum temperature during the winter of 7.9°C and a maximum of 22.4°C during summer. As in other semiarid-arid regions of the world, the soils of the PTRA present high risk of eolic erosion, compaction, as well as low fertility (Burkart *et al*., 1999; Silenzi *et al*., 2012). Additionally, this region receives low inputs of fertilization and control of weeds, insects, and diseases.

The diversity of native herbaceous legumes is low and perennial grasses dominate the grasslands of the PTRA (Busso, 1997). Naturalised populations of woolly burr medic (*Medicago minima* L.) show a notorious adaptation in about 10 million hectares (Fresnillo, 1990). However, this species shows fast senescence, low fodder production and very low drought tolerance (Covas, 1978). Moreover, traditional perennial pasture legumes, such as lucerne (*Medicago sativa* L.), white clover (*Trifolium repens* L.), and red clover (*Trifolium pratense* L.) show lack of adaptation to dry conditions and are not used in the PTRA (Iurman *et al*., 2008).

Scientific information about the benefits of legumes in cereal rotations is extensive. The Australian farming systems, known as ´ley farming´ and ´phase farming´, are based on legume crops. Australian ´ley farming´ practice, which stands for the inclusion of an annual pasture legume in a cereal crop rotation, is a well-known solution to revert environmental and economic problems derived from monoculture practices (Nichols *et al*., 2007, 2012). Traditionally, the most common species used for this purpose were subterranean clover (*Trifolium subterraneum* L.) and annual medics (*Medicago* spp.) (Loi *et al*., 2005).

The western Mediterranean-Asiatic region is a major area of legume biodiversity which includes a wide range of annual and perennial species, currently grown under semiarid-arid environments. Among the diverse range of adapted pasture legume species, vetches (*Vicia* spp.) and vetchlings (*Lathyrus* spp.) are considered the most agronomically important species. The *Onobrychis, Hedysarum* and *Ornithopus* genera have great potential as future pasture legumes (Hayot Carbonero *et al*., 2011; Ates *et al*., 2013).

Despite all the scientific advances generated for the conditions in Australia and the Mediterranean basin, no studies have yet been conducted in the PTRA. In this region, legume adaptation also presents some difficulties mainly associated with drought and high rainfall variability. *Vicia villosa* Roth is an annual legume species, well adapted to different semiarid-arid temperate areas of the world. It is considered as a reference legume in the PTRA due to its confirmed adaptation and a large number of references for its use and management (Renzi *et al*., 2017, 2019).

The current work aims to explore opportunities for developing new pasture legumes to complement *V. villosa* (Renzi and Cantamutto, 2013). A key requirement for farmers to adopt a new pasture is that it should present an acceptable biomass production during the first year of establishment. The objective of the present work was to assess the suitability of different legume species compared to *V. villosa* during the first year of establishment in the PTRA.

MATERIALS AND METHODS

Field experiment

Legume germplasm was provided by the Gene Bank of the Research Station Hilario Ascasubi (62º37'W, 39º23'S), located in the south of Buenos Aires province. Twenty-seven legume accessions were cultivated in the area of influence of the Research Station from 2015-2019. The selected legumes include 5 species of the genus *Medicago*, thirteen of *Trifolium*, and one of *Onobrychis*. All species showed antecedents of autumnal sowing and use as forage. Most of them are annual species, except for *T. fragiferum*, *T. hybridum*, *T. medium*, *T. montanum*, *T. ochroleucon*, *T. pannonicum*, and *O. viciifolia* (Kaiser, 2015). Within the evaluated accessions, the cultivars (cv) indicate a greater breeding effort compared to the rest of the accessions (table 1).

A field experiment was performed each year from 2015-2019 with similar sowing dates (10th of April 2015, 27th of April 2016, 11th of April 2017, 22th of March 2018, 5th of April 2019). The accessions evaluated, taxonomic names, common names, references for cultivars and sowing rates are listed in table 1.

The experiment was arranged in a randomized block design (n=3). The experimental units were 4x1.4m. A small plot cone seeder or row manual seeder was used for sowing at 20cm row spacing. During the growing season, observations of flowering time were recorded (2015, 2016, and 2019). Growing degree days (GDD; threshold temperature = 0°C) for the duration between sowing and flowering were estimated. A single cut per year of the forage dry matter (DM) production was measured at the end of winter (mid-September) and in late spring (November) by cutting plant shoots at ground level in 0.16 m^2 samples. The aboveground biomass harvested was oven-dried at 65°C for 72 hours.

