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Bio-agronomic traits of *Psoralea bituminosa* and *P. morisiana* accessions collected in Sardinia

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Abstract. *Psoralea* genus (Leguminosae) includes some species with promising features as pasture legume in Mediterranean areas prone to drought. Eight Sardinian and 7 Spanish accessions of *P. bituminosa* were evaluated during 2009-2011. Forage production and its furocoumarin content, seed yield and mass were observed. The total forage yield ranged from 68.4 g DM plant^{-1} in Loculi to 252.6 g DM plant^{-1} in Albo-H-TolFrio, with the accessions showing differences in the seasonal production also. The 1000 seed weight varied from 15 g in Llano del Beal to 35 g in Monte Gonareddu, while Llano del Beal and Monte Rosello showed the highest seed yield (about 30 g pl^{-1} year^{-1}). A high variability was observed for furocoumarin content, too. These preliminary results are very encouraging in view of the valorisation of *Psoralea* as perennial forage legume for marginal rainfed areas.

Keywords. *Psoralea* – Seed characteristics – Forage production – Furocoumarin content.

Evaluation bio-agronomique des accessions de* Psoralea *collectées en Sardaigne

Résumé. *Le genre* Psoralea *comprend des espèces avec des caractéristiques prometteuses comme légumineuses de pâturage dans les zones méditerranéennes exposées à la sécheresse. Huit accessions de Sardaigne et 7 accessions espagnoles de* P. bituminosa *ont été évalués au cours des années 2009-2011. La production de fourrage et de son contenu de furocoumarine, le rendement grain et le poids de mille grains ont été observés. La production total variait de 68,4 g MS par plante en Loculi à 252,6 g MS par plante, pour Albo-H-TolFrio; aussi les accessions ont présentes des différences en termes de production saisonnière. Le poids de mille grains variait de 15 g en Llano del Beal à 35 g pour Monte Gonareddu. Le rendement grain était le plus élevé pour Llano del Beal et Monte Rosello (environ 30 g per plant). Une grande variabilité a été aussi observée pour le contenu des furocoumarines. Ces résultats préliminaires sont très encourageants en vue de la valorisation de le genre* Psoralea *comme légumineuse fourragère pérenne pour les zones marginales pas irriguées.*

Mots-clés. Psoralea *– Caractéristiques des semences – Production de fourrage – Contenu des furocoumarin.*

I – Introduction

Psoralea is a legume that grows in Mediterranean basin and Macaronesia and has a potential as pasture fodder for ruminants in semiarid Mediterranean environments but only few selected ecotypes are cultivated in Canary Islands and Morocco (Muñoz *et al*., 2000). In Sardinia Island two species of *Psoralea* can be found in natural stands: *P. bituminosa* (L.) Stirt. and the endemic *P. morisiana* Pignatti et Metlesics. They can be easily distinguished on the basis of some morphological traits and for the presence of a typical bitumen smell in *P. bituminosa* (Camarda, 1981). Both species have an appreciable content of furocoumarins, compounds that have been related to a low palatability of fresh fodder (Pecetti *et al*., 2007) but that can be used in PUVA therapy (Viola *et al*., 2009). In this work we describe a part of a larger screening done among Sardinian populations of *P. morisiana* and *P. bituminosa* and Spanish accessions of *P. bituminosa,* in order to discriminate within populations with low furocoumarin content useful for fodder production in Mediterranean environments or for furocoumarin extraction and pharmaceutical utilisation.

II – Materials and methods

Plant establishment. Five accessions of *P. morisiana* (Monte Gonareddu, Punta Giglio, Siliqua, Bitti, Burcei) and three accessions of *P. bituminosa* (loculi, monte Rosello, Siniscola) collected in Sardinia during 2009 were evaluated in an experimental field at the Centre for the Conservation and Valorisation of Plant Biodiversity, Alghero, Italy (40° 35' N, 8° 22' E) together with 7 accessions of *P. bituminosa* belonging to var. *albomarginata* (AlboH-TolFrio, Famara), var. *crassiuscula* (Crassiuscula, Vilaflor) and var. *bituminosa* (Calnegre, Llano del Beal, Tenerife) from Canary Islands and Spain. The site has a Mediterranean climate, with an average annual rainfall of 540 mm and a dry season during summer. The soil is alluvial and calcareous (pH 6.9). The experiment was carried out in plots with 12 plants per accession in a completely randomized design with three replicates. Plants were grown from scarified seeds sown in jiffy pots in a greenhouse then transplanted in field in February 2010. Fertilization was done before planting with 46 kg ha^{-1} of P_2O_5. Occasional irrigation was supplied to plants when necessary from late spring to early summer in the first year. The following observations and analysis were done:

Phenology. Plants were observed weekly at the beginning of the flowering, 50% flowering, 100% flowering, end of flowering, first ripe fruit, plant senescence and re-flowering stages.

Seed yield and 1000 seed weight. Seeds were collected weekly during ripening in 2010 and 2011. Seed production was observed on 3 plants per plot. The 1000 seed weight was calculated from the average weight of subsamples of 100 seeds (4 replications).

