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Seed production of *Bituminaria bituminosa*: Size, production, retention and germination capacity of the legumes

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SUMMARY – *Bituminaria bituminosa*, a Mediterranean and Macaronesian pasture legume, presents a large genetic diversity, with 3 botanical varieties (*bituminosa*, *albomarginata* and *crassiuscula*). A breeding and screening process started to recombine desirable traits currently dispersed in pre-selected populations and natural hybrids of the three before-mentioned varieties. Additionally, the domestication of *B. bituminosa* as a new pasture legume will require producing seed at the lowest cost. To achieve this objective, it is necessary to search for plant diversity in relation to seed production, fruit retention and germination capacity of the harvested seeds. Preliminary data showed that *B. bituminosa* produces large quantities of seeds, but in general, they shed easily and present low germination capacity (about 20% without scarification). However, selected accessions such as Calnegre, Tahonilla and Cañadas Teide, present good seed retention (50-70% after 50 days of fruit ripening) and germination values improved to 80% when scarified.

Keywords: *Bituminaria bituminosa*, seed production, legume retention, germination.

RESUME – *"Production de graines chez Bituminaria bituminosa : taille, production, rétention et capacité germinative de ces légumineuses".* Bituminaria bituminosa *est une légumineuse fourragère de la région méditerranéenne et de la Macaronésie, qui présente une grande diversité génétique avec 3 variétés botaniques (*bituminosa, albomarginata *et* crassiuscula*). Des recherches ont commencé dans le but d'améliorer et de recombiner les caractères désirables actuellement dispersés dans les populations et hybrides naturels des trois variétés citées. En plus, la domestication de* B. bituminosa *comme nouvelle légumineuse de parcours dépend de la production de semences à bas prix. Pour atteindre cet objectif, il est nécessaire de rechercher la diversité génétique en relation avec la production et la rétention des graines ainsi que la germination des semences. Des résultats préliminaires ont démontré que* B. bituminosa *produit une grande quantité de graines, mais en général, elles restent peu de temps dans les gousses, dégrainant très facilement, et les semences présentent un faible taux de germination (20%). Toutefois, certaines populations sélectionnées comme Calnegre, Tahonilla et Cañadas Teide, retiennent 50-70% de leurs graines après 50 jours de maturité, et les taux de germination peuvent atteindre 80% quand les graines sont scarifiées.*

Mots-clés : Bituminaria bituminosa*, production de semences, rétention des graines, germination.*

Introduction

Bituminaria bituminosa (L.) Stirt., is a pasture legume with a large geographical distribution in the Mediterranean basin and Macaronesian islands. In the Canary Islands the species presents a large diversity with 3 botanical varieties found in habitats ranging from the coastal semiarid areas on Lanzarote Island with an annual rainfall of 150-300 mm (var. *albomarginata*) to the high elevation subhumid area (1700-2200 m, 500 mm) of Tenerife (var. *crassiuscula*) (Méndez *et al*., 1990). The third var. *bituminosa* has a wide adaptation across the Canary Islands (300-1000 mm) and is the only one present in the Mediterranean basin. In the Iberian península it is found in environments ranging from 250-1000 mm of rainfall and up to 1250-1500 m of altitude. Currently it is used as hay for milking goats in the Canary Islands and grazed as a native component in Galilea scrubland, Israel. Recent research indicates it has potential as a pasture legume for low-rainfall Mediterranean environments.

The self-pollinated breeding system of *B.bituminosa* has been confirmed by Juan *et al*. (2003, 2004). Accessions of *B. bituminosa* from the three varieties set seed inside insect-proof cages; however, the presence of insect pollination improved seed production. Pods (legumes) contain only one seed (Coca *et al*., 2004).

During the last decade several Mediterranean teams (Spain, Italy, Israel, Greece and Australia) have been working with *B. bituminosa* and some already cooperate to accelerate the breeding process. A key issue to develop any cultivar of *B. bituminosa* is to evaluate and select accessions with a large seed production and a good germination capacity, so that seed production could be developed at commercial scale and sowing *B. bituminosa* could be done at low cost. In this paper we present preliminary results showing enough genetic variability to select lines with high seed production, seed retention and seed germination.

Materials and methods

Pod size. The pod weight was calculated from data of years 2000, 2003 and 2007; 10 Spanish populations; 2 reps of 100 seeds/population; weight of pods dried at 30ºC; pods harvested from plots cultivated at La Alberca, Murcia, but originally collected in the Canary Islands and peninsular Spain. The seed and beak size were calculated from photos of legumes on a millimetre paper background (Fig. 1).

