AGRICULTURE ISSUES AND POLICIES

LEGUMES

TYPES, NUTRITIONAL COMPOSITION AND HEALTH BENEFITS

HIROTO SATOU
AND
REN NAKAMURA
EDITORS

New York

Additional color graphics may be available in the e-book version of this book.

Library of Congress Cataloging-in-Publication Data

Legumes: types, nutritional composition and health benefits / editors: Hiroto Satou and Ren Nakamura.
p. cm.
Includes index.
ISBN: 978-1-62808-281-4 (eBook)
1. Legumes. 2. Legumes--Composition. 3. Legumes--Health aspects. 4. Legumes as food. I. Satou, Hiroto. II. Nakamura, Ren.
SB177.L45L48 2013
633.3--dc23
2013021117

Published by Nova Science Publishers, Inc. † New York

CONTENTS

In: Legumes
Editors: Hiroto Satou and Ren Nakamura
ISBN: 978-1-62808-280-7

Chapter 1

LEGUMES FOR GRAZING AND HEALTH: THE CASE OF *BITUMINARIA BITUMINOSA*

María Pazos-Navarro[1], Mercedes Dabauza[1], Enrique Correal[1], David J Walker[1], José A del Río[2], Ana Ortuño[2], Pilar Méndez[3], Arnoldo Santos[4], Segundo Ríos[5], Vanessa Martínez-Frances[5] and Daniel Real[6,7,8,9]

[1]Department of Natural Resources, IMIDA, Murcia, Spain
[2]Department of Plant Biology, Faculty of Biology, University of Murcia, Espinardo, Murcia, Spain
[3]Unidad de Producción Animal, Pastos y Forrajes Instituto Canario de Investigaciones Agrarias (ICIA), Tenerife, Islas Canarias, Spain
[4]Unidad de Botánica, ICIA, Tenerife, Islas Canarias, Spain
[5]Centro Iberoamericano de la Biodiversidad (CIBIO), Universidad de Alicante Carretera San Vicente del Raspeig, Alicante, Spain
[6]Department of Agriculture and Food Western Australia, WA, Australia
[7]Future Farm Industries Cooperative Research Centre, The University of Western Australia, Crawley, WA, Australia
[8]School of Plant Biology, The University of Western Australia, Crawley, WA, Australia
[9]Centre for Legumes in Mediterranean Agriculture, The University of Western Australia, Crawley, WA, Australia

ABSTRACT

Legumes have been used for centuries for different purposes as human food (grain legumes such as lentils, chickpeas, etc.), animal feed (pasture and forage legumes) and as sources of secondary metabolites with health, medical or nutraceutical benefits (pharmaceuticals, cosmetics and chemicals). Due to this relevance, some of these species are the subject of breeding programmes for selecting hybrids with desirable traits. To aid in these programmes, Plant Biotechnology offers multiple methods for conservation and

commercial propagation of valuable plants for different objectives, such as the pharmaceutical industry or pasture production.

Bituminaria bituminosa (L.) C.H. Stirton (syn. *Psoralea bituminosa* L., *Fabaceae, Psoraleeae*; Stirton 1981) is a perennial legume widely distributed in the Mediterranean Basin and Macaronesia. The plants are grazed by small herbivores, but are mainly used for hay to feed milking goats, mostly in the Canary Islands. Moreover, *B. bituminosa* plants are a source of secondary metabolites of pharmaceutical and medicinal interest, such as furanocoumarins (FCs, psoralen and angelicin) and pterocarpans (bitucarpin A and B). So, their possible physiological functions and pharmacological properties are being analysed.

Our research group began to search for alternative perennial pasture legumes for low-rainfall Mediterranean environments at the end of the 1980‘s. *B. bituminosa* showed desirable traits (tolerance to cold and/or drought), that draw it as a good option. So, the first step was to select new germplasm with different traits to increase genetic variability for starting a breeding programme, with the most promising varieties, accessions and natural hybrids to deliver cultivars in the minimum time. To achieve this objective, conventional crossing between cultivars and wild accessions, and techniques for interspecific crossing breeding were carried out, in order to obtain new hybrid lines with a combination of desirable traits. Then, for a commercial approach, studies based on seed production of *B. bituminosa* selected cultivars have to be carried out and also research on the alternative uses of this species, based on its content of bioactive components with pharmaceutical potential.

As a result of this breeding programme, our research group has selected several hybrid and non-hybrid lines showing high levels of expression of, or a combination of desirable traits such as high forage quality, tolerance to biotic or abiotic stresses, or high contents of FCs. Therefore, the development of biotechnological tools such as micropropagation or *in vitro* plant regeneration protocols allowed the possibility of selecting somaclonal variants, obtaining pollen-derived haploid plants for optimising plant breeding programmes, and regenerating genetically transformed plants to control the biosynthesis of FCs. Moreover, these techniques offer attractive approaches for the large-scale propagation and conservation of germplasm resources of these plants.

In the present chapter, we describe this species, show its potential uses and the strategies that may be followed to carry out a traditional breeding programme in *B. bituminosa* and the tools developed to assist in it.

1. INTRODUCTION

The legume family (*Fabaceae*) grows in a wide range of agro-ecological conditions, and the plant morphologies vary from herbs to giant forest trees; including major grain legumes, oilseed, forage, ornamental and medicinal crops, and agro-forestry species (Lewis et al. 2005). This family has been divided into 3 major subfamilies, with unequal distribution: *Papilionoideae, Caesalpinioideae* and *Mimosoideae* (Singh et al. 2007).

Legumes are economically important in world trade because of their uses as human food and animal feed and in other commercial applications (pharmaceutical products, soap, paints, resins, coatings, cosmetics and chemicals among others) (Singh et al. 2007). The *Fabaceae* are second only to the *Gramineae* in agricultural importance. While cereals are an excellent source of energy in the form of carbohydrates, legumes are the major source of dietary protein for a large proportion of the human population. Legumes also provide essential amino acids and minerals required by humans (Grusak 2002). They are also important forages in temperate (e.g. alfalfa, clover) and tropical (*Stylosanthes* spp., *Desmodium* spp.) regions.

Legume biomass is also used as fodder, as cover crops and as green manure. A wide range of annual and perennial forage legumes are used in Mediterranean areas because of the different environmental conditions and farming systems. Annual (*Trifolium subterraneum* L.and *Medicago* spp.) or biennial (*Hedysarum coronarium* L.) legumes (Lombardi et al. 2001; Roggero et al. 2001) are established as pure or mixed swards to increase fodder production or the nutritive value of pastures. Perennial legumes provide numerous agro-environmental benefits due to continuous soil cover. In Mediterranean areas, summer drought favours plants with adaptations to protect meristematic tissues and for dehydration delay, dehydration tolerance and summer dormancy. Drought problems for legumes are likely to worsen with the projected rapid expansion of water-stressed areas of the world (Graham and Vance 2003).

There is an absence of drought-tolerant herbaceous perennial forage legume and herb options other than lucerne (*Medicago sativa* L.) for environments with Mediterranean-like climates, common in extensive areas of southern Australia, the Mediterranean basin and Chile (Dear et al. 2001; Li et al. 2008). Clearly, there is great deal of material available from dry areas in the Mediterranean basin and elsewhere. For example, there is a great diversity of perennial legumes in the southernmost parts of Italy; hence, in the future other semi-arid parts of the Mediterranean basin should be explored (Coks 2003). Alternative perennial legumes have the potential to complement and expand the feed base for grazing livestock and provide biologically-fixed N and an opportunity to control weeds in cropping rotations (Real et al. 2011). These novel species would assist in combating soil degradation attributable to deep drainage, by using more out-of-season rainfall compared with predominantly-winter-growing annual pastures and cropping systems, and be sufficiently persistent to incrementally dry the soil profile over successive years (Dear and Ewing 2008; Sandral et al. 2006). Although there is a great number of legume species available from dry areas in the Mediterranean basin (Cocks 2003), frequently, those which are adapted (Mediterranean flora) have not been selected and the selected commercial varieties are not adapted (Lorenzetti and Falcinelli 1987).

Among their multiple characteristics, legumes also synthesise secondary compounds that protect the plant against the attack of pathogens and pests (Dixon et al. 2002; Ndakidemi and Dakora 2003). These compounds also have health-promoting activities relevant to humans. So, the moderate consumption of legumes may help to prevent strokes, cardiovascular, Parkinson‘s, Alzheimer‘s and Huntington‘s diseases, liver ailments and even some forms of cancer (Singh 2005, 2007; Maurich et al. 2004, 2006). They have a blood cholesterol-reducing effect (Andersen et al. 1984) and are also included in the diet of insulin-dependent diabetics (Jenkins et al. 2003). Certain legumes, however, produce anti-nutritional factors, such as trypsin inhibitors and phyto-hemaglutinins (Gupta, 1987) and allergens, the latter being a severe problem in peanut (Spergel and Fiedler 2001). Genomics approaches, including metabolomics and proteomics, are essential to understanding the metabolic pathways that produce these health-promoting or anti-nutritional secondary compounds and for enhancing or eliminating, respectively, their production in the plant.

Our botany and agronomy research group (ICIA-Tenerife and IMIDA-Murcia) has worked on the screening of perennial legumes well adapted to dry Mediterranean conditions prevalent in the Canary Island and South-east mainland Spain agricultural areas. After screening for drought tolerance between native shrubby and herbaceous perennial legumes (species from genera *Chamaecytisus*, *Teline*, *Medicago*, *Colutea*, etc), we finally ended up selecting *Bituminaria bituminosa* (L.) C.H. Stirton, a multipurpose perennial legume widely distributed in the Mediterranean basin and Macaronesia (Correal et al. 1994, 2006; Méndez 1993; Méndez and Fernández 1992; Méndez et al. 2000; Pascual et al. 1991; Ríos et al. 1993, 1992; Sánchez et al. 1996). Thus, *B. bituminosa* is well adapted to high temperature and low rainfall and has a high retention of leaves when moisture stressed, therefore providing valuable feed over summer (Real et al. 2011; Real and Verbyla 2010). Other authors reported that *B. bituminosa* was different from other species evaluated; it showed a very high vigour both in spring and in summer. It has been shown to be a robust species that can survive in dry areas (Pagnotta et al. 2003). The *B. bituminosa* plants are grazed by cattle in the range of northern Israel (Sternberg et al. 2006) and used as hay to feed milking goats in the Canary Islands. In addition, it could be used for the phyto-stabilisation of former mining areas contaminated by heavy metals (Walker et al. 2007). Moreover, this species also synthesises bioactive compounds which have current and potential uses for modern medicine, such us pterocarpans and furanocoumarins (FCs, psoralen and angelicin) (Martínez et al. 2010).

Due to the large morphological and genetic diversity found in *B. bituminosa* and its potential relevance for Mediterranean pasture-crop systems and the pharmaceutical industry, our research group in Spain, in collaboration with Australian researchers, is carrying out a breeding programme and we have been able to select lines with high forage quality, tolerance to biotic or abiotic stresses or high contents of FCs. These plants are valuable for the pharmaceutical or livestock industries, which are interested in developing new or cheaper drugs or new drought-tolerant perennial legume cultivars for Mediterranean-like climates. To aid in this programme and to evaluate different agronomic traits (stress tolerance or FC content), hydroponic culture, controlled-freezing chamber and field studies have been carried out. We have evaluated the physiological response of *B. bituminosa* to heavy metals (Martínez-Fernández et al. 2011, 2012) and have related FC accumulation to indicators of stress (water relations and proline) (Walker et al. 2012). We have also developed *in vitro* culture systems (micropropagation and plant regeneration by organogenesis or somatic embryogenesis) and genomic tools for (i) mass propagation of selected genotypes (germplasm conservation, animal feeding purposes or large-scale production of FCs), (ii) production of synthetic seeds by encapsulation of breeding line somatic embryos, (iii) genetic engineering to study the FC biosynthesis pathway and (iv) genetic characterisation of the diversity within *B. bituminosa* breeding programmes (Pazos-Navarro et al. 2011, 2012, 2013)

The potential role of *B. bituminosa* in extensive grazing systems in Mediterranean climatic zones and as a source of pharmaceutical products will be discussed in this chapter.

2. Geographic Distribution and Taxonomy

2.1. Botanical History

B. bituminosa has been known from ancient times due to its bituminous odour and trifoliate leaves. It was mentioned for the first time in Herodotus's work, according to Ríos (present chapter). Later, it was described by Dioscórides (1st century AD) in his famous book

Materia Medica, according to the first copy known, the Codex Vindobonensis (Bizancio, 512 AD) – where it is mentioned as *Trifolium asphaltites.* In the same way, it was reported and described by several botanists from the XVI and XVII centuries, such as P.A. Mattioli (1544), A. Laguna (1555) and J. Gerard (1597, 1633), sometimes together with a drawing with flowers (Gerard) or without flowers (Mattioli, Laguna).

