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in

López-Francos A. (ed.), Jouven M. (ed.), Porqueddu C. (ed.), Ben Salem H. (ed.), Keli A. (ed.), Araba A. (ed.), Chentouf M. (ed.).
Efficiency and resilience of forage resources and small ruminant production to cope with global challenges in Mediterranean areas

Zaragoza : CIHEAM
Options Méditerranéennes : Série A. Séminaires Méditerranéens; n. 125

2021
pages 547-550

Influence of different *Rhizobium sullae* strains and soil fertility on the agronomic performance of Sulla (*Sulla coronaria* L.)

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Abstract. Sulla is widely used as a forage legume in several Mediterranean regions, however only a limited area of Sardinia is cultivated to Sulla. Its cultivation is manly confined to areas where its native specific rhizobium is available and soil pH is alkaline. Four farms were selected in the southern part of Sardinia with slightly acid soils and where the Sulla had never been sown. Intensive and large-scale trials were conducted to assess the adaptation of Sulla to different soil types and to assess the effectiveness of a range of elite Sulla rhizobial strains. The un-inoculated control treatments had significantly lower nodule numbers at all sites, indicating the presence of a very low Sulla rhizobial background and stressing the importance to inoculate Sulla in the region. Some of the paddocks had a challenging fertility for the growth of Sulla; however the use of an effective and robust rhizobial strain such us WSM1592 and 79N2 was of paramount importance to successful growth the species.

Keywords. Forage species – Legume – Dairy sheep – Mediterranean environment.

***Influence de différentes souches de* Rhizobium sullae *et de la fertilité du sol sur les performances agronomiques de Sulla* (Sulla coronaria *L.*)**

Résumé. *Sulla est largement utilisé comme légumineuse fourragère dans plusieurs régions méditerranéennes, mais seule une zone limitée de la Sardaigne est cultivée en Sulla. Sa culture est confinée à des zones où son rhizobium natif spécifique est disponible et où le pH du sol est alcalin. Quatre fermes ont été sélectionnées dans le sud de la Sardaigne avec des sols légèrement acides et où Sulla n'avait jamais été semé. Des essais intensifs et à grande échelle ont été menés pour évaluer l'adaptation de Sulla à différents types de sol et pour évaluer l'efficacité de toute une gamme de souches rhizobiennes d'élite. Les traitements témoins non inoculés présentaient un nombre de nodules significativement plus faible sur tous les sites, ce qui indique la présence d'un très faible précédent milieu rhizobien de Sulla et souligne l'importance d'inoculer Sulla dans la région. Certaines des prairies présentaient une fertilité difficile pour la croissance de Sulla; cependant, l'utilisation d'une souche rhizobienne efficace et robuste, telle que WSM1592 et 79N2, était d'une importance primordiale pour la réussite de la croissance de l'espèce.*

Mots-clés. *Espèces fourragères – Légumineuses – Brebis laitières – Environnement méditerranéen.*

I – Introduction

Sulla (*Sulla coronaria* L.) is a short-lived perennial, deep-rooted pasture legume that is grown throughout Mediterranean countries where it is fed green, used for hay or as silage (Foote, 1988). The microsymbionts of Sulla display a high level of specificity for nodulation and nitrogen fixation (Yates *et al*., 2015). When effectively nodulated, Sulla plants can fix large amounts of nitrogen and produce adequate green biomass (Sulas *et al*., 2009). It is commonly found in clay calcareous soils with alkaline pH and sometimes in slightly acidic soils. Sulla is widely used as a forage legume in several Mediterranean regions, however only a limited area of Sardinia is cultivated with Sulla. Its cultivation is manly confined to areas where its native specific rhizobium is available and soil pH is alkaline. However, the results from recent research showed that seed inoculation can successfully extend Sulla cultivation in soils not traditionally recommended (Sitzia *et al.,* 2018). The unsuccessful cultivation of Sulla on these latter soils was likely due to the use of poor inoculation methods and/or the presence of competitive and persistent, yet ineffective, rhizobial populations. Root-nodule bacteria isolated from nodules are commonly evaluated for traits such as plant infection, optimum N fixation, acid tolerance, persistence, broad spectrum compatibility within host species and competitiveness with an established background population of rhizobia (Howieson *et al.,* 1999). The focus of selection for one or more of these traits is related to the systems and environments where the strains are likely to be used. The standard process involves screening experiments in controlled environments and under field conditions. The aim of this research was therefore to investigate the effectiveness of several rhizobia isolated from Sulla, i.e. different *Rhizobium sullae* strains, under different edaphic conditions and to study the suitability of Sulla to Sardinian soils where traditionally it is not grown by farmers.