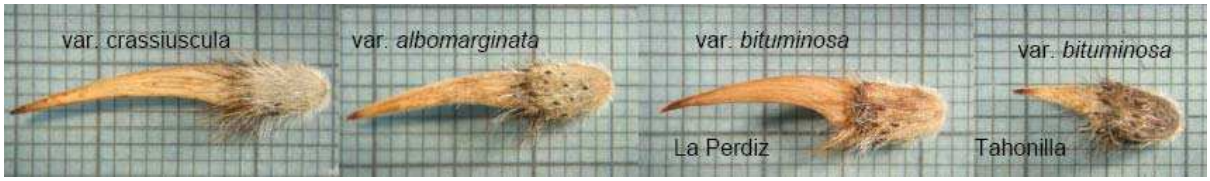

Fig. 1. Photos of pods (legumes) of the three varieties of *Bituminaria bituminosa*.

Seed production. Plants cultivated at La Alberca, Murcia; years 2003, 2004, 2006 and 2007; seed harvested in g/plant; mean, range and maximum values for 3 varieties and 10 populations. A total of 267 plants were harvested during the 4 years. Part of the seed produced fall down before harvesting; hence, data obtained was an underestimation of the total seed produced.

Estimation of flower heads/plant. The number of flower heads was counted on individual plants from six populations, as well as the flower number per head in 3 heads/plant.

Seed retention. Data spring 2007; La Alberca, Murcia; period 16th April to 1st August; 10 populations of the 3 varieties; 5 populations from the Canary Islands (Boca de Tauce, Famara, Vinamar, Tahonilla, Guimar), 3 from peninsular Spain (Calnegre, Mijas, Llano del Beal) and 2 from other Mediterranean countries (Cerdeña, Galilea); 3 flower heads per population; counts every two weeks of: (i) number of flowers; (ii) green pods; and (iii) dry mature pods retained.

Seed germination. Data Dec 2004 (Western Australia); 10 populations; germination in growth chamber; 400 seeds/population; 100 seeds/treatment; 2 reps 50 seeds/treatment; 4 treatments; a 2 x 2 experiment (scarified *vs* non scarified seed) x (constant temperature 25ºC *vs* alternate temp. 15ºC/25ºC 16/8 h); seeds scarified rotating against a cylinder covered with sand paper during a fixed time, adjusted previously depending on seed size; alfalfa seeds were used as control; seeds were placed between two layers of saturated moist filter paper and within plastic bags (ISTA, 2004).

Results and discussion

Pod size. The mean pod weight for the 10 populations was 2.6 g/100 pods (Table 1). The largest pods corresponded to populations of var. *crassiuscula* 3 g/100, partly due to its large seed beak (3 times the seed´s length; seed 0.5 cm, beak 1.5 cm). Populations of var. *albomarginata* and *bituminosa* had a similar mean weight (2.5 g/100), but it was more variable in var.*bituminosa* (1.7-2.9 g/100), which has a beak (0.6-1.3 cm) about 2 times longer than the seed (0.5 cm).

Table 1. Mean values of seed weight and seed harvested from the 3 varieties of *B. bituminosa*

Variety	Number of populations	Weight (g) 100 seeds			Seeds harvested (g/plant)		
		Mean	Range	n*	Mean	Maximum	n**
Crasiusscula	2	3.00	2.8-3.1	8	38	190	34
Albomarginata	3	2.47	2.1-2.7	20	50	175	98
Bituminosa	5	2.45	2.0-2.9	40	43	127	135
Mean values		2.64			44		

n* number data evaluated (populations, years, replications); n** number of individual plants harvested.

Seed production. The mean seed production for the 10 Spanish populations was 44 g/plant, ranging from 4-190 g/plant. Populations from variety *albomarginata* (50 g/plant) produced more seed than the other two varieties (38-43 g/plant) (Table 1). In general, Canarian populations produced more seed than peninsular ones. Tenerife cv., Vinamar, Tahonilla and Teno were on a mean basis, the most productive populations (50-71 g/plant); however, at the individual plant level, the maximum values were obtained for selected plants of populations Cañadas del Teide (190 g/plant), Famara (175 g/plant) and Calnegre (127 g/plant).