Likewise, it was described in several works of pre-Linnean botanists (XVII century and the first half of the XVIII century) such as Bauhin, Morison or Lobelius, most of them indicated by Linnaeus (Hortus Cliffortianus, 1753). When Linnaeus published his famous work *Species Plantarum* (1753), he described for the first time the genus *Psoralea* with 16 species, but these are currently, distributed in several genera: *Bituminaria*, *Cullen*, *Cyamopsis*, *Dalea*, *Indigofera*, *Otholobium*, *Pediomelum*, *Psoralea* and *Rhynchosia* (Jarvis 2007). Among the described species was *Psoralea bituminosa* currently included in the *Bituminaria* genus. This species had been previously mentioned, as *Trifolium*, under polynomic description, in the *Hortus Cliffortianus*. This was the first scientific description of *Psoralea bituminosa* according to the new nomenclatural system proposed by Linnaeus. In the same work (1753), the distribution of this species was mentioned as –Habitat in Siciliae, Italiae, Narbonae collibus maritimus‖, all belonging to the Mediterranean region.

The genus *Bituminaria* Heist, ex Fabricius (Enum., ed. 2: 165, 1759) is based on *Psoralea bituminosa* L., Sp. Pl. 763 (1753), lectotipified by Jafri in Jafri & El-Gadi, Fl. Libya 86: 39 preserved in the Linnaean herbarium (LINN) with the number N. 928.19, according to Jarvis (2007: 776). Medik (1787) established the genus *Aspalthium***,** which is considered by Stirton as a synonym of *Bituminaria*. Several authors, such as Stirton or Lewis et al. (2005), enclose this accepted genus in the Family *Fabaceae*, subfamily *Papilionoideae* and Tribe *Psoraleeae*.

Stirton (Bothalia, 317-318, 1981) accepted the genus *Bituminaria* Heist. ex Fabricius (*Psoralea* pro parte), which is considered to be related to *Pediomelum* Rydb. from North America. He recognized two subgenera (*Bituminaria* and *Christevenia*), each of them with only one species, respectively: *B. bituminosa* (L.) C.H. Stirton based on *Psoralea bituminosa* L. and *Bituminaria acaulis* (Stev.) C.H. Sirton based on *Psoralea acaulis* Stev. apud Hoffm, in Comm. with type from –iberia occidentalis‖ [sic], West Georgia (Turkey). These two species are also the only ones recognized in the Flora Iberica by S. Talavera (Vol. VII(I), 1999), who mentions the paternity of the latter as *B. acaulis* Hoffm., a species present in central-western Asia, reaching Anatolia.

Stirton (Bothalia, 317-318, 1981) reported the extreme variability of *B. bituminosa*, suggesting that it should be investigated. He proposed the possible differentiation of *Psoralea morisiana* Pignatti & Metlesics, from Cerdeña (Italy), later accepted and combined as *Bituminaria* by Greuter (1986), as *B. morisiana* (Pignatti and Metlesics) Greuter.

S. Talavera (Vol. VI I(I), 1999), did not mention *B. morisiana*, although he reported the existence of populations in the Iberian Peninsula, with some differences that could be related to *Psoralea plumosa* described by Rch. in the Fl. Germ. Excursion 869 (1832), which must be a synonym of *Psoralea palaestina* Gouan, Ill. Observ. Bot.: 51 (1773) and, in any case, included in *B. bituminosa* variability.

Another species recently combined by Greuter (Willldenowia, 16 (1): 108, 1986) was *Psoralea flaccida* Nábelek, as *Bituminaria flaccida* (Nábelek) Greuter, described for Tunisia and also mentioned by Boulos (1999) for Egypt, but not mentioned in the Flora Iberica.

Rencently a new species (*B. basaltica*) has been described by Minissale et al. (2013) for the islet of Filicudi (Sicily, Italia).

2.2. Geographic Distribution

After Linnaeus, several works reported more information about the distribution of *B. bituminosa*, not only in European Mediterranean countries but also those of Mediterranean Africa (Flora Libyca, Flora of Morocco, Flora of Egypt,…) and the near East. The present known distribution for *B. bituminosa* includes not only Mediterranean areas, but also southern areas of temperate Europe and the northern border of Saharo-Arabian areas. It is not present in the northwest of the Iberian Peninsula but does grow in the Macaronesian islands of Madeira (Portugal) and the Canaries (Spain).

B. bituminosa, which is widely distributed, is a polymorphic taxon well adapted to different habitats, regarding both altitude and climatic conditions; thus, *B. bituminosa* is present between latitudes 44º and 29ºN and longitudes 15ºW and 40ºE; its presence in high-elevation areas may extend up to 1300 m in the Mediterranean basin, and in the Canary Islands and Morocco up to 1800-2000 m. For this reason several species, subspecies, varieties and forma have been described, most of them not accepted currently.

R. Maire in 1927 proposed the new variety *atropurpurea* (as *Psoralea bituminosa*), characterised by its dark-violet flower and located in the Moroccan High Atlas; although this species has not been recognized in flora catalogues of Morocco, such as Fenanne et al. (1999), Fennane and Tatou (2005) or Valdés et al. (2002), it may be because the intense colour of its petals is not exclusive and can be observed also in other localities along its distribution. Nevertheless, according to our own observations, it is possible that in other parts of Morocco, such as the Anti Atlas Mountains, well-differentiated cryptic taxa could exist, but have not yet been described, or were observed by Jahandiez and Maire (1934, 1941) but not recognized by the previously-mentioned authors.

Jahandiez and Maire (1934) not only accepted the var. *atropurpurea* (*Psoralea bituminosa*) but also the var. *latifolia* Moris reported at Chaouen (Rif) by Emberger and Maire. Later, these authors (1941) reported other varieties and forma in the Catalogue of the Moroccan Flora:

Psoralea bituminosa var. *typica* Fiori f. *decumbens* S. et Ma., nom. nudum,
P. bituminosa var. *angustifolia* (Guss.) Strobl. (*P. b.* f. *linearifolia* S. et Ma., nom nudum),
P. bituminosa var. *rotundata* Maire and,
P. bituminosa var. *laxa* Maire et Weiller.

It is not known if these taxa have been recognized and combined into *B. bituminosa*. R. Negré (1961), in his work about the little flora of arid regions of western Morocco, did not distinguish varieties within *P. bituminosa*. Jafri (1980), in his Flora of Libya, did not mention varieties for *P. bituminosa*, although he pointed out the variability of the studied material, indicating the high diversity not only for leaves but also for stipules, calyx, fruit beak, etc.; however, Ozenda (1991), did not report the presence of *P. bituminosa* in his –Flora and vegetation of Sahara‖ but only *Cullen plicata* as *Psoralea plicata*; however, none of the current flora accept the mentioned –infra-taxa‖.

For Egypt, L. Boulos (1991) listed two species: *B. bituminosa* and *B. flaccida*. He considered that the latter could be only an extreme variation of the former, having the terminal lobe of lowers leaves from 0.5 to 1.5 cm and floriferous heads with 3-8 flowers (10-25 for the typical plants).

2.3. Taxonomy

We are listing some of the taxa described as *Bituminaria* or *Psoralea* (belonging to *Bituminaria*) not only at the specific level but also the infraspecific one. Those in bold type have been accepted by different authors.

2.3.1. Mediterranean-South temperate Europe-East Asia

Bituminaria acaulis (Steven) C.H. Stirton.

B. bituminosa ssp. *pontica* (A. P. Khokhr.) Zernov (Northen Caucasus and Crimea). This subspecies was published as *Psoralea pontica* and combined later to the subspecific rank. If were to be recognized as a taxon, the rest of the infrataxa should be included in *B. bituminosa* ssp. *bituminosa*.

B. bituminosa (L.) C.H. Stirton var. ***bituminosa.***

B. bituminosa var. *atropurpurea* [as *Psoralea*] Maire (Morocco, High Atlas).

B. bituminosa var. *brachycarpa* (Felmann) A. Danin, as *Psoralea*, presents small flowers and fruits. It is located in Israel and probably in Palestine.

B. bituminosa var. *communis* (Webb) [*B.b*. var. *bituminosa*].

B. bituminosa var. ***hulensis*** Felmann. It is located in wet areas of Hula Valley (Israel) and probably in Palestine.

B. bituminosa var. *latifolia* Moris (1837-1859) [reported by Muñoz and Correal 1998].

B. bituminosa var. *palestina* (Gouan) Ralf L. Jahn.; plants very hairy, located in Palestine.

B. bituminosa var. *prostrata* Zoh.; prostrate plants located in Palestine.

B. bituminosa f. *angustifolia* Grenier and Godron (1847-1850) (Muñoz and Correal 1998).

Pau (1916), according to Coca (2003), divided the species (*P. bituminosa*) into two infraspecific taxa: f. *latebracteata* and f. *parvibracteata*. Fiori (1923-1929) proposed the var. *typica* from Italy. More recently, Arnot (1985) reported a partial revision without recognizing infraspecific taxa, probably due to the limited amount and diversity of the studied material.

Bituminaria flaccida (Nábelek) Greuter, Willdenowia 16 (1) 108, 1986 [described from Tunisia according to the Index Kewensis as *Psoralea*] (in Muñoz and Correal 1998)

Bituminaria morisiana (Pignatii & Metlesics) Greuter, syn.: *P. morisiana* Pignatii and Metlesics.

Psoralea taurica Ledeb ex Nym. [*Bituminaria bituminosa* according to Stirton]

2.3.2. Macaronesian area

Madeira archipelago (Portugal)

B. bituminosa (L.) C.H. Stirton var. *bituminosa*. No infraspecific taxa have been described from these islands.

Canary Islands (Spain)

B. bituminosa (L.) C.H. Stirton var. *bituminosa*

B. bituminosa var. ***crassiuscula*** (Méndez et al. (1991)

B. bituminosa var. ***albomarginata*** (Méndez et al. (1991) [Cited by Webb & Berhtel., 1842, as var. *palaestina*]

Some investigations are currently in progress to differentiate between Canarian populations (var. *bituminosa)* and the Mediterranean ones. So, new names, not only for the common *B. bituminosa* in the Canary Islands but also for some populations with very narrow leaflets of the dry southern areas (especially on Tenerife Island), should be proposed. These considerations are supported by molecular studies such as those of Coca et al. (2003), Muñoz et al. (2000) and Pazos-Navarro et al. (2011). These studies point out genetic differences between populations of *B. bituminosa* from the western Mediterranean basin and those of Macaronesia – particularly those of the Canary Islands.

In general, the different subspecies, varieties and forma described for the *B. bituminosa* complex may belong to variations related to the typical variety (*bituminosa*). It is possible that some taxa, previously described, might have enough characteristics to be accepted as true taxa; however, further investigations on the species distribution area, particularly in the northwest African region, are necessary.

3. GENETIC VARIABILITY STUDIES OF *BITUMINARIA BITUMINOSA*

Until recently, *B. bituminosa* was considered a ‗genomic orphan‘ species (Varshney et al. 2009), with almost no genomic resources available. The few genetic studies developed for this species have shown genetic and morphological differences between *B. bituminosa* accessions from Mediterranean basin and Macaronesian locations.

Firstly, large morphological differences found between *B. bituminosa* accessions at different locations in the Canary Islands gave origin to the description of two new varieties, *albomarginata*, from very dry habitats, and *crassiuscula*, from 2000 m altitude (Méndez et al. 1990-91), beside the common variety *bituminosa*, which is present throughout the Canary Islands, and several ecotypes related to particular geographic locations of the Canary Islands.

Later, Muñoz et al. (2000), using PCR and morphological analysis, separated accessions of the Canary Islands from those of mainland Spain. Molecular analysis also identified differences within Canarian and peninsular accessions, the Canarian accessions being more diverse than those from mainland Spain. These initial results reflected the large intraspecific diversity of the species.

A second study by Juan et al. (2005), based on AFLP analysis, confirmed previous results published by Muñoz et al. (2000); thus, mainland Spain and Mediterranean populations of var. *bituminosa* were separated in one cluster, and populations of the Canary Islands in a second cluster containing the varieties *albomarginata*, *crassiuscula* and *bituminosa*.

Walker et al. (2006) determined the 2C nuclear DNA content of *B. bituminosa* for populations from the Canary Islands, mainland Spain and Sardinia, being in the range of 1.00 – 1.09 pg (2n = 2x = 20). Although no significant differences were found, there was true intraspecific variation in DNA content, related to the geographic origin of the population, being lower for the Canarian populations.

In a recent study using DNA sequencing technologies, Pazos-Navarro et al. (2011) established an efficient method to develop molecular markers (SSR) for *B. bituminosa*. The results showed clear differences between the three varieties (*albomarginata*, *bituminosa* and *crassiuscula*). Moreover, differences between Canarian and Mediterranean *bituminosa* accessions were observed and a new botanical division within this variety could be evaluated. Other authors had already pointed out these differences, based on genetic, morphological and physiological analyses (Muñoz et al. 2000; Coca et al. 2004). Additionally, results obtained by Pazos-Navarro et al. (2011) showed evidence of cross-pollination between the three varieties in the IMIDA-Murcia *B. bituminosa* breeding programmes.