Forage production. Forage samples were collected in October 2010, December 2010, March 2011 and October 2011 from 3 plants per plot, when plant height exceeded 15 cm. Plant samples were oven-dried at 60 °C until a constant weight was achieved.

Extraction and determination of furocoumarins. In October 2011, fresh leaves (100 g) were extracted by maceration using cold MeOH/HCl 2M (800 ml) and kept under stirring for 20 hours at room temperature. The extracts were filtered and concentrated under vacuum at a temperature below 50°C in a rotary evaporator, then dissolved in distilled H_2O (250 ml) and the extraction repeated three times with 45 ml of $CHCl_3$. The organic extract was concentrated under vacuum at 30°C and the residue used for the furocoumarin analysis. Furocoumarins were analysed by a GC (Hewlett Packard 5970) equipped with a ZB5 column (Phenomenex, 60 m, 0,25 m) coupled with a MS instrument (Hewlett Packard GMD) and quantified by the Standard Addiction Method. All analysis were repeated three times.

Except for furocoumarin content, all data were subjected to ANOVA and mean values separated by LSD test. Statistical analysis were performed with the software package R.

III – Results and discussion

Flowering, seed weight and seed production. Sardinian accessions showed an earlier flowering than Spanish accessions, especially in 2010 (Table 1). Flowering was earlier and shorter for almost all populations during 2011, when plants were totally under rainfed conditions. The 1000 seed weight ranged between 15.0 g and 35.1 g and no statistical differences were found between year 2010 and year 2011. Seed production varied from 0.2 g pl^{-1} to 33.9 g pl^{-1}. Sardinian populations showed a very low production of seeds in the first year, except for Monte Rosello. In the second year, however, the seed production of the Sardinian accessions Bitti, Loculi, Monte Rosello, Siliqua and Siniscola attained the most productive Spanish accession, Llano del Beal.

Famara did not flower in the first year and the seed yield was negligible in the second year also; on the contrary, Calnegre produced a low amount of seed only in the first year.

No statistical differences were found for seed production between *P. morisiana* and *P. bituminosa* accessions, but within *P. bituminaria* some differences were found for var. *bituminosa* vs *crassiuscula* and *albomarginata*, showing a seed mean production of 16.1, 7.9 and 4.2 g pl^{-1} respectively.

Table 1. Day of first flower appearance (DoY), flowering duration (days), 1000 seed weight (g) and seed production (g pl^{-1}) in 15 accessions of *P. bituminosa* and *P. morisiana*

Species/ Variety	Population	Start of flowering (DoY)		Flowering duration (days)		1000 seed weight (g)		Seed production (g pl^{-1})	
		2010	2011	2010	2011	2010	2011	2010	2011
Albomarginata	Albo-H-TolFrio	185^{cde}	132^{ef}	48	59	24.6^{bc}	26.8^{b}	2.0^{d}	13.9^{b-e}
	Famara	–	175^{a}	–	14	–	17.7^{fgh}	–	1.1^{e}
Crassiuscula	Crassiuscula	196^{bc}	147^{cd}	56	44	23.2^{bc}	24.8^{bc}	4.3^{d}	6.1^{de}
	Vilaflor	190^{bcd}	150^{c}	69	44	24.3^{bc}	25.3^{bc}	14.6^{bc}	6.7^{de}
Bituminosa	Calnegre	229^{a}	–	31	–	16.2^{ef}	–	0.2^{d}	–
	Llano del Beal	156^{fg}	138^{de}	84	63	15.0^{f}	15.4^{h}	33.9^{a}	32.0^{a}
	Tenerife	210^{ab}	161^{b}	42	18	22.6^{cd}	20.4^{def}	1.1^{d}	0.6^{e}
	Loculi	126^{h}	116^{g}	98	63	16.3^{ef}	16.4^{gh}	4.0^{d}	28.2^{ab}
	Monte Rosello	153^{g}	132^{ef}	60	59	24.4^{bc}	20.6^{def}	28.0^{a}	24.6^{ab}
	Siniscola	130^{h}	125^{fg}	87	63	19.1^{de}	18.0^{e-h}	16.6^{b}	32.0^{a}
Morisiana	M. te Gonareddu	157^{fg}	137^{de}	90	56	35.1^{a}	32.2^{a}	2.7^{d}	8.7^{cde}
	Punta Giglio	170^{d-g}	146^{cd}	119	69	23.6^{bc}	21.1^{de}	16.7^{b}	14.3^{b-e}
	Siliqua	177^{c-f}	145^{cd}	78	83	26.6^{b}	23.0^{cd}	5.9^{cd}	17.8^{a-d}
	Bitti	165^{efg}	131^{ef}	77	84	26.9^{b}	23.2^{cd}	6.5^{bcd}	23.8^{abc}
	Burcei	154^{g}	144^{cd}	88	73	21.4^{cd}	19.1^{efg}	5.7^{cd}	13.0^{b-e}

Different letters in the same column indicate values statistically different at $p \leq 0.05$. Dates were expressed using the sequential Day of the Year (DoY), starting with day 1 on January 1st.