In general, Canarian accessions flowered earlier than peninsular ones. Additionally, plants of Canarian populations Tahonilla and cv. Tenerife, of var. *bituminosa*, also flowered in autumn, indicating adaptation to mild winter environments. By contrast, accessions from the Iberian Peninsula flowered and produced mature seed later, indicating an adaptation to cold winter environments.

Estimation of flower heads/plant. The mean number of flower heads/plant was about 2500. Each flower head had a mean of 28 flowers, making a total of 70,000 flowers/plant. Assuming that 56% of flowers give rise to pods at day 30 after full flowering, 40,000 seeds are produced per plant. If the mean weight of 100 seeds is 2.6 g, then about 1 kg of seed/plant could be produced (Table 2). Considering that for the 10 populations studied we collected a mean of 44 g/plant, that is equivalent to 5% of the potential seed produced; using the maximum weight collected (190 g/plant), then we were still harvesting only a 19% of the potential.

Table 2. Estimations of seed production for the 3 varieties of *B. bituminosa*

Variety	n	Flowers/plant			% seeds retained at day 30	Seeds produced	
		Number of heads	Flowers per head	Number flowers		number	g/plant
Crasiusscula	1	1783	34	60,622	71%	43,042	1291
Albomarginata	1	1686	25	42,150	48%	20,232	500
Bituminosa	4	4009	25	100,225	50%	50,112	1228
Mean values		2493	28	67,665	56%	37,795	1006

Ants harvest large amounts of pods during the spring and early summer. Doves have been seen picking seeds from the soil during the period when pods ripen and fall to the ground.

Seed retention. Populations of varieties *albomarginata* (Famara, Vinamar) and *crassiuscula* (Cañadas del Teide) present higher seed retention values (48-71% at day 30; 40-56% at day 50; 9-23% at day 100) than most populations of var. *bituminosa* (Guimar, Mijas, Cerdeña, Galilea; Llano del Beal), but exceptions like Calnegre (82%, 68% and 41% at days 30, 50 and 100) and Tahonilla (79%, 58% and 13% at days 30, 50 and 100) had excellent seed retention values (Table 3).

Germination capacity. The germination capacity was low (17-21%), but it was improved four times (77-87%) when seeds were scarified. In most cases, seeds germinate better at constant

temperatures. The population Calnegre gave the best results, with a mean germination rate of 81% for the 4 treatments. Variability within plants was detected in all populations, that in part might be due to natural hybridising between varieties. A natural hybrid between var. *bituminosa* x var. *crassiuscula* included in Table 4 gave a mean germination of 90% for the 4 treatments.

Table 3. Retention of seeds in *B. bituminosa*: Effect of time, variety and population

Variety	Population	Number of flowers/head	Number of seeds/head		
		Day 0	Day 30	Day 50	Day 100
Crasiusscula	Cañadas Teide	34	24	19	3
Albomarginata	Famara	26	18	11	6
	Vinamar	25	12	10	4
Bituminosa	Tahonilla	24	19	14	3
	Guimar	20	4	0	0
	Calnegre	22	18	15	9
	Mijas	28	5	1	0
	Llano del Beal	31	7	6	4
	Cerdeña	22	4	0	0
	Galilea	21	6	0	0

Table 4. Germination rates (%) of B. bituminosa seeds: effect of variety, population, scarification (scarified S *vs* non scarified NS) and temperature (constant C *vs* alternate A)

Variety	Population	n*	Mean germination rates (%)				Mean	Range
			Scarified		Non scarified			
			SC	SA	NSC	NSA		
Crasiusscula	Cañadas del Teide	3	91	93	4	5	48	2-99
Albomarginata	Famara	4	88	93	7	5	48	0-100
	Teno	2	91	80	8	11	47	3-100
Bituminosa	Tahonilla	1	99	95	8	5	52	5-99
	cv. Tenerife	2	93	73	13	2	45	0-98
	Galilea	1	93	82	17	10	50	10-93
	Llano del Beal	3	96	82	18	14	52	11-98
	La Perdiz	2	62	35	8	10	29	6-87
	Mijas	2	69	46	55	39	52	3-83
	Calnegre	1	91	94	70	71	81	70-94
Bitum x *crass*	PN F37-A8	1	100	97	89	75	90	75-100
	mean values		87	77	21	17	50	0-100
Control	*Medicago sativa*	1	80	-	-	94	87	80-94

n*: number of seed lots of individual plants/populations

Conclusions

Preliminary results indicate that there is enough variability in *B. bituminosa* to select lines with high seed production, retention and germination. The differences found were mostly of genetic origin, but biotic and environmental factors also had an effect.

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