After these genetic studies, *B. bituminosa* can no longer be considered a genomic orphan species, having now a large (albeit incomplete) repertoire of expressed gene sequences (432,306 mRNA molecules) that can serve as a resource for future genetic studies. Moreover, the expressed gene repertoire discovered in this work could be useful for follow-up experiments targeting biochemical pathways and/or important agronomic traits.

4. Uses of *Bituminaria bituminosa*

4.1. Traditional Livestock Use in the Canary Islands

The presence of livestock in the Canary Islands has been known since pre-Hispanic times (Álvarez et al. 1977). Aboriginal people arrived with goats, sheep, pigs, dogs and cats, plus some cereal and legume species (Morales et al. 2009). Cattle were introduced after the Spanish conquered the islands, partly from Africa, but mostly from the Iberian Peninsula (Capote et al. 2004).

All the aboriginal island communities carried out intense livestock activity from the middle of the 1st millennium BD until the annexation by the Castilian Spanish (XV century), the herds of goats, sheep and pigs being one of the main contributors to the economy (Pérez 1963). The grazing was conducted throughout the whole of the Canary Islands, moving livestock from coastal areas to the top of the high-altitude areas according to the season of the year, so that an optimal utilisation of the grazing resources could be obtained along a vertical transhumance.

Tedera, the local name for *B. bituminosa*, has traditionally been used as a forage plant in the Canary Islands, where it grows spontaneously from sea level to over 2000 m, both in natural and altered environments, preferably in disturbed soils of waysides and roads but also in abandoned farmlands with slightly-compacted soils of poor quality. Tedera is usually not cultivated as a forage crop but collected from natural populations and offered to livestock as hay. The first known reference in the islands to the word ―tedera‖ is from a seventeenth century document (Corrales and Corbella 2001). Subsequently, other authors cite this species as forage of interest in the islands (Pérez and Sagot 1867; Bolinaga 1915). Mr. Bolinaga, the Higher Gardener of La Orotava Botanic Garden (Tenerife Island) wrote a press note about the interest in cultivating tedera on the best soils, like alfalfa, but as a rainfed crop. He also indicated that many seeds were collected annually for this purpose, not specifying by who or where. There are also references to tedera seed collected from La Palma, ordered by the Agriculture and Industrial Plants Office of the United States Department of Agriculture (1928) and carried out by David Fairchild (1930) (Francisco Ortega et al. 2012). In his article, Fairchild refers to the importance of goats in the Canarian agriculture, tedera together with other endemic shrub legumes like tagasaste (*Chamaecytisus proliferus* ssp. *proliferus* var. *palmensis*) and gacia (*Teline stenopetala* ssp. *stenopetala*) being the main feed resource for small ruminants on La Palma Island. Currently, there are no data about the presence of tedera in archaeological excavations carried out in the Islands, as is the case for the ruderal flora, involuntarily introduced by the pre-Hispanic population, such as *Malva parviflora*, *Amaranthus* sp, *Solanum nigrum* and *Chenopodium murale*, which established as weeds on cropland. Though this does not mean that tedera was not present in the Islands before human settlement because archaeological research has focused mainly on human feeding (Morales et al. 2009). However, because grazing was one of the most-important activities of the aboriginal population and tedera is one of the few species remaining green during long periods of the summer and autumn, it is probable that it was used in pastures.

There are no data available on how tedera arrived to the Canary Islands, but its colonisation of each island could have occurred at different times; thus, considering that some of the oldest rocks in the islands are present in Fuerteventura –more than 20 million years- some populations could be very old (pers.com. A. Santos).

Several studies of the aboriginal language have proposed that the word –tedera‖ is of indigenous origin, deriving from the Berber language (Wölfel 1965). It is also interesting to note that the term Tarada (which sounds like Tedera) is the name given by Tuaregs to *Cullen plicatum* (syn. *Psoralea plicata*) (Benchelah et al. 2000), a species with some morphological similarities to *B. bituminosa*, and which is appreciated by camels, gazelles and mouflons. Guinea (1947) also refers to *Cullen plicatum* as excellent fodder, besides being used as a substitute for tea in central Sahara and Algeria. All these facts suggest that knowledge of this plant as a fodder species could have been brought along by the first inhabitants of the islands. Other opinions point out the Hispanic origin of the term (Alvar 1959; Llorente 1987); thus, considering that it is a bitumen plant and therefore could burn easily, we cannot discard the hypothesis that the plant name was a combination of two Spanish words, "tea" (resin-impregnated wood used to illuminate or to start a fire) and "asphalt" (bitumen).

More recent technical reports on the livestock development possibilities on the Islands (E.D.E.S. 1969, Ramos-Rodríguez and de la Puente-Jiménez 1983) considered tedera as an excellent fodder for ruminants, usually in the form of hay, but also grazed directly or mixed with cereal straw or other herbs. These reports also mention that on La Palma Island, tedera is sown in early autumn, with the arrival of the first rains, but with no tillage or control of seeding rates. In the 1990s, tedera was also used by the Nature Conservation Institute of Tenerife to control soil erosion and, although the project was abandoned, even today its effectiveness for controlling soil losses can be seen in some areas.

4.2. Current Research into the Use of *B. bituminosa* as Livestock Feed in the Canary Islands

In 1980s, the pasture and forage research team of the ICIA (Canarian Institute for Agricultural Research) started prospecting the variability of *B. bituminosa* and other forage legumes of interest present in the archipelago, and, at the same time, collecting germplasm and plant material of native pasture legumes (Guma et al. 2010). As a result of this, two endemic varieties of *B. bituminosa* were described: var. *albomarginata* and var. *crassiuscula*, which together with var. *bituminosa* are the three infraspecific taxa accepted for the moment (Méndez et al. 1990-91).

Populations of var. *albomarginata* thrive in environments having low rainfall (< 300 mm) but high relative humidity, because it is usually present in areas not far from the sea (300-600 m above sea level, on the potential areas of the thermophiles forest). The var. *bituminosa* is broadly distributed across the Canary Islands (350-800 mm), with significant differences between biotypes. The var. *crassiuscula* is adapted to annual precipitation of around 500 mm, part of it as snow. Its natural populations are located to 1800-2200 m above sea level on the potential areas of the pine forest. In the Canary Islands, tedera populations are adapted to a broad range of soils, from deep sandy to stony shallow soils; thus, it can be found on soils with pH from 4.7 to 8.5, textures from sandy to clay loam (according to USDA nomenclature -USDA, 1978-) and electrical conductivities of 0.5-4.6 dS m^{-1}. Mycorrhizal studies with the three varieties showed that var. *albomarginata* was the most dependent on the mycorrhizal symbiosis, but without significant differences from the other two varieties (Jaizme et al. 2001).

Agronomic trials to evaluate the forage production, nutritional value and plant-livestock interface of Canarian tederas focused mainly on populations of var. *bituminosa* (common-tedera) and var. *albomarginata* (albo-tedera). These were done using milking goats, initially measuring the quantity and quality of hay produced by common-tedera and albo-tedera in comparison with lucerne hay (Méndez et al. 2000); later, the seasonal grazing value and grazing recovery of the three Canarian tedera varieties were evaluated (Ventura et al. 2009; Méndez et al. 2011). Variety *crassiuscula* is considered an important genetic resource, but not a good forage species since it is less productive, less persistent and does not grow well in the warm climatic conditions prevailing in the majority of the Canary Island livestock farming areas. Work on sheep has been done by Western Australia (WA) colleagues and work with beef cattle by Israeli colleagues.

Because of its adaptation to dry climatic conditions, the Canarian tedera is a herbaceous perennial tolerant to drought and has a relatively-high net photosynthetic rate and, hence, good potential as a forage legume (Pn:14-18 µmol m^{-1} s^{-1}): var *albomarginata* has the highest water-use efficiency (WUE) of the three varieties, with a value close to 5 mmol CO_2 mol^{-1} H_2O (Méndez et al. 2000).

Dry matter yields of common-tedera cultivated during 4 years under irrigation in experimental plots at the ICIA (Tenerife) were respectively 7.5, 13.5, 7.7 and 5.5 Tm ha^{-1} of hay, with a mean water consumption of 1,600 m^3 ha^{-1} in a warm area receiving 350 mm of annual rainfall; the seeding rate was around 35 kg ha^{-1} (Méndez 2009; Méndez et al. 2008). The *albomarginata* pure variety took longer to yield enough biomass for a first cutting and so it could not be exploited until the second year, and it was cut once per year; hence, forage yields of *albomarginata* were about 50% lower than those of common-tedera; however, its edible biomass yield (leaves and stems with a diameter less than 5 mm) was 10 – 20% higher than common-tedera, and it needs less water to produce similar amounts of forage. Additionally, it was observed that natural hybrids with one *albomarginata* parent were more productive and persistent than common-tedera (unpublished data, P. Méndez).

When the forage yields (green matter, GM) of individual plants were compared, the highest values were for mainland Spain populations (2058 kg GM plant^{-1}) and for some Canarian tedera of var. *bituminosa* (1700-1900 kg GM plant^{-1}) (±30-35 % Dry Matter, DM), followed by *crassiuscula* accessions and other ecotypes of var. *bituminosa* (1100-1400 kg GM plant^{-1}), var. *albomarginata* and common-tedera (1000-1090 kg GM plant^{-1}) (±25% DM). The lower yields corresponded to ecotypes related to var. *bituminosa* and to some ecotypes of var. *albomarginata* (350-900 kg GM plant^{-1}).

The nutritive value of Canarian common-tedera, evaluated by Álvarez et al. (2004, 2007) and Ventura et al. (2004), gave the following results: Crude Protein content between 15-20%, *in vivo* Dry Matter Digestibility (DMD), Organic Matter Digestibility (OMD) and Crude Protein Digestibility (CPD) of 60.0, 61.6 and 51.4%, respectively, an energy content of 0.74 MFU (milk fodder units), high contents of Ca, P and K and a daily intake of 0.54 kg DM animal^{-1}, which corresponds to an intake of 31 DMD kg^{-1} W^{0,75}; the *in vitro* methods for Protein Ruminal Degradability give higher values (72.2%). A diet based on common-tedera hay had a positive influence on milk and cheese production of Canarian goat breeds (Álvarez et al. 2007; 2008).

During palatability trials with Canarian milking goats, the animals preferred common-tedera hay over alfalfa hay, and the amounts consumed of tedera were significantly higher than for alfalfa; furthermore, alfalfa yields under irrigation were 2.5-times higher than tedera yields, but alfalfa consumed 4-times more water than tedera (Méndez 2000). Moreover, the three Canary tedera varieties (*bituminosa*, *albomarginata* and *crassiuscula*) had crude protein and digestible energy contents slightly higher than a medium-quality alfalfa; however, when tedera was cut in summer and offered fresh to goats, it was rejected, but not if offered as hay (Ventura et al. 2000; 2009).

To study the effect of goat grazing, in a grazing trial (Méndez et al. 2011a) common-tedera had a good recovery when goats consumed less than 90 % of its edible biomass. The results suggest a use for tedera as a perennial legume for grazing that could maintain and even extend the grazing period in areas of semi-arid Mediterranean climate, it being possible to get milk yield and quality values similar to those obtained with alfalfa (Méndez et al. 2011b).

The furanocoumarins psoralen and angelicin are present in the leaves, stems and fruits of this species with the total concentration varying from 100 to 8000 mg kg^{-1} (Innocenti et al. 1997; Martínez et al. 2010; Méndez et al. 2001; Tava et al. 2007; Zobel et al. 1991); however, angelicin is not detected in the pure Canarian *albomarginata* variety from Lanzarote Island, or it is only present in traces in some individuals (less than 8 mg kg^{-1}) (Méndez and Fernández 1990, Méndez et al. 2001; 2006). The predominance of angular FCs (angelicin) could be related to phylogenetically-new taxa and to more-advanced biosynthetic pathways (Berembaum and Feeny 1981); this, if relevant, could indicate that var. *albomarginata* is the most-ancestral group.

4.3. Traditional Medicine

In the oldest medicinal plant treaties is described, quite accurately and in detail, a trifoliate and smelly legume called, by Herodoto of Halicarnaso (5th century BC), *Triphyllon* (Forster 1942; Rivera et al. 2012) and later, by Dioscórides (1st century AD), *Trifolium asphaltites*. These classical authors emphasized two unequivocal features of the plant: (1) the presence of trifoliate leaves and (2) the strong smell of asphalt or bitumen when it is collected.

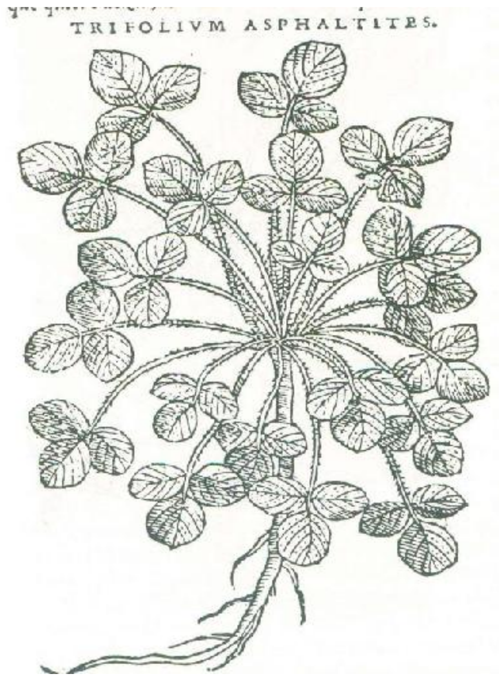


Figure 1. *Bituminaria bituminosa* picture extracted from *Materia Medica* (Mattioli 1544).