The legume seed was inoculated with a commercially available rhizobial inoculum. Seed germination testing was previously carried out according to ISTA rules (International Seed Testing Association, 2019) to adjust the sowing rates (table 1). Fertilizers were avoided to exclude chemical interactions with bacterial activity. The seedbed was disked lightly and then levelled in the late summer before sowing the plots. Small grains were cultivated before establishing the field experiments with legumes.

The soil surface layer (0-20 cm) of the experimental sites were *entic haplustoll*, sandy loam, slightly alkaline (pH = 7.9±0.2), with a low organic matter content (1.7±0.5%) and a high P content (15.6±5.9 ppm P Bray & Kurtz). Weather data from each year were gathered from a meteorological station located less than 800 m from the experimental site (http://inta.gob.ar/documentos/informes-meteorologicos).

Statistical analysis

A linear mixed model (LMM) was performed to test if the accessions differed in the winter and spring DM production, and GDD. We considered the accession as fixed factors and the year as a random factor. The blocks were nested within the year. The LMM was performed with R version 3.6.3 using Infostat software (Di Rienzo *et al*., 2019) interface to R. The coeffi-

Species	Common name	Accession or cultivar	Seed weight (mg)	Sowing rate**	Inoculant group***	Year
Medicago lupulina L.	Black medic	SVKVEF-004	1.57±0.23	10	AM	15´; 19´
M. minima (L.) Bart.	Woolly burr medic	CZENIJ-104	1.20±0.04	10	AM	15´
M. rigidula L.	Tifton medic	SRBIST-104	4.45±0.13	10	AM	15´
M. polymorpha L.	Burr medic	cv. Scimitar	2.80±0.17	10	AM	15´; 16´; 17´; 18´; 19´
M. truncatula Gaertn.	Barrel medic	cv. Chetah	4.37±0.06	10	AM	15´; 16´; 17´; 18´; 19´
		cv. Jester	3.47±0.06	10	AM	15´; 16´; 17´; 18´; 19´
Onobrychis viciifolia Scop.	Sainfoin	SRBIST-059	20.8±1.02*	42	Special#	15´; 16´; 17´; 18´; 19´
		Advanced Line INTA (AD-INTA)	22.9±1.76*	40	Special#	16´; 17´; 18´; 19´
Trifolium ochroleucon Huds.	Sulphur clover	CZENIJ-112	2.17±0.15	12	B	15´
T. arvense L.	Rabbit-foot clover	SRBIST-033	0.47±0.06	6	B	15´
T. fragiferum L.	Strawberry clover	CZENIJ-111	1.77±0.21	12	B	15´
T. hybridum L.	Alsike clover	SRBIST-032	0.48±0.13	15	B	15´
T. michelianum Savi.	Balansa clover	cv. Taipan	1.10±0.10	6	C	15´; 16´
		cv. Viper	1.07±0.12	5	C	15´; 16´; 17´; 19´
		cv. Frontier	0.90±0.05	5	C	16´; 17´; 18´; 19´
T. medium L.	Zigzag clover	SRBIST-008	2.09±0.11	12	B	15´
T. montanum L.	Mountain clover	SVKVEF-016	0.68±0.06	10	B	15´
T. retusum L.	Teasel clover	SRBIST-106	0.13±0.06	5	B	15´
T. striatum L.	Knotted clover	SRBIST-101	2.13±0.15	10	B	15´
T. pannonicum L.	Hungarian clover	SRBIST-068	2.90±0.07	10	B	15´
T. resupiratum L.	Persian clover	cv. Nitro	0.57±0,06	10	C	15´; 16´; 17´; 18´
T. subterraneum L.	Subterranean clover	cv. Goulburn	4.90±0.36	15	C	15´; 16´; 17´; 18´
		cv. Riverina	4.97±0.15	17	C	15´; 16´
		cv. Urana	4.83±0.29	15	C	15´; 16´; 17´; 18´
		cv. Bindoon	4.77±0.21	15	C	15´; 16´; 17´; 18´
T. vesiculosum Savi.	Arrowleaf clover	cv. Zulú II	1.37±0.06	8	C	15´; 16´; 17´; 18´; 19´
Vicia villosa Roth	Hairy vetch	cv. Patagonia INTA	31.2±0.74	20	E	15´; 16´; 17´; 18´; 19´