II – Materials and methods

Four dairy sheep farms, located in the Southern part of Sardinia, were selected to grow Sulla (note that Sulla was never cultivated in these farms before). Before sowing Sulla, soil pH, organic matter content total N (Kjeldahl method), available P (Olsen method) and exchangeable K were determined as described in Margesin and Schinner (2005). Soil texture was also investigated. For soil physico-chemical analyses, 10 soil samples (0-20 cm depth, approx. 1 kg each) were randomly collected from each plot, pooled together and sieved to <2mm in the laboratory to obtain a bulk sample. Triplicate soil samples from this latter were then used for each physico-chemical determination. Two experiments were carried out at each farm.

Sulla was inoculated in both experiments few hours before sowing with selected *R. sullae* strains using peat inoculants. Two rhizobial strains, i.e. 79N2 and 852N3, and the Australian commercial strain WSM1592 (Yates *et al*., 2015) were separately used for seed inoculation. Peat slurries were prepared by mixing the peat (containing the selected rhizobia) with the seed coating adhesive (250 g of peat inoculant, 1000 ml adhesive) and used to inoculate 30 kg of Sulla seeds. These were mixed with the slurry and then pelleted with fine lime. Uninoculated seeds were also included in both experiments.

Experiment I: the infectiveness of the three *R. sullae* strains was addressed during spring 2018 by assessing the nodulation numbers on plants grown in each farm. The nodulation assessment was carried out by assessing individual Sulla plants sampled from 1.5 m long rows organized in a randomized block design that included 3 rhizobial strains and an uninoculated control. Five months after seeding, a total of 40 plants (10 x replicate) per treatment were removed, and roots carefully washed. The total number of nodules was then recorded.

Experiment II: each farm paddock, was divided into 4 plots of 2500 m^2 each. Plots were sown in autumn 2017 with 30 kg ha^{-1} of commercial seeds (cv. Bellante). Plant establishment (i.e. plant number) was recorded approximately 45 days after seeding in 30 quadrats (900 cm^2 each) randomly positioned in each plot. In spring 2018, biomass production was measured by cutting 4 quadrats (0.5 m^2 each) per plot. Botanical composition was recorded separating Sulla from the weed species, then the Sulla component was dried at 60 °C and weighted. The plots during the year were grazed by dairy Sarda ewes when the sward reached 10 cm height.

Data were analyzed by GLM model. Differences between treatments were assessed by Tukey T tests ($P < 0.05$, SAS Institute, 2002).

III – Results and discussion

Three of the four sites (A, F and P) were slightly acidic with pH ranging from 5.9 to 6.8. Total amount of N for the four sites was over 1 mg/kg of soil, with the exception of site F that recorded 0.45 mg/kg of soil. The P content was suitable at sites C and P, although at sites A and F it was far below the critical levels for pastures. The organic matter content was similar for the four sites and ranged from 1.87 and 2.61% (Table 1).

Table 1. Soil characteristics at 0-10 cm depth for the 4 experimental sites

Site	GPS coordinate	Soil type[2]	pH	N g/kg	P mg/kg	K mg/kg	Organic g/kg	Matter C/N
A (Santadi)	39°03'N/8°43 'E	CL	5.9	1.37	1.97	96	2.61	19.63
C (Iglesias)	39°16'N/8°34'E	SCL	7.5	1.18	9.48	117	2.28	19.19
F (Flumentepido)	39°10'N/8°27'E	SCL	6.3	0.45	3.23	146	1.87	41.74
P (Iglesias)	39°18'N/8°34'E	SCL	6.8	1.20	13.02	132	2.38	19.99
w α=0.051			0.2	0.11	2.81	4.75	0.20	2.06

[1]Tukey's Significant Difference. [2]CL: clay loam; SCL: sandy clay loam.

During the trial, total annual rainfall was similar to the climatological value, but an erratic distribution was detected in all sites. In particular, a dry autumn was followed by an unusually wet spring (rainfall climatological value of May were 38.4, 41.6 and 42.8 in A, C and P, and F site, respectively; Table 2).