Just for these characteristics, the pre-Linnean botanists (Caspar Bauhin, Tournefort, Ray, etc.) maintained the meaning of that definition under the name *Trifolium bituminosum redolens*. Later, Linnaeus separated this plant from the rest of the clovers, creating the genus *Psoralea* (from the Greek Ψοραλεος *Psoraleos* = one grain (Gledhill 2008), keeping the reference to the asphalt smell in the specific epithet *bituminosa*. Finally, in relatively-recent times, Stirton (1981), because it has an indehiscent legume, segregated it into a new genus, *Bituminaria* (*bitumen, bituminis* = tarry, clammy, adhesive, smelling of tar (Gledhill 2008), thus highlighting doubly the smelly characteristic present in the Mediterranean strains of this species.

Knowledge of this medicinal plant has been accurate since the times of Dioscórides, which is not always common. There are many references confusing it with other medicinal species. The Italian translation of *Materia Medica* (Mattioli 1544) presents a picture in which appears, perfectly-drawn, a non-flowering rosette of *B. bituminosa* (Figure 1). In this translation and in later editions, such as the Castilian edition of Andres Laguna (1566), it appears next to other clovers under the name *Trifolium asphaltites*, always differentiated by its smell and medicinal virtues from other trifoliate "simples" (*Trifolium sensu stricto*).

From an ethnobotanical point of view, it is considered that the greater the number and types of popular names that a plant collects, the more traditional knowledge exists or existed about it (Rivera and Obon 1991).

In Spain, the tradition of medicinal plants is directly influenced by the great medical and pharmaceutical development achieved during the Muslim times in Al-Andalus (centuries VIII-XV). In the case that concerns us, this is shown by the many names under which this "simple" was compiled in the Spanish Moorish books preserved (Asín Palacios 1943; Bustamante et al. 2007). The relationship between Arabic, Mozarabic or Romance names and those registered in Castilian and Catalan ethnobotanical works (Table 1) is very evident, many of them being mere translations of the previous oldest. Among the names recorded, 60% of them describe one or two of the defining characteristics in *Bituminaria*: trifoliate leaves and bituminous odour (Table 1). Some names allure to the smell to goats or to its similarity to another plant which also has an intense odour, the rue (*Ruta graveolens*). There is also a coincidence in the medicinal uses between rue and *B. bituminosa*, especially those related to female physiology.

However, two of the most-common names in Spain, "angelote" (angel-like) and "cecinegra" (black-browed), have a different meaning, probably corresponding to a close observation of its corolla, which presents a black spot in the keel. This detailed popular knowledge highlights the importance of this medicinal plant. Similarly, another name, "contraruda" (Green et al. 2008), although not kept frequently, reveals the great value that our traditional medicine placed on *B. bituminosa* as an antidote.

Despite being a plant present in medicine since ancient times, its absence in modern Pharmacopoeias and Phytotherapy treatises is striking (Vanaclocha and Cañigueral 2003; Cañigueral pers.com.). This may be due to the phototoxicity caused by the furanocoumarins (FCs) present in the plant and, probably, the absence of pharmacological studies and clinical trials (that could support the many uses it had in the past), which exclude this plant from our modern medicines. But its enormous potential as a source of active compounds to cure different diseases is supported throughout history by its presence from Dioscórides to Hispano-Arab physicians (Bustamante et al. 2007) and in Arabic and Latin texts, which were propagated across Europe through translations into Italian (Mattioli 1544), Castilian (Laguna 1566) and other European languages. These medicinal uses remain in the Spanish tradition, as evidenced by over 30 medicinal uses reported in recent ethnobotanical works (Table 2).

Since ancient times, its more-referenced therapeutic uses are as vulnerary applications and for different processes related to women‘s health (menstruation, abortion and post-partum phase), although the diversity of therapeutic indications is very large (Table 2). Some less-frequent therapeutic indications cited since Dioscórides are for fluid retention or as treatment of certain nervous diseases (epilepsy, hysteria). There exists also a curious coincidence between the doctrine of Dioscórides and Ayurvedic medicine; thus, in Europe, the administration of seeds (beans) - crushed or swallowed - to combat fevers is advised, something that is currently done in India with seeds of *Psoralea corylifolia*. Recent studies have shown a significant antimicrobial activity of *B. bituminosa* flower extract against *Mycobacterium phlei* and a moderate activity against *Staphylococcus aureus* and *Candida albicans* (Miro, 1981). From ancient references, veterinary uses (mostly abortive) and some magical uses are reported also (Forster 1942; Rivera et al. 2012).

Table 1. Popular names of *Bituminaria bituminosa* acquired in different languages

Popular names of *Bituminaria bituminosa*		Emphasizes names							References
	English meaning	**stinky smell**	**good smell**	**leaves morph.**	**medicinal uses**	**heliotropism**	**other analogies**	**unknown**	
Names of the classics greco-roman tradition									
trifolium asphaltites	bituminous clover	x		x					Laguna (1566)
asphaltio	like asphalt	x							Laguna (1566)
triphyllon	three leaves			x					Rivera et al. (2012)
Names of Al-Andalous tradition (arabic and romances)									
buţdah, budah, yarbah buţdah	stinky, foul smell	x							Bustamante et al. (2007; 2011a; 2011b)
aţrifulūn, ţarīfulun	three leaves			x					Bustamante et al. (2007; 2011a; 2011b)
ğasdat harrān	small fig						x		Bustamante et al. (2007; 2011a; 2011b)
puţda, puda, yerba puţda	stinky, foul smell	x							Asín Palacios (1943)
ţūrnah šūl	sunflower-like					x			Bustamante et al. (2007; 2011a; 2011b)
yarbah rubdah	stinky, rue-like smell	x							Bustamante et al. (2007; 2011a; 2011b)
šgîriya	unknown							x	Bellakhdar (1997)
Botanical prelinaean names									
trifolium bitumen redolens	bituminous clover	x		x					Tournefort (1700)
trifolium bituminosum	bituminous clover	x		x					James (1747)
Names in Spanish languages									
Castilian names									
angelote, angelota	angel-like						x		Font i Quer (1985); Reyes Prosper (1915); Lázaro Ibiza (1906); Obón and Rivera (1991); Rivera et al. (1994; 2008); Bellakhdar (1997); Cano and Ríos (1996)
cecinegra, cejinegra	black-browed						x		Font i Quer (1985); Reyes Prosper (1915); Obón and Rivera (1991);

Table 1. (Continued)

Popular names of *Bituminaria bituminosa*		Emphasizes names							References
	English meaning	stinky smell	good smell	leaves morph.	medicinal uses	heliotropism	other analogies	unknown	
									Rivera et al. (1994; 2008); Cano and Ríos (1996)
cecinegra, cejinegra	black-browed						x		Font i Quer (1985); Reyes Prosper (1915); Obón and Rivera (1991); Rivera et al. (1994; 2008); Cano and Ríos (1996)
contrarruda	antidote				x				Verde et al. (2008)
hierba cabruna, yerba cabruna, hierba cabrera	goat-scented herb	x							Font i Quer (1985); Reyes Prosper (1915); Lázaro Ibiza (1906); Bellakhdar (1997); Pascual (1978)
hierba de las quemaduras, hierba de los quemaos	heal burns herb				x				Fajardo et al. (2007); Verde et al. (2008)
hierba de los granos	pimple's herb				x				Molero et al. (1992)
hierba teresa	teresa herb							x	Verde et al. (2008)
higueruela	small fig						x		Font i Quer (1985); Rivera et al. (2008); Bellakhdar (1997)
pestosa, mata pestosa, hierba pestosa, apestosa	stinky, foul smell	x							Pers. Com. S.Ríos; Obón and Rivera (1991); Rivera et al. (1994; 2008); Cano and Ríos (1996)
ruda, rudón, ruda basta, ruda de las cabras	rue-like herb	x							Obón and Rivera (1991); Rivera et al. (1994; 2008); Verde et al. (2008)

Popular names of *Bituminaria bituminosa*		Emphasizes names							References
	English meaning	**stinky smell**	**good smell**	**leaves morph.**	**medicinal uses**	**heliotropism**	**other analogies**	**unknown**	
trébol bituminoso	bituminous clover	x		x					Font i Quer (1985); Reyes Prosper (1915)
trébol hediondo, trébol heliondo, helionda	bituminous clover	x		x					Font i Quer (1985); Reyes Prosper (1915); Lázaro Ibiza (1906); Bellakhdar (1997); Pascual (1978)
Catalan names									
cabruna, herba cabruna	goat-scented herb	x							Font i Quer (1985); Moll (2003)
camabruna, calambruna, calabrulla, cabrulles, cabruja	corruption derived from cabruna?						x		Font i Quer (1985); Parada et al. (2002)
girasol, girasol de marge, girasol bord	sunflower-like					x			Font i Quer (1985); Bellakhdar (1997); Pellicer (2000)
herba bruna	brown herb						x		Font i Quer (1985)
pedrenca	rocky soil ecology?						x		Font i Quer (1985); Bellakhdar (1997)
trèvol pudent, herba pudenta	stinky, foul smell	x							Font i Quer (1985); Bellakhdar (1997); Pellicer (2000)
Others European names									
psoralée bitumineuse (fr.*)	bituminous clover	x							Bonnier and Layens (1964)
trevullu malu, trivozu malu, truvullu malu (sard.*)	bad clover	x							Congia (1998)
trifoglio bituminoso (it.*)	bituminous clover	x		x					Congia (1998)
stinking trefoil (eng.*)	bituminous clover	x		x					James (1747)

*Note: (fr. French, sard. Sardinian Languages, it. Italian, eng. English).

Table 2. Medicinal uses of *Bituminaria bituminosa* reported by numerous authors

Medicinal uses	Plant parts used	Form of administration	References
Immune system, allergies			
anti-inflammatory	entire plant	infusion	Mulet (1991)
blood cleansing	entire plant	infusion	González-Tejero (1989)
Respiratory apparatus			
pleurisy	seeds/fruits (legumes)	decoction	James (1747)
Infectious and contagious diseases			
antiseptic	root	decoction	Mulet (1991); Villar et al. (1987)
antimicrobial	flowers	experimental data	Miró (1981)
Digestive system			
stimulant	fresh leaves	infusion	Bellakhdar (1997); Villar et al. (1987)
phlegmatic tumors and others	fresh leaves	cataplasm	Bustamante et al. (2004)
Kidney and urinary disorders			
urine retention	fresh leaves, seeds/fruits (legumes)	infusion, decoction	James (1747); Gómez de Ortega (1784); Laguna (1566)
dropsy	fresh leaves, seeds/fruits (legumes)	infusion, decoction	James (1747); Gómez de Ortega (1784); Laguna (1566)
Proctology			
antihemorroidal	fresh leaves, root	cataplasm	Mulet (1991)
Cardio-vascular system			
hemostasis	leaves and twigs	decoction	Mulet (1991)
aphrodisiac	seeds/fruits (legumes)	decoction	Alliota et al. (2003); Rivera et al. (2012)
Nervous system/fevers			
coral gout or epilepsy	seeds/fruits (legumes)	infusion	Fournier (1948); James (1747); Laguna (1566)
hysteria	seeds/fruits (legumes)	decoction	Gómez de Ortega (1784)
antispasmodic	seeds/fruits (legumes)	decoction	Fournier (1948);
palsy or paralysis	seeds/fruits (legumes)	pressed oil	Gómez de Ortega (1784)
chills	seeds/fruits (legumes)	milled and ingested	Dioscórides
antipyretic	seeds/fruits (legumes)	decoction	Alliota et al. (2003); Fournier (1948); Rivera et al. (2012)
Women´s diseases			
promote postpartum	seeds/fruits (legumes)	infusion	Alliota et al. (2003); James (1747); Rivera et al. (2012)
abortion	seeds/fruits (legumes)	infusion/ decoction	Alliota et al. (2003); Bellakhdar (1997); Rivera et al. (2012); (Villar et al. (1987); Verde et al. (2008)
provoke menstruation	seeds/fruits (legumes)	infusion/ decoction	Alliota et al. (2003); Fournier (1948); James (1747); Gómez de Ortega (1784); Laguna (1566); Rivera et al. (2012)

Medicinal uses	Plant parts used	Form of administration	References
Muscular system			
rib pain	leaves and fruits (legumes)	infusion	Gómez de Ortega (1784); Laguna (1566)
rheumatism	entire plant	decoction	Alliota et al. (2003); Rivera et al. (2012)
Skin problems			
treat wounds	flowers, leaves and twigs	decoction, unguent	Bellakhdar (1997); Cano and Ríos (1996); Font i Quer (1985); Mulet (1991); Obón and Rivera (1991); S. Ríos (Pers. Com.); Rivera et al. (1994; 2008)
burns	leaves fried in olive oil	unguent	Fajardo et al. (2007); Verde et al. (2008)
removal of verrucas	entire plant	cataplasm, powder	Bustamante et al. (2004)
treat sores	fresh leaves with salt	cataplasm	Bustamante et al. (2004)
Antidotes			
against snake and other venoms	entire plant	decoction	Dioscórides; James (1747); Gómez de Ortega (1784); Laguna (1566)
Other uses			
Veterinary			
sheep diseases	leaves and twigs	decoction	Verde et al. (2008)
Pastures and fodder crop			
forage	entire plant	fresh and dried (Heno)	Pellicer (2000); S. Ríos (Pers. Com.); Rivera et al. (2008)
Magical			
was used by the Magians	ash of entire plant	strewing over sacrificial victims	Forster (1942); Rivera et al. (2012)

The fruits or the whole plant are often used for medicinal purposes, being used less frequently the flowers or roots. For internal administration, the infusions or decoctions are the most-classical form of administration. Externally, poultices and baths with fresh plant material or powders have also been used, but ointments are less frequent (Table 2).