Forage production and furocoumarin content. The total forage yield per plant ranged from 68.4 g in Loculi to 252.6 g in Albo-H-TolFrio. The Sardinian accessions Monte Gonareddu, Bitti, Punta Giglio, Siliqua, and Siniscola produced a total forage yield comparable to the best Spanish accessions (Table 2). A marked seasonality in forage production was observed. Most of Spanish accessions produced a good amount of forage in autumn; on the contrary, most of Sardinian accessions produced new sprouts in spring. Calnegre, after a respectable forage production in December 2010, suffered for winter cold and showed a high plant mortality.

In the early autumn cut (Oct 2011), several accessions showed a high furocoumarin content, in particular three Sardinian population (*P. morisiana*: Monte Gonareddu, Punta Giglio, *P. bituminosa*: Monte Rosello). All accessions showed a higher angelicin than psoralen content, except for Monte Gonareddu, where psoralen prevailed. In general, Sardinian accessions of *P. morisiana* showed a low content of psoralen, as well as the Spanish accessions of *P. bituminosa* belonging to the var. *albomarginata* and *crassiuscula*. These results are similar to those reported by Pecetti *et al*. (2007) for *P. bituminosa* germplasm of Central Italy, where environmental conditions are less stressful than in Southern Italy. Among *P. bituminosa*, Monte Rosello showed the highest content of angelicin. It is noteworthy that angelicin is studied in a new application for the treatment of thalassemia (Lampronti *et al*., 2003).

Table 2. Dry matter yield (g DM pl^{-1}) and furocoumarin content (mg kg^{-1} leaf FW) in the various Sardinian accessions of *P. bituminosa* and *P. morisiana* and Spanish accessions of *P. bituminosa*

Species/ Variety	Accession	Yield (g DM pl^{-1})					Furocoumarins (mg kg^{-1} leaf FW)	
		Oct 2010	Dec 2010	Mar 2011	Oct 2011	Total yield	Oct 2011	
							Ang	Pso
Albomarginata	Albo-H-TolFrio	81.8^{ab}	77.1^{ab}	–	93.6^{a}	252.6^{a}	202.7	57.2
	Famara	18.8^{ef}	–	34.4^{de}	22.1^{bc}	75.3^{d}	88.4	29.0
Crassiuscula	Crassiuscula	74.0$^{a\text{-}d}$	78.2^{ab}	–	54.0^{ab}	206.2^{ab}	65.1	39.5
	Vilaflor	80.8^{ab}	46.7^{bc}	–	58.3^{ab}	185.8^{ab}	44.6	44.6
Bituminosa	Calnegre	109.2^{a}	93.8^{a}	–	3.3^{c}	206.3^{ab}	288.8	48.7
	Llano del Beal	63.9$^{a\text{-}e}$	–	51.9^{cde}	66.9^{ab}	182.7^{abc}	142.2	90.7
	Tenerife	50.7$^{b\text{-}f}$	55.7^{bc}	–	21.8^{bc}	128.1^{bcd}	70.4	37.3
	Loculi	10.4^{f}	–	25.7^{e}	32.3^{bc}	68.4^{d}	127.5	70.0
	Monte Rosello	76.1^{abc}	39.2^{c}	–	57.3^{ab}	172.5$^{a\text{-}d}$	751.8	108.7
	Siniscola	37.1$^{b\text{-}f}$	–	60.3^{cd}	50.2^{abc}	147.6$^{a\text{-}d}$	245.7	54.6
Morisiana	Monte Gonareddu	32.1$^{c\text{-}f}$	–	97.9^{a}	42.8^{bc}	172.9$^{a\text{-}d}$	154.3	339.4
	Punta Giglio	40.1$^{b\text{-}f}$	–	70.7^{bc}	39.4^{bc}	150.2$^{a\text{-}d}$	244.2	116.1
	Siliqua	36.4$^{b\text{-}f}$	–	90.0 ^{ab}	57.4^{ab}	184.8^{ab}	195.0	89.5
	Bitti	30.3^{def}	31.0^{c}	–	69.8^{ab}	131.1^{bcd}	105.7	13.7
	Burcei	21.3^{ef}	–	33.3^{e}	22.3^{bc}	76.9^{cd}	55.0	00.0

Ang = angelicin; Pso = psoralen. Different letters in the same column indicate values statistically different at $p \leq 0.05$.

IV – Conclusions

An interesting variability among the accessions of *P. morisiana* and *P. bituminosa* was found. Some of them showed an interesting seasonal distribution of forage production, especially in early autumn. These preliminary results are very encouraging in the view of the valorisation of *Psoralea* as perennial forage legume for marginal rainfed areas. Growing *Psoralea* in permanent stands may provide alternative sources of natural fodder protein, especially in late spring, early summer or early autumn, reducing costs at farm level for supplements, and also in terms of fertilizers and soil tillage. Some of the studied varieties showed interesting amounts of furocumarins, therefore it is possible consider them as a valuable sources of bioactive compounds to be used in different therapies. On the other hand, the effect of furocoumarins on animal health must be better investigated as well as their content in conserved forages.

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