Table 2. Monthly rainfall (mm) during the experimental period (data from ARPAS Sardegna) in 2017

Site	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total
A	18.6	21.2	59.6	58.8	24.8	54.8	127.6	30.8	199.4	55.8	0	651.4
C	22.6	3.6	72	160.4	28.6	119	206.6	47.2	158.2	54.4	0	872.6
F	20.4	10	64	81.4	24.4	81.8	153	38.6	169	22.4	0	665
P	22.6	3.6	72	160.4	28.6	119	206.6	47.2	158.2	54.4	0	872.6

The experiment I clearly highlighted the importance of *R. sullae* on plant nodulation and growth (not shown). The uninoculated control had significantly lower nodule numbers at all sites, particularly when compared to strain WSM1592 (Table 3). The lowest nodule numbers were recorded at site A which had the lowest pH and P content.

Table 3. Total nodule numbers on Sulla plants inoculated with different *R. sullae* strains and on Sulla uninoculated plants at four Sardinian sites (A, C, F and P)

Site	WSM1592	79N2	852N3	Uninoculated
A	2.28^{A}	0.28^{B}	0.28^{B}	0.28^{B}
C	6.34^{A}	4.13^{B}	3.90^{B}	1.07^{C}
F	3.07^{A}	2.40^{AB}	1.50^{B}	0.03^{C}
P	4.30^{A}	1.58^{B}	0.47^{B}	0.32^{B}

$P<0.05$ between treatments.

Similar results were observed at the plot scale (experiment II). Plant establishment ranged between 117(±10.24)/m^2 at F site to 221(±15.89)/m^2 at site A. The dry matter yield of the uninoculated treatments was always lower, and sometime negligible such as at P and F sites, compared to inoculated ones (Fig. 1). Overall, WSM1592 and 79N2 were the most effective strains allowing Sulla to produce adequate amount of biomass across three sites (A, C and P). The dry matter production at site F was the poorest and this was probably due to a poor strain adaptation to soil conditions (low P and pH).

It is important to note that the lowest nodulation score recorded for the three strains at site A (Table 3), had no effect on the dry matter production as much as for site F. Early nodulation sampling and slow formation of the nodules in low fertility soil may be the reason for this discrepancy. However, the sole nodulation score can be misleading when assessing the symbiotic performance of a rhizobial strain in the field. A parallel assessment of plant yield should be always recommended to investigate the suitability of plant-rhizobial combinations in different pedo-climatic conditions.

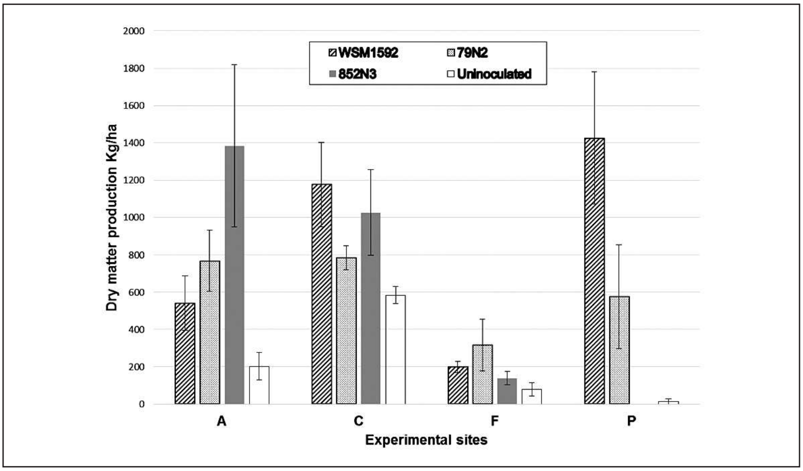

Fig. 1. Pasture dry matter production of Sulla inoculated with different *R. sullae* strains and of Sulla uninoculated at four Sardinian sites (A,C,F and P).

IV – Conclusions

The presence of *R. sullae* is fundamental for Sulla establishment where it was never cultivated before. The use of effective rhizobial strains such as WSM1592 or 79N2 can allow the successful growth of Sulla in these latter soils, unless the minimum requirements of soil fertility are respected.

Acknowledgments

The authors are grateful to A. Pintore, S. Mastinu and S. Fancellu for their technical contribution. This work was funded by the Regione Sardegna “Risorse microbiche del Sulcis Iglesiente per l’innovazione dei sistemi agropastorali” delibera CIPE n:31 del 20.02.

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