In conclusion, *B. bituminosa* presents a rich medicinal and pharmaceutical history, from the beginning of Western medicine until relatively-recent times, which has remained alive in the Mediterranean folk medicine and particularly in the Spanish tradition. Its absence from modern pharmacopoeias may be due to the phototoxicity of its FCs, or simply the lack of clinical trials that confirm its uses; however, its high content of FCs (psoralen and angelicin mainly) could ensure the return of this plant to the drug industry in the near future.

4.4. Potential Use for Modern Medicine

A study of the composition of *B. bituminosa* (Tava et al. 2007) revealed the presence of different terpenic compounds, such as isopentenyl alcohol, furanmethanol tetradecanol, hexadecanol and caryophyllene, phenolic compounds (vanillic acid ethyl ester, plicatin B, etc.) and furanocoumarins such as angelicin and psoralen. Other studies (Maurich et al. 2006;

Pistelli et al. 2003) have shown the presence of the pterocarpans, erybraedin C and bitucarpin A.

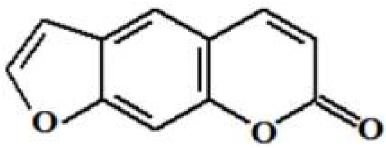
4.4.1. Furanocoumarins

Furanocoumarins are a sub-group of phenolic compounds included in the coumarins group. Coumarins are lactones with the basic structure of 1,2-benzopyrone and an oxygenation at the C-7 position. The FCs can be grouped into the linear type, where the furan ring is attached at carbons 6 and 7, and angular type, where this ring is attached at carbons 7 and 8 of the coumarin structure, and these are sub-divided into linear FCs (psoralens), such as psoralen (1), and angular ones (angelicins) like angelicin (2) (Figure 2). Linear FCs have been identified in a great variety of plant families, and the highest concentrations have been found in the *Umbelliferae* (*Apiaceae*), *Rutaceae*, *Leguminosae* (*Fabaceae*) and *Moraceae*. The angular FCs are distributed less widely and confined principally to the *Apiaceae* and *Leguminosae* (Bourgaud et al. 1989). In the case of *B. bituminosa*, different authors have described the presence of FCs, among them psoralen and angelicin (Innocenti et al. 1991, 1997; Martínez-Fernández et al. 2011; Méndez et al. 2001; Tava et al. 2007; Walker et al. 2012).

4.4.2. Therapeutic Activity of furanocoumarins

The FCs have been used in folk medicine for a long time. The Indian sacred book "Athara Veda" describes the treatment of leukoderma (vitiligo) using a poultice from a plant now known as *Psoralea corylifolia*, and the ancient Egyptians used *Ammi majus* for the same disorder. The first FC, 5-methoxypsoralen (5-MOP), was isolated in 1838 by Kalbrunner from bergamot oil.

These compounds are a therapeutically-important sub-type and have various clinical applications. The most-outstanding property of FCs is their great ability to sensitise cells to visible light, sunlight and, especially, near-ultraviolet (UV) light. This results in strong toxicity, mutagenicity and possibly carcinogenicity. The mechanism of action is well known. After intercalation into the double helix of the DNA and molecular complexing, the light-activated FCs react with the pyrimidine bases, especially thymine. The FCs are typical phototoxic compounds that produce photodermatitis in combination with UV light exposure, as well as cytotoxic and mutagenic disorders (Gupta and Anderson 1987; Stern 2007). At the molecular level, FCs bind to cellular constituents such as proteins and lipids, damaging lysosomes, leading to the formation of reactive oxygen species and contributing to the formation of novel antigens by covalent modification of proteins. The FCs are well known for interfering with drug metabolism, in particular with cytochromes P450 (CYP). Since the discovery of FCs and other structurally-related biomolecules, extensive research on different aspects of therapeutic interest has been carried out due to the singular behaviour of these compounds when irradiated with UV light (Curini et al. 2006). These properties have made possible the use of these molecules in PUVA therapy (psoralen plus UV-A radiation) or extracorporeal photopheresis for the treatment of many skin diseases such as vitiligo or psoriasis, and also for several autoimmune diseases (systemic lupus erythematosus, Crohn‘s disease, type 1 diabetes mellitus or multiple sclerosis) as well as for the treatment of cutaneous T-cell lymphoma, solid organ transplant rejection and graft versus host disease. Importantly, these compounds - alone or in combination with other drugs - represent promising candidates to develop new therapies or improve the existing ones (Hönigsmann et al. 2008; Scarisbrick et al. 2008).


1

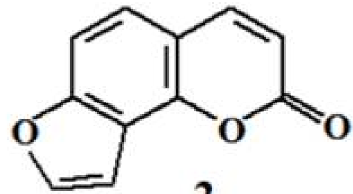

2

Figure 2. Structures of furanocoumarins present in *B. bituminosa*: psoralen (1) and angelicin (2).

4.4.3. Parts Used for Medicinal Purposes

Although FCs are found in all plant parts, the maximum concentration is found in the fruits (legumes containing one seed), buds and leaves, especially in the younger leaves, since FC synthesis is thought to occur here and in the outer skin of the plant. Factors that can bring about an increase in the concentration of FCs in various plant structures include seasonal changes, with FC levels increasing in the summer; disease-causing pathogens; mechanical damage to plants, including air pollutants and other environmental conditions; and plant size (Hagemeier et al. 1999; Hamerski and Matern 1998; Vasconsuelo and Boland 2007).

4.4.4. Furanocoumarins as phytoalexins and allelochemicals

The FCs are recognised as potent phytoalexins. Most plants accumulating these compounds possess a highly-inducible biosynthetic pathway that can be triggered by various biotic and abiotic stresses. Since FCs are strongly phototoxic, their presence in plants has been demonstrated to be a protective mechanism against phytopathogenic microorganisms and herbivores. So, when roots of *Glehnia littoralis* (*Apiaceae*) were inoculated with *Pseudomonas cichorii* the production of four linear FCs (psoralen, xanthotoxin, bergapten and demethylsuberosin) was induced as phytoalexins. Cells of *Petroselinum crispum* (*Apiaceae*) treated with elicitors derived from different species of the genus *Phytophthora* expressed defence-associated genes and the production of FC phytoalexins. Similar results were observed in *A. majus* cells treated with fungus elicitors (Heathpagliuso et al. 1992; Masuda et al. 1998).

Many phototoxic plants grow preferentially in sunlit habitats and hence maximise the exposure of herbivores to photoactivating wavelengths. Plant chemistry can have deleterious effects on insect parasitoids, including reduction in body size, increased development time and increased mortality. The linear FCs psoralen, 5-MOP (bergapten) and 8-methoxypsoralen (8-MOP, xanthotoxin) have considerable biological activity against herbivores, including humans. Increased dietary concentrations of each FC significantly decreased insect larval weight, extended generation time and induced higher mortality. Xanthotoxin was the most toxic, followed by psoralen and bergapten. At low concentrations they have proven deadly to many insect species, thus providing significant protection against insect herbivores. Even arthropods that have developed some tolerance to these compounds can show reduced performance upon ingestion, including delayed development that may be associated with antifeeding responses and reduced survival (Li et al. 2007; Stevenson et al. 2003).

5. Breeding

The breeding technique for *B. bituminosa* was established in 2007 by Real et al. which - combined with the molecular tools developed by Pazos-Navarro et al. (2011) - permitted the identification of hybrids, either natural or the result of hand-crosses between elite material.

The genetic variability already observed in *B. bituminosa* can be summarised in some of the following desirable plant traits: a) a broad soil and climatic adaptation and hence, a large potential seed market for commercial varieties; b) a wide seed yield range to select lines with high seed production, retention and germination (Correal et al. 2008); differences found were mostly of genetic origin, but biotic (pollinators, ants, birds) and environmental factors (rain and temperatures) should be considered; c) a large variability in its FC content (psoralen and angelicin), with higher levels in var. *bituminosa* than in var. *albomarginata,* makes it an ideal source for the extraction of these compounds for pharmaceutical purposes (Del Río et al. 2010), and also for screening plant material with lower FC contents for animal production.

Due to the potential advantages of *B. bituminosa* for Mediterranean-like areas of southern Australia, an agreement led by Dr. D. Real was established on July 2008 for the research, breeding and commercialisation of *B. bituminosa* between three Spanish institutions (ICIA-Tenerife, IMIDA-Murcia, CIBIO-Alicante) and the national research consortium Future Farm Industries Co-operative Research Centre (FFI CRC) of Australia. This agreement benefited from the previous breeding experience of the IMIDA-Murcia and ICIA-Tenerife with *B. bituminosa*, providing native accessions and natural hybrids for the start of an Australian breeding programme on tedera, to cross the best parent plants in order to combine elite material into one or several cultivars, selecting in the breeding process homozygous plants with the combination of desirable characters.

Since the agreement was signed, the Spanish team has done work on the following topics: germplasm characterisation; drought, cold and heavy metals tolerance; *in vitro* propagation; molecular markers, root nodule bacteria and breeding for high secondary compounds content for pharmaceutical purposes.

To simplify the description of the different *B. bituminosa* varieties, we shall use the following common names:

a. *B. bituminosa* var. *albomarginata* (Canary Islands): albo tedera
b. *B. bituminosa* var. *crassiuscula* (Canary Islands): Teide tedera
c. *B. bituminosa* var. *bituminosa* (Canary Islands): tedera
d. *B. bituminosa* var. *bituminosa* (Mediterranean basin): Mediterranean tedera.

Two Spanish research teams - ICIA-Tenerife and IMIDA-Murcia - started in the early 1980s the work on characterisation, screening and breeding of tedera; in the 2000s, a third team -CIBIO-Alicante- joined also, and from this work important results were obtained:

> Accessions of tedera from the Mediterranean basin are generally of low forage value and persist mostly for two years under the conditions of the southeast of mainland Spain.

Accessions of tedera from the Canary Islands are generally more productive, of better forage quality and perennial, but they do not tolerate the low winter temperatures prevalent at high altitude in Mediterranean environments.
A future research objective should be to produce hybrids of higher yield, quality, persistence and tolerance to cold winters.

In order to differentiate between Canarian and Mediterranean accessions, a large number of morphological and behavioural characteristics were used, such as habitat, growth habits, persistence and stress tolerance. Some of them are summarised as follows:

5.1. Mediterranean Tedera Populations (var. *bituminosa*)

They grow in autumn-winter with a rosette of leaves; some accessions tolerate cold winters (with absolute minimum temperatures as low as -10ºC).
They have long stems and narrow leaves during flowering at the end of spring and they shed leaves in summer.
They are present mostly in alkaline soils but also in acid soils.
They are present in environments with mean rainfall ranging from 300-1000mm.
They are biennial (short lived), but exceptionally, 1-5% of perennial plants were found in certain populations like Calnegre and Llano Beal, both accessions from Murcia.

5.2. Canarian Tedera Populations.

5.2.1. Tedera or common tedera (var. bituminosa)

It is traditionally harvested from native stands and consumed as hay by milking goats.
It is present in alkaline and acid soils and in areas with 300-1000mm of mean annual rainfall.

5.2.2. Teide tedera (var. crassiuscula)

It grows at high altitudes (3ºC minimum in coldest months of growth) in sub-humid habitats (500-700 mm rainfall), but becomes dormant in winter, escaping cold temperatures.
It does not survive at 1000 m in NW Murcia (1-3ºC minimum in coldest month) (Muñoz and Correal 1999).
It presents low perennity when cultivated in Tenerife and Murcia.

5.2.3. Albo tedera (var. albomarginata)

A drought-tolerant plant; –green‖ in summer; native to coastal, semi-arid habitats having 150-300 mm annual rainfall

It is a dwarf shrub presenting a cushion growth habit, a high leaf/shoot ratio, woody basal stems and high perennity.