Table 1. Sample size and serology results.
Analysis of the 3'UTR of *SLC11A1* bovine gene
*unhulled seed
** kg of clean, germinable seed ha^{-1}
***Inoculant group: AM: *Sinorhizobium medicae*, B and C: *Rhizobium leguminosarum* bv. *trifolii*, E: *R. leguminosarum* bv. *viciae*, and Special: *Rhizobium* sp.
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cient of variation (CV%) for each accession was calculated only in those that were evaluated for more than one year (table 1).

The relationship between spring DM and GDD was plotted using GraphPad Prism Software version 8.0 (GraphPad, San Diego, California, USA).

RESULTS

Pampa-Patagonia transitional region of Argentina displays strong climate variability with favourable periods for plant growth during autumn and spring. The annual precipitation from 2015-2017 was similar to the long-term mean (>400 mm), while <400 mm $year^{-1}$ was registered in 2018 and 2019 (table 2).

Medicago rigidula, *Trifolium arvense*, *T. montanum*, *T. retusum* and *T. striatum* L. failed to establish (death of seedlings) during 2015. Therefore, they were not evaluated in subsequent years. Similarly, *M. minima*, *T. ochroleucon, T. fragiferum, T. hybridum*, *T. medium* and *T. pannonicum* showed very low winter DM yield during 2015 (figure 1a), thus they were excluded from further evaluations.

Both winter and spring DM mean values are presented in figure 1a, showing a wide range of oscillation (0 to 2.1 t ha^{-1} and 0 to >6 t ha^{-1} during winter and spring seasons, respectively).

Month	Precipitation (mm)					PET (mm)	Temp. (°C)
	2015	2016	2017	2018	2019		
January	69	133	4	5	19	270±21	23.5±1.3
February	113	74	44	35	5	202±14	22.7±1.2
March	20	30	96	33	119	198±18	19.1±0.9
April	73	26	58	31	3	97±26	15.7±1.9
May	7	67	27	44	38	44±19	11.5±1.2
June	1	15	25	11	30	42±16	8.4±1.1
July	45	11	22	44	2	54±36	7.5±0.9
August	34	23	86	2	4	76±9	9.7±1.0
September	15	10	7	24	18	103±14	12.1±1.0
October	58	55	8	36	44	131±22	14.2±1.1
November	64	37	61	59	72	194±27	18.1±1.6
December	116	15	1	35	30	240±21	21.4±0.9
Annual	614	495	439	359	384	1652±72	15.4±0.6
Historic			446			1695	14.9

Table 2. Monthly precipitation (mm), average monthly potential evapotranspiration (PET, mm) and temperatures (°C) during the experiments in comparison to the historic average (1966-2018).

Interaction between accessions per year was observed both in winter ($F_{46,127}$ = 4.9; P<0.001) and spring DM ($F_{46,127}$ = 12.6; P<0.001). Figure 1b and c show the relationship between the coefficient of variation and winter and spring DM among accessions as measured of stability between years.

V. villosa (cv. Patagonia INTA) had a higher winter DM followed by *M. truncatula* (cv. Jester and Chetah) and *T. subterraneum* (cv. Goulburn) (figure 1b). Spring DM production contributed approximately 80% of the total DM per year under PTRA conditions. The best performance in spring DM were *V. villosa* (cv. Patagonia INTA), *O. viciifolia* (AD-INTA>ARHA-059), and *T. vesiculosum* (cv. Zulu II) (figure 1c). However, the latter showed low stability (> CV%) due to reducing the spring DM yield when annual rainfall was < 400 mm (figure 1c).

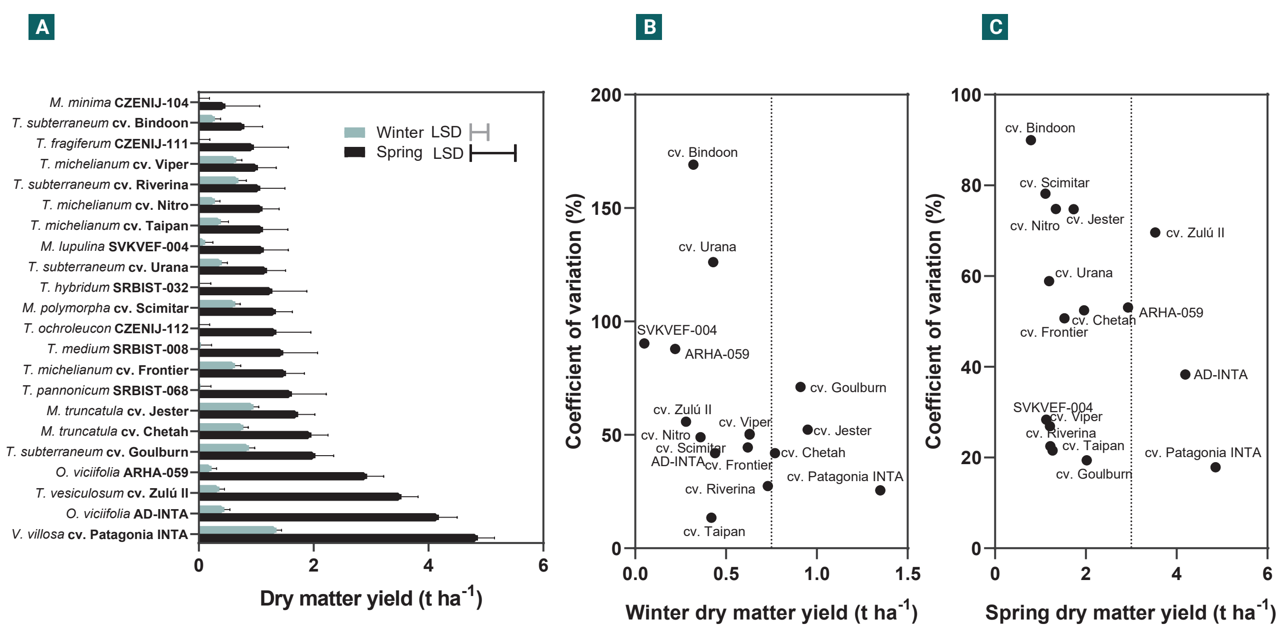

Figure 1. Winter and spring dry matter (DM) yield in different legume accessions (a), and relation to the coefficient of variance (%) (b and c). Least significant differences (LSD) (p <0.05) were used to indicate differences among accessions.

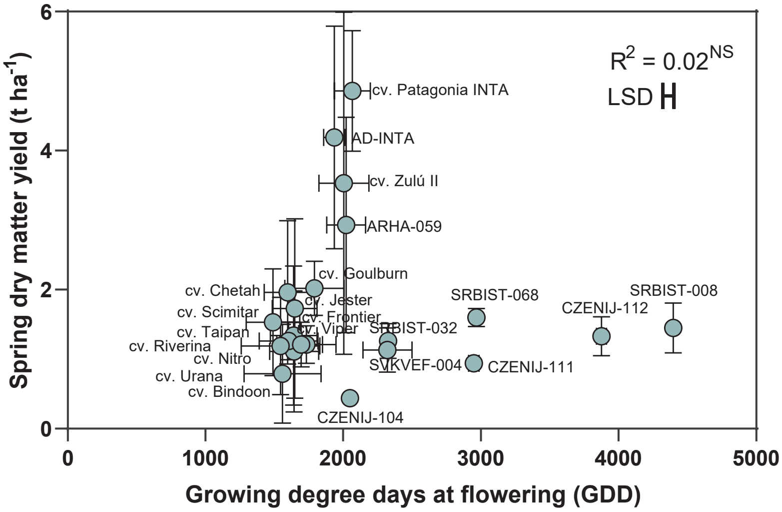

Figure 2. Spring dry matter (DM) yields as a function of growing degree days (above 0ºC) necessary to reach flowering. Least significant difference (LSD) (p<0.05) was used to indicate differences among accessions.

No significant relationship between flowering time (GDD) and spring DM was observed, although mid-maturing species, as the case of *V. villosa*, *T. vesiculosum*, and *O. viciifolia* (≈ 2000°Cd) showed higher spring DM production, compared to early-maturing (< 2000°Cd) and late-maturing accessions (> 2000°Cd) (figure 2).

DISCUSSION

This study explored opportunities for developing new pasture legumes to complement the *V. villosa* (Renzi *et al*., 2019). *M. truncatula* and *T. subterraneum* L. (cv. Goulburn) showed high winter dry matter production, similar to *V. villosa*, but a poor biomass production in spring. Spring dry matter contributed ≈80% of the total biomass per year. We found evidence that *O. viciifolia* performs well for spring DM production and moderate stability (CV%) during the establishment (figure 1c). *O. viciifolia* is a drought tolerant pasture legume with many positive agronomic, environmental, nutritional and nutraceutical attributes, and currently, there has been a resurgence of interest in it (Mora-Ortiz and Smith, 2018). Cultivation as a pasture legume is increasing in semiarid-arid environments, like the western United States (Hybner, 2013; Sintim *et al*., 2015) and northeast Spain (Delgado *et al*., 2002; Cirujeda *et al*., 2019). This situation supports the possibility of its development as a new pasture legume under PTRA conditions.

While in some legume accessions the DM production was very low, these experiments were valid for accession comparison but not for generalizing about the species performance as a whole. However, like in the dryland of West Asia and North Africa, it would seem that vetches would appear to have greater potential use than annual medicines (Ates *et al*., 2013; Cicek *et al*., 2020). *V. villosa* was the best species in DM production due to its breeding and development locally, and *O. viciifolia* could be a new option for PTRA.

A common outcome in this type of study is the need for earlier accessions for semiarid-arid Mediterranean climates, based on the fact that delaying flowering is counterproductive because it is exposed to terminal drought (Thomson *et al*., 1997; Berger *et al*., 2002). Moreover, in Mediterranean climates most rainfall occurs at the same time as the low evaporative demand, improving the rainfall-use efficiency and pasture growth (Cobon *et al*., 2019). Our results show that mid-maturing accessions present the best performance under PTRA conditions. The mid-maturing accessions would perform better than the early flowering types in low and unpredictable rainfall environments because they show a greater ability to modify growth according to the occurrence of rainfall events. The good adaptation of *V. villosa* does not correspond to a greater tolerance to drought periods, being higher in *O. viciifolia* (Schvachsa, 2018). In *V. villosa*, the shallow root system with good lateral branches as well as the mid-maturity cycle could take advantage of the low and occasional rain events (Renzi and Cantamutto, 2013). *V. villosa* is well adapted to PTRA farming systems, possibly due to different factors, such as an autumn–spring adjusted growing season, cold hardiness, a long vegetative phase that maximizes productivity, phenotypic plasticity with the ability to modify growth according to the occurrence of rainfall events, and excellent nodulation (Renzi *et al*., 2017). *O. viciifolia* shows good behaviour both in the spring DM production and tolerance to drought, with a higher survival rate under the evaluated conditions.

Based on a derivation from current experiments, *O. viciifolia* is included as a crop for persistence evaluation and on-farm weed control trials. We observed that *O. viciifolia* with a range of 0.9±0.3 to 5.8±2.0 t DM ha^{-1} $year^{-1}$, depending on rainfall occurrence (232-to-575 mm $year^{-1}$), could persist during more than three years under PTRA (unpublished data). As *O. viciifolia* has shown adaptation to dryland conditions, it is imperative to evaluate different genotypes for further use in breeding programs for developing cultivars. Also, annual pasture legumes, like *V. villosa*, could be sown in mixtures with deep-rooted *O. viciifolia* to improve the forage seasonal distribution, water use, and increase the resilience of pastures to environmental stresses (Dear 2003; Cicek *et al*., 2020). This study can be useful for a similar semiarid-arid environment considering also the predicted climate change scenarios.

CONCLUSIONS

This study explored the dry matter production of 19 selected legume species during the first year of establishment. Besides the well-known hairy vetch, we found evidence to show that *O. viciifolia* (sainfoin) demonstrated good performance for spring DM production and drought tolerance. This is the first exploratory study of the potential use of sainfoin as a pasture in the PTRA.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no competing interests

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in zenodo at https://doi.org/10.5281/zenodo.5363431.

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