It is only native to Lanzarote but related populations are present also in Fuerteventura, Gran Canaria, Tenerife and La Gomera in the geologically oldest areas.

It does not tolerate frost.

On Tenerife Island, where one ecotype close to albo-tedera is present in dry coastal areas, common tedera in intermediate-altitude zones and Teide-tedera in high-altitude areas, P. Méndez compared the agronomic performance of populations of the three varieties at the ICIA field station and observed vigorous tedera plants growing between plant rows in the second year of cultivation. Additionally, the first tedera cultivar from Tenerife, introduced into Murcia in the early 1980s, performed as a vigorous hybrid when compared to lower-yielding Mediterranean populations of peninsular Spain.

Something similar to what happened in Tenerife was observed at the IMIDA-Murcia after cultivating populations of the three tedera varieties during several years (in the 1990s and 2000s); however, in the case of Murcia, Mediterranean populations were present and new, natural hybrids between Canarian and Mediterranean tedera were identified. Natural hybrids were recognised by the presence of a recombination of morphological and physiological traits (vigour, perennity and tolerance to abiotic stresses) from the different varieties co-existing in the same field station of La Alberca.

From breeding work carried out during the 2000s (Juan et al. 2003; 2004; Real et al. 2008), more results were obtained and can be summarised as follows:

B. bituminosa is an inbreeding species that also outcrosses.

The three tedera varieties produce self-pollinated seed, but seed production is increased when pollinators visit the flowers.

Environmental conditions and pollinators have an effect on legume production.

In addition, the Spanish teams worked during the 2000s on screening and selection for specific plant traits such as (1) tolerance to cold winters and dry summers, (2) growth adaptation to mild winters and long, dry summers and (3) high pharmaceutical compounds production. In this sense, several interesting results were obtained. The different botanical varieties have differing frost tolerance. The least tolerant is albo-tedera (var. *albomarginata*) that is burnt by mild frost of short duration; however, the plant is often able to recover. The Teide-tedera (var. *crassiuscula*) is present at the top of the Cañadas area, growing at altitudes of 2000 m above sea level close to Teide Mountain in Tenerife, and some populations are under snow for several weeks each year. The Mediterranean tedera (var. *bituminosa*) from high-altitude sites is the most frost-tolerant, being able to survive through severe winters, as was the case of populations La Perdiz (hybrid P6-E7P-F23A12) and Llano del Beal (hybrid P3-E7P-F28A15) from Murcia and Mikropolis from Greece, during a field trial carried out in El Chaparral-Cehegín (Murcia), at 800 m altitude, during the years 2009-2011, with minimum temperatures between -4 and -7^{o}C. Additionally, natural hybrids between Mediterranean tedera and Canarian tederas (albo-tedera, Teide-tedera and common tedera) were also tested and some were frost-tolerant; however, controls of Canarian pure albo-tedera and common tedera were frost-sensitive, as was seen during their first introduction into follow a pedigree selection process in which the best-performing individuals of the 25-30 plants of an F3 progeny were selected by particular screening criteria, and planted again in families of plant rows in an F4 and so on. However, since 2008, we have used hand crossing to recombine plant traits of some of the best breeding material, such as the already-mentioned cold-tolerant breeding lines.

Thus, our breeding programme has consisted mostly of following natural crossings with hybrid vigour, progeny selection and recurrent selection of successive plant progenies, and ultimately, in hand-crossings between prominent tedera varieties and accessions, plus progeny selection and breeding according to particular plant traits. Beside the already-mentioned desirable tedera plant traits like persistence and high forage yield under environmental stresses such as drought and cold, we did some screening for high legume-seed retention, high seed production and high seed germination.

No toxicity has been reported for *B. bituminosa*. There are only reports that some accessions from the Mediterranean basin are unpalatable due to their smell. Also, goats reject the plants when the FC content is higher than 0.5-1.0 %; this, together with the rapid excretion, prevents toxicity problems (Ramos et al. 1998). After the ingestion by goats of 10 mg kg^{-1} xanthotoxine (8-methoxypsoralen), rapid metabolism and excretion were observed. So, human toxicity resulting from the ingestion of the milk or its derivates is improbable (Ivie 1987; Pangilinan et al. 1992). Condensed tannins expressed as catechin equivalents ranged from 0.1% for var. *albomarginata* to 0.5% for var. *bituminosa*, and total phenols expressed as tannic acid equivalents ranged from 0.9% for var. *albomarginata* to 1.4% for var. *bituminosa* (Ventura et al. 2012).

As a result of this breeding programme, our research group has selected several breeding lines showing high forage yield, tolerance to abiotic stresses or high FCs content. Some of the results obtained in this breeding programme may be compared with those obtained by ICIA-Tenerife or WA: for example, tedera (1) is able to grow all-year-round if not limited due to extreme drought during summer or very-low temperatures during winter, (2) forage yields (Méndez et al. 2006) ranged from 400-2000 g GM $plant^{-1}$ (20-25% Dry Matter content) in experiments carried out at ICIA-Tenerife with the three tedera varieties, the more-productive accessions being Mediterranean tedera from mainland Spain (e.g. Llano del Beal) and tederas from the Canary Islands (e.g. Tenteniguada), (3) forage yields ranged from 200-450 g DM $plant^{-1}$ in experiments carried out at IMIDA-Murcia (Correal et al. 2003), Mediterranean accessions also being more productive. Moreover, (4) in both locations (Tenerife and Murcia) natural hybrids between accessions of Mediterranean tedera and Canarian tederas gave forage yields of 1-4 kg DM $plant^{-1}$, that is, 5-8 times higher than those obtained from native accessions. Finally, (5) similar results (3 Mg DM ha^{-1}) were obtained with hybrids in WA, where large-scale tedera trials are being carried out to evaluate its productivity under sheep grazing.

6. TOOLS TO ASSIST THE *BITUMINARIA BITUMINOSA* BREEDING PROGRAMME

Once breeding lines have been selected, it is necessary to develop tools in order to assist in germplasm conservation, evaluation of tolerance to stresses and FC content evaluation.

Plant biotechnology, in its broadest sense, offers multiple methods, including plant tissue culture, hydroponic culture, cell biology and molecular biology, for the evaluation, germplasm conservation and commercial propagation of selected breeding lines valuable for the pharmaceutical industry (large-scale production of interesting secondary metabolites), or for pasture production (Wink 1999).

6.1. Hydroponic Culture

The cultivation of plants in hydroponic culture (i.e., in aerated nutrient solution) is a useful tool for the study of the effect of external factors, individually or in combination, on all aspects of plant growth: net biomass production, physiology, biochemistry and anatomy (Figure 3). It also gives rise to clean roots, which can be analysed easily. We have employed it to study the response of *B. bituminosa* to external stresses: salinity, waterlogging (hypoxia), water deprivation, heavy metals and elevated temperature.

Teakle and Real (2010) grew *B. bituminosa* accessions in saline (200 mM NaCl) and/or stagnant nutrient solution, to simulate the conditions in waterlogged, saline soils of pastures in southern Australia. After 27 days of exposure to these conditions, root and shoot growth declined (relative to control conditions of non-saline, aerated solution) in the order: stagnant, non-saline solution > aerated, saline solution > stagnant, saline solution. There was variation among the accessions; for example, the accession most sensitive to –waterlogging‖ was the only one which did not develop root aerenchyma. The results indicate that it will be possible to select elite plants with elevated tolerance of transient waterlogging in saline soils. Unpublished, preliminary work showed that exposure of *B. bituminosa* to moderate salinity (90 mM NaCl) produced increases of up to 30% in the tissue levels of the FCs, angelicin and psoralen.

Hydroponic assays in which polyethylene glycol (PEG) was included in the nutrient solution in order to lower its water potential were performed in order to examine the physiological response of *B. bituminosa* to water deprivation. They showed that, as expected, the root hydraulic conductivity of water decreased in the presence of PEG while the root:shoot biomass ratio increased; this is a common phenomenon which minimises the water loss/uptake ratio. The two accessions analysed did, however, differ in their accumulation of proline (an indicator of water deprivation) and the results - when combined with those of pot and field assays - gave valuable insights into the drought tolerance of this species (Martínez-Fernández et al. 2012).

The effects on plants of heavy metals present in nutrient solution, singly or in combination, can be studied in detail, since commercially-available computer programs permit the chemical speciation of the metals to be determined. Thus, Martínez-Fernández et al. (2011) studied the effects of high nutrient solution zinc (Zn) concentrations on the growth and physiology of two contrasting populations of *B. bituminosa* (one, –C2‖, from a Zn-contaminated site and another from a –clean‖ site). Although the growth of the plants of population C2 was more Zn-resistant, the two populations had similar tissue levels of Zn and organic acids (which may detoxify intracellular Zn) as well as similar water relations. These authors also found that exposure to elevated cadmium (Cd), copper (Cu) or Zn disrupted plant water relations and increased the tissue accumulation of angelicin and psoralen, which protect stressed plants against infection and feeding.

Hydroponic culture of plants does have certain drawbacks. For example, the size and age of plants that can be grown are usually limited. Walker et al. (2012) found that plants of *B. bituminosa* grown hydroponically in the vegetative stage increased their leaf FC accumulation when the day/night temperature regime was changed from 22/17 ºC to 33/22 ºC. However, in the field, mature plants from the same accessions had lower leaf FC levels in early summer (mean maximum temperature of 30 ºC) than in the autumn (20 ºC), attributable to greater FC accumulation in seeds and roots in early summer, where their protective effects help to ensure the survival of the population or individual plant.

6.2. *In vitro* Plant Tissue Culture

The development of biotechnological tools by *in vitro* tissue culture systems, initiated from cells, tissues and organs of *B. bituminosa*, offers an attractive approach for industrial purposes: large-scale propagation, conservation of germplasm, the possibility of selecting somaclonal variants, obtaining anther or microspore-derived haploid plants and regenerating genetically-transformed plants to control the biosynthesis of FCs, and other bioactive compounds in a short span of time, for industrial uses.

Since no previous work on *in vitro* culture had been carried out with this species, we firstly established micropropagation protocols for several clones of tedera and inter-varietal hybrids resulting from crosses among var. *bituminosa* (either Mediterranean or Canarian) and the other two varieties (*albomarginata* and *crassiuscula*) selected in the breeding programmes for their potential as forage or as sources of FCs. By using apical and nodal explants cultured on a modified Murashige and Skoog (MS) medium (1962) with organic additives and plant growth regulators (PGRs), differences among genotypes were observed not only in the frequency of sprouted shoots but also in the rooting frequency of shoots. The micropropagated plants were established *ex vitro* in the field and the FC levels were equal to or even higher than those in the non-micropropagated mother plants. Moreover, they maintained the original characteristics of the mother plants, showing that the micropropagation protocols established are a good method for the multiplication of selected plants (Pazos-Navarro et al. 2012).

Plant regeneration from explants by *de novo* shoot or embryo formation via organogenesis (Pazos-Navarro et al. 2013) or somatic embryogenesis (unpublished data) has been evaluated. A high frequency of plant regeneration via organogenesis using different explants of tedera has been achieved (Pazos-Navarro et al. 2013). Three explant types from *in vitro* plants were used: leaflets, petiole-leaf-attachment (PLA) and petioles (Figure 3). For callus induction we use a modified MS medium supplemented with organic additives and different concentrations and combinations of plant growth regulators (PGRs): Naphthalene-acetic acid (NAA) or Indole-acetic acid (IAA; 0.5, 5 M) combined with Benzylaminopurine (BA; 5, 10, 20 M). For shoot development, 0.5 M NAA or IAA was combined with 5 M BA. After 4 weeks of culture on callus induction medium, 100% organogenic calli was obtained independent of the explant type and culture medium assayed; the aspect of the calli was compact and green (Figure 3). However, on the shoot development medium, the average number of developed shoots per callus depended on the explant type and the previous callus induction medium. For leaflet and PLA explants, the previous culture on 5 M IAA + 10 M

BA induction medium was better, whereas for petioles 0.5 M NAA + 10 M BA was better. Independent of the explant type, the developed shoots rooted on 10 M NAA + 1 M GA_3. The *ex vitro* acclimatisation was successful, achieving 96.4 % survival in the greenhouse (Figure 3) and 100 % in the field (Figure 3).

The FC content (i.e., psoralen, angelicin and total content) was examined along the plant regeneration process, from organogenic calli, *in vitro* regenerated shoots and *ex vitro* plants (grown in the greenhouse and grown in the field for 1 or 4 months), and compared with cultivated plants in the experimental field. The accumulation of FCs depended on the level of cellular organisation (the lowest FC content was in calli), the developmental stage of the plants and their exposure to environmental factors, being higher in *ex vitro* plants in the field, as Diwan and Malpathak (2010) observed in *in vitro cultures* of *R. graveolens* (another FC producer species).

The plant regeneration via embryogenesis has also been achieved from different explants of tedera and albo-tedera (unpublished data). The results show that explant type is a key factor in the frequency of embryogenesis and in the number of embryos per callus.

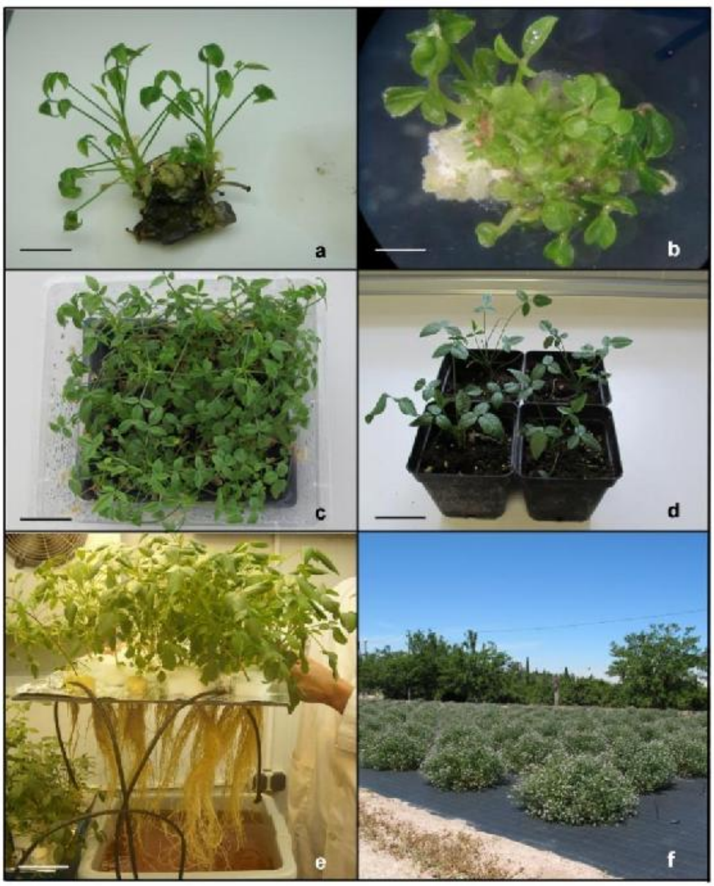


Figure 3. a. Micropropagation of *Bituminaria bituminosa* plants from nodal segments, b. organogenic callus with multiple shoots, c. regenerated plants form organogenic calli, d. acclimatisation of plants, e. plants in hydroponic culture, and f. micropropagated plants growing in the experimental field. Bar: 20 mm (a, c); 5 mm (b); 50 mm (d); 100 mm (e).

Genetic characterisation of *B. bituminosa*, elicitation experiments involving abiotic and biotic factors and cryopreservation of meristems are currently in progress, in collaboration with several research groups from Western Australia or Spain.

6.3. Screening of Plant Cold Tolerance Using a Controlled-freezing Chamber

For young plants grown in pots, the use of a controlled-freezing chamber is a simple way to screen their cold (freezing) tolerance and hence select lines appropriate for cultivation in frost-prone areas. The plants are placed in the chamber (set at ambient temperature) and then the temperature is reduced slowly (1-4ºC $hour^{-1}$) to the desired temperature (for *B. bituminosa*, between -5 and -10ºC); the plants are then removed and replaced in their original place of cultivation. They are monitored for up to one month, while visual symptoms of freezing damage (leaf and shoot meristem necrosis) appear. The populations or breeding lines showing least damage can be selected for planting at the chosen field site. The –selection temperature‖ is chosen according to the harshness of the winter at this site.

For plants already growing in the field, measurement of the freezing-induced electrolyte leakage from detached leaves, using a conductivity meter, is a simple method of assessing their cold tolerance before the onset of winter. This leakage arises due to damage caused to the cell plasma membrane by freezing, particularly due to loss of intracellular water to ice on the leaf surface, down a water potential gradient (Xin and Browse 2000). The leaves are misted with distilled water (to allow the formation of ice crystals on the surfaces) before being placed in the controlled-freezing chamber. The temperature is then reduced slowly, with a period of one hour at -1ºC to allow ice formation, to the desired temperatures (for *B. bituminosa*, between -2 and -12ºC); the temperature is maintained at each of these temperatures for 30 minutes and then a sample of the leaves is removed and stored at 4ºC. After thawing-out and development of membrane damage (typically 24 hours), the leaves are incubated in water and the electrical conductivity (EC) of the –extract‖ measured; the leaves are then heated to 90 ºC before the EC (assumed to represent the maximum value, after destruction of the membranes) is measured again. Expression of the post-thawing EC as a percentage of the maximum permits calculation of the temperature at which 50% membrane damage occurred; this LT_{50} value can be used to compare plants, populations and acclimation. The performance of these assays at different points of the plant growth cycle (for example, in early winter, when the plants are acclimated to cold, and in spring, following de-acclimation) allows correlation of the *in vivo* cold tolerance of the leaves with physiological parameters measured at the same time. In the case of *B. bituminosa*, Walker et al. (2010) demonstrated a relationship between cold tolerance and osmotic adjustment in the leaves, related to the accumulation of soluble sugars and amino acids.

Discrepancies may arise between the apparent cold tolerance determined with plants grown in pots or detached leaves and the behaviour of plants in the field during winter. This is because it is impossible to recreate natural conditions (which represent a combination of different, simultaneous stresses) for pot-grown plants (which may also be subjected to freezing tests after little or no acclimation). Also, for –quick‖ (24 hours) assays with detached leaves, membrane injury is assessed but damage which takes longer to develop (such as oxidative stress and necrosis) is not. However, freezing-chamber assays accelerate the screening process.

CONCLUSION

Thanks to this multidisciplinary research group (IMIDA-ICIA-CIBIO-MU-WA) and other international groups (Italy, Israel, Greece and Turkey), *B. bituminosa* has been shown to be an important legume with two potential uses: (1) forage and (2) pharmaceutical. In this sense, breeding lines have been selected showing desirable traits such as high forage yield, tolerance to abiotic stresses or high FC content. However, efforts are still needed to collect germplasm from cold Mediterranean environments, to identify the core collection in each Mediterranean region for the important breeder‘s plant traits and to develop molecular markers for key plant traits. So, as future priorities, we need to (1) establish a Mediterranean breeding programme to develop high-yielding and quality tedera cultivars adapted to cold environments, (2) foster international cooperation for the efficient management of genetic resources and human knowledge, (3) develop new genetic tools in order to select new individuals for pharmaceutical purposes and (4) search for new germplasm from different origins, to incorporate into plant breeding programmes so as to enrich the lines already obtained.

REFERENCES

Alliota, G; Piomelli, D; Pollio, A, Touwaide, A. *Le Piante Medicinalle del Corpus Hippocraticum*. Geurini e Associati. Napoli, 2003.

Alvar, M. *El español hablado en Tenerife*. CSIC. Madrid, Spain, 1959.

Álvarez, J. Leyenda erudita sobre la población de Canarias con africanos de lenguas cortadas. *Anuario de Estudios Atlánticos*, 1977, 23, 51-81.

Álvarez, S; Fresno, M; Darmanin, N; Briggs, HR; Méndez, P; Castro, N. Native forages Assessment for the improvement of milk production in goats. *Journal of Animal Veterinary Advances*, 2007, 6 (1), 94-98.

Álvarez, S; Fresno, M; Méndez, P. In: Hopkins A, Gustaffson T, Bertilsson J, Dalin G, Nilsdotter-Linde N, Spórndly E. *Biodiversity and Animal Feed. Future Challenges for Grassland Production. 22nd General Meeting European Grassland Federation*. Uppsala-Sweden; 2008, 72.

Asín Palacios, M. *Glosario de voces romances. Registradas por el botánico anónimo hispano-musulmán (Siglos XI-XII).* CSIC/Escuela de Estudios Árabes. Madrid-Granada; 1943.

Bellakhdar, J. *Mèdecine arabe ancienne et savoirs populaires. La pharmacopée marocaíne traditionelle.* Ed.Dumas. Paris, 1997.

Bolinaga, J. *Pastos para secano*. La Región; 1915, 6, 7-9.

Bonnier, G; Layens, G. *Flore complète portative de la France, de la Suisse et de la Belgie.* Ed. Librairie Générale. Paris; 1964.

Bourgaud, F; Allard N; Guckert A; Forlot P. Natural sources of furocoumarins (psoralens). In: *Psoralens, Past, Present and Future of Photochemoprotection and other biological activities*. John Libbey Eurotext, Montrouge; 1989, 219-229.

Bustamante, J; Corriente, F; Tilmatine, M. Abulhayr Al 'Išbīlī (s.V/XI) "*Kitā*bu 'Umdati *Ţţabīb Fī Ma 'Rifati Nnabāt Likulli Labīb*" (*Doctor Basical Book for Botany Knowledge for all expert*). Vol. II. CSIC Madrid, 2007.

Bustamante, J; Corriente, F; Tilmatine, M. Abulhayr Al 'Išbīlī (s.V/XI) "*Kitā*bu 'Umdati *Ţţabīb Fī Ma 'Rifati Nnabāt Likulli Labīb*" (*Doctor Basical Book for Botany Knowledge for all expert*). Vol. III. T.1. CSIC Madrid, 2010.

Bustamante, J; Corriente, F; Tilmatine, M. Abulhayr Al 'Išbīlī (s.V/XI) "*Kitā*bu 'Umdati *Ţţabīb Fī Ma 'Rifati Nnabāt Likulli Labīb*" (*Doctor Basical Book for Botany Knowledge for all expert*). Vol. III. T.2. CSIC Madrid, 2010.

Cano, F; Ríos, S. 8. Etnobotánica. In: Ríos, S. & Alcaraz, F. Editors. *Flora de las riberas y zonas húmedas de la cuenca del río Segura.* Ed. Serv. Publ. Universidad de Murcia, 1996.

Capote, J; Tejera, A; Amills, M; Argüello, A; Fresno1, M; López, JL. Influencia histórica y actual de los genotipos canarios en la población caprina americana (FAO) *Animal Genetic Resources Information*, 2004, 35, 49-60.

Cianfrogna, JA; Zangerl, AR; Berenbaum, MR. Effects of furanocoumarins on feeding behavior of parsnip webworms *Depressaria pastinacella. Journal of Chemical Ecology*, 2002, 28, 1365-1375.

Coca, B; Ríos, S; Juan, A; Méndez, P; Santos, A; Correal, E. Germoplasma de tedera (*Bituminaria bituminosa*): caracterización y descriptores. In: García B editor. *Pastos y Ganadería Extensiva.* Salamanca (Spain): Sociedad Española para el Estudio de los Pastos. CSIC, 2004, 123-127.

Cocks, PS. The adaptation of perennial legumes to Mediterranean conditions. In: Bennett SJ editor. *New perennial legumes for sustainable agriculture*. University of Western Australia Press, 2003, 224.

Congia, P. *Dizionario Botanico Sardo.* Ed. Zonza. Cagliari, 1998.

Corrales, C; Corbella, D. *Diccionario histórico del español en Canarias*. ICE. Colección Documentos de la Gomera, Spain; 2001.

Correal, E; Hoyos, A; Ríos, S; Méndez, P; Real, D; Snowball, R; Costa, J. Seed production of *Bituminaria bituminosa*: size, production, retention and germination capacity of the legumes. In: *Proceedings 12th Meeting FAO-CIHEAM Inter-regional Cooperation Research and Development Network on Pastures and Fodder crops*. Elvas (Portugal): CIHEAM; Options Méditerranéennes, Series A, 2008, 79, 379-383.

Correal, E; Moñino, I; Méndez, P; Ríos, S. Valoración agronómica de siete poblaciones de *Bituminaria bituminosa*: efecto de la sequía estival y siega sobre su persistencia y producción de hoja. In: Robles AB, editor. *Pastos, Desarrollo y Conservación (SEEP).* Junta de Andalucía, Consejería de Agricultura y Pesca. 2003, 35-40.

Correal, E; Robledo, A; Ríos, S; Rivera, D. Mediterranean dryland sheep-cereal systems. In: Lloveras J, González-Rodríguez A, Vázquez-Yañez O, Piñeiro J, Santamaría O, Olea L, Poblaciones MJ, editors. *Grassland Science in Europe*. EGF. Badajoz; 2006, vol 11, 14-26.

Curini, M; Carvotto, G; Epifano, F; Giannone, G. Chemistry and biological activity of natural and synthetic prenyloxycoumarins. *Current Medicinal Chemistry*, 2006, 13, 199-222.

Dear, BS; Ewing, MA. The search for new pasture plants to achieve more sustainable production systems in southern Australia. *Australian Journal of Experimental Agriculture*, 2008, 48, 387-396.

Dear, BS; Moore, GA; Hughes, SJ. Adaptation and potential contribution of temperate perennial legumes to the southern Australian wheat belt: A Review. *Australian Journal of Experimental Agriculture*, 2003, 43, 1-18.

Del Río, JA; Ortuño, A; Pérez, L; Bennet, RG; Real, D; Correal, E. Furanocoumarin content in *Bituminaria bituminosa* varieties and *Cullen* species. In: Porqueddu C, Ríos S, editors. *13th Meeting of the FAO-CIHEAM Sub-Network on Mediterranean Pastures and Fodder Crops. The Contributions of Grasslands to the Conservation of Mediterranean Biodiversity*. Alicante (Spain). Zaragoza: CIHEAM/CIBIO/FAO/SEEP; Options Méditerranéennes, Series A: Mediterranean Seminars; 2010, 92, 67-70.

Dioscórides, P. In: Laguna, A. *Pedacio Dioscórides Anazarbeo. Acerca de la Materia Médica Medicinal y de los venenos mortíferos.* (ed. facsimil). Biblioteca de Clásicos de la Medicina y de la Farmacia Española. Madrid; 1566.

E.D.E.S. *Estudio de las posibilidades de desarrollo agrícola en la isla de El Hierro*. Informe (inédito). 1969.

Fajardo, J; Verde, A; Rivera, D; Obon, C. *Etnobotánica en la Serranía de Cuenca. Las plantas y el hombre.* Diput. de Cuenca; 2007.

Font i Quer, P. *Plantas medicinales. El Dioscórides renovado*. Ed. Labor. Barcelona; 1985.

Forster, E. Trees and plants in Herodotus. *The Classical Review*, 1942, 56 (2), 57-63.

Fournier, P. *Le livre des plantes médicinales et vénéneuses de France*. Ed. Paul Lechevalier. Paris, 1948.

Gómez de Ortega, C. *Continuación de la Flora Española o Historia de las plantas de España, que escribía Joseph Quer.* T.VI. Madrid; 1784.

González-Tejero, Mª. *Investigaciones etnobotánicas en la provincia de Granada.* Tesis doctoral (inéd.) Facultad de Farmacia. Universidad de Granada; 1989.

Graham, PH; Vance, CP. Legumes: importance and constraints to greater use. *Plant Physiology*, 2003, 131, 872-877.

Guinea, E. 1947. Catálogo razonado de las plantas del Sahara español. *Anales del Jardín Botánico de Madrid*, 8(1), 357-442.

Guma, IR; Santos, A; Reyes, A; Padrón, MA; Méndez, P; González, R. Perennial forage legumes endemic to the Canary Islands: Collection and *ex situ* conservation. *Genetic Resources and Crop Evolution*, 2010, 58, 181-187.

Gupta, AK; Anderson, TF. Psoralen photochemotherapy. *Journal of American Academy of Dermatology*, 1987, 17, 703-734.

Hagemeier, J; Batz, O; Schmidt, J; Wray, V; Hahlbrock, K; Strack, D. Accumulation of phthalides in elicitor-treated cell suspension cultures of *Petroselinum crispum*. *Phytochemistry*, 1999, 51, 629–635.

Hamerski, D; Matern, U. Elicitor-induced biosynthesis of psoralens in *Ammi majus* L. suspension cultures. *European Journal of Biochemistry*, 1998, 171, 369–375.

Heathpagliuso, S; Matlin, SA; Fang, NB; Thompson, RH; Rappaport, L. Stimulation of furanocoumarin accumulation in celery and celeriac tissues by *Fusarium oxysporum* f sp apii, *Phytochemistry*, 1992, 31, 2683-2688.

Hönigsmann, H; Szeimies, RR; Knobler, R. Photochemotherapy and Photodynamic Therapy. In: *Fitzpatrick Dermatology in General Medicine*. McGraw-Hill Professional, New York, NY, USA; 2008; 2249-62.

Innocenti, G; Cappelletti, EM; Caporale, G. Furocoumarin contents in the vegetative organs of cultivated *Psoralea* species. *International Journal of Pharmacognosy*, 1991, 29 (4), 311-316.

Innocenti, G; Piovan, A; Filippini, R; Caniato, R; Cappelletti, EM. Quantitative recovery of furanocoumarins from *Psoralea bituminosa*. *Phytochemical Analysis*, 1997, 8, 84-86.

Jaizme, MC; Méndez, P; Flores, H. Efecto de la micorrización sobre la tedera (*Bituminaria bituminosa*) en las primeras fases de cultivo. In: *Biodiversidad en Pastos*, 2001, 181-187.

James, R. *Pharmacopeia Universalis: or a new universal English dispensatory*. London; 1747.

Juan, A; Coca, B; Crespo, MB; Ríos, S; Correal, E. Datos preliminares sobre variabilidad molecular poblacional en el agregado de *Bituminaria bituminosa* (*Leguminosae*). In: de la Roza B, editor. *Producciones agroganaderas: gestión eficiente y conservación del medio natural*. Sociedad Española para el Estudio de los Pastos. XLV Reunión Científica. Gijón, Asturias (Spain), 2005, Vol II, 971-977.

Juan, A; Moñino, I; Correal, E; Crespo, MB; Coca, B. 2003. Producción de frutos en poblaciones canarias e ibéricas de *Bituminaria bituminosa*. In: Robles AB, editors. *Pastos, Desarrollo y Conservación (SEEP)*. Junta de Andalucía, Consejería de Agricultura y Pesca. 2003, 41-46.

Juan, A; Moñino, I; Correal, E; Méndez, P; Crespo, MB. 2004. Comparación de las tasas de fructificación de *Bituminaria bituminosa* (*Leguminosae*) bajo condiciones de cultivo en Canarias y la Península Ibérica. In: García B, editor. *Pastos y Ganadería Extensiva*. Salamanca (Spain): Sociedad Española para el Estudio de los Pastos. CSIC, 2004, 111-115.

Laguna, A. *Pedacio Dioscórides Anazarbeo. Acerca de la Materia Médica Medicinal y de los venenos mortíferos*. (ed. facsimil). Biblioteca de Clásicos de la Medicina y de la Farmacia Española. Madrid; 1566.

Lázaro Ibiza, B. *Botánica descriptiva. Compendio de la Flora Española*. T.I. Madrid, 1906.

Lewis, G; Schrire, B; Mackinder, B; Lock, M. *Legumes of the world*. Royal Botanical Gardens, Kew, Richmond, Surrey, UK (Ed); 2005.

Li, GD; Lodge, GM; Moore, GA; Craig, AD; Dear, BS; Boschma, P. Evaluation of perennial pasture legumes and herbs to identify species with high herbage production and persistence in mixed farming zones in southern Australia. *Australian Journal of Experimental Agriculture*, 2008, 48, 449-466.

Li, X; Schuler, M; Berenbaum, M. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology*, 2007, 52, 231-253.

Llorente Maldonado de Guevara, A. *El léxico del tomo I del "Atlas lingüístico y etnográfico de las islas Canarias"*. Universidad de Extremadura; 1987.

Machado, A. La biodiversidad de las Islas Canarias. Capítulo 7. In: Pineda FD, de Miguel JM, Casado MA, Montalvo J, editors. *La diversidad biológica de España*. Madrid (Spain): Pearson Educación, S.A., 2002; 89-99.

Macías Hernández, A. El sector pecuario en la economía canaria, 1400-1970. Algunas notas para su estudio. *Reunion SEEP, Tenerife;* 1995.

Macías Hernández, A. La economía de los primeros isleños. In: Bethencourt Massieu, A. editor, *Historia de Canarias*, Las Palmas de Gran Canaria, 1995.

Martínez, S; Correal, E; Real, D; Ortuño, A; Del Río, JA. *Bituminaria bituminosa*: A source of furanocoumarins of pharmaceutical interest. In: Awaad AS, Govil JN, SinghVK editors. *Encyclopedia of Medicinal Plants. Drug Plants I*. Houston, TX, USA.: Stadium Press LLC, 2010, 307–322.

Martínez-Fernández, D; Walker, DJ; Romero, P; Correal, E. The physiology of drought tolerance in Tedera (*Bituminaria bituminosa*). In: Porqueddu C, Ríos S, editors. *13th Meeting of the FAO-CIHEAM Sub-Network on Mediterranean Pastures and Fodder Crops. The Contributions of Grasslands to the Conservation of Mediterranean Biodiversity*. Alicante (Spain). Zaragoza: CIHEAM/CIBIO/FAO/SEEP; Options Méditerranéennes. Series A: Mediterranean Seminars; 2010, 92, 155-159.

Martínez-Fernández, D; Walker, DJ; Romero-Espinar, P; Flores, P; Del Río, JA. Physiological responses of *Bituminaria bituminosa* to heavy metals. *Journal of Plant Physiology*, 2011. 168, 2206-2211.

Martínez-Fernández, D; Walker, DJ; Romero, P; Martínez-Ballesta, MC; Correal, E. The response of the leguminous fodder plant *Bituminaria bituminosa* to water stress. *Journal of Agronomy and Crop Science*, 2012, 198 (6), 442-451.

Masuda, T; Takasugi, M; Anetai, M. Psoralen and other linear furanocoumarins as phytoalexins in *Glehnia littoralis*. *Phytochemistry*, 1998, 47, 13-16.

Maurich, T; Iorio, M; Chimenti, D; Turchi, G. Erybraedin C and bitucarpin A, two structurally related pterocarpans purified from *Bituminaria bituminosa*, induced apoptosis in human colon adenocarcinoma cell lines MMR- and p53-proficient and –deficient in a dose-, time-, and structure-dependent fashion. *Chemico-Biological Interactions*, 2006, 159, 104-116.

Méndez, P. Forage potential of Canary Island Legumes. In: FAO REUR Technical Series 28. *Management of Mediterranean Shurblands and Related Forage Resources.*Chania-Greece. 1993; 141-144.

Méndez, P. El heno de tedera (*Bituminaria bituminosa*): un forraje apetecible para el caprino. In: *III Reunión Ibérica de Pastos y Forrajes*. Bragança-A Coruña-Lugo; 2000, 411-414.

Méndez, P. La tedera. Un forraje conocido pero poco valorado. *Agropalca*, 2009, 4, 19.

Méndez, P; Álvarez, S; Fresno, M. Nutritive value and yield under irrigation of tedera hay (*Bituminaria bituminosa*) an alternative forage for warm semi-arid to sub-humid climate. In: Hopkins A, Gustaffson T, Bertilsson J, Dalin G, Nilsdotter-Linde N, Spórndly E. *Biodiversity and Animal Feed. Future Challenges for Grassland Production. 22nd General Meeting European Grassland Federation*. Uppsala-Sweden; 2008, 52.

Méndez, P; Álvarez, S; Morales, E; Bermejo, L. Quantity and quality of goat milk from a herd grazing on a cultivated pasture with tedera (*Bituminaria bituminosa*) and barley as main constituent. *Advances in Animal Biosciences*, 2011b, 2 (2), 495.

Méndez, P; Bermejo, L; Morales, E; Álvarez, S. Effect of goat grazing on tedera pasture (*Bituminaria bituminosa*). *Advances in Animal Biosciences*, 2011a, 2 (2), 375.

Méndez, P; Díaz, E; Rivero, R. Contenido en cumarinas del forraje verde de tedera (*Bituminaria bituminosa*). In: Crespo MB, Juan A, Ríos S, editors. *Biodiversidad en pastos. Actas de la XLI Reunión Científica de la SEEP* – Foro Iberoamericano de Pastos, CIBIO, Alicante, España; 2001, 335-339.

Méndez, P; Fernández, M. Interés forrajero de las variedades de *Bituminaria bituminosa* (L.) Stirton (tedera) de Canarias. *Pastos*, 1990, nº extraordinario, 264-271.

Méndez, P; Fernández, M. La producción forrajera de las islas Canarias. *El Campo. Boletín de Información Agraria*; 1992, 124: 66-70.

Méndez, P; Fernández, M; Santos, A. Variedades de *Bituminaria bituminosa* (L.) Stirton (*Leguminosae*) en el archipiélago canario. *Pastos*, 1990-91, vol XX-XXI, 157-166.

Méndez, P; Peters, J; Morales, D; Jiménez, MS. Características del intercambio gaseoso de la tedera (*Bituminaria bituminosa*). In: Consellería de Agricultura, Ganadería e Política Agroalimentaria. *III Reunión Ibérica de Pastos y Forrajes.* Bragança-A Coruña-Lugo; 2000, 91-96.

Méndez, P; Santos, A; Correal, E; Ríos, S. Agronomic traits as forage crop of nineteen populations of *Bituminaria bituminosa*. In: Lloveras J, González-Rodríguez A, Vázquez-Yañez O, Piñeiro J, Santamaría O, Olea L, Poblaciones MJ, editors. *Grassland science in Europe*, EGF, Badajoz; 2006, vol.11, 300-302.

Minissale, P; Brullo, C; Brullo, S; Del Galdo, GG; Sciandrello, S. *Bituminaria basaltica* (Fabaceae), a new species from Italy. *Phytotaxa*, 2013, 98 (1), 1-15.

Miró, M. *Screening fitoquímico en plantas superiores. Detección de actividad antiinflamatoria y antibacteriana*. Memoria doctoral (ined.). Facultad de Farmacia. Universidad de Granada; 1981.

Molero, J; Pérez Raya, F; Valle, F. (Eds.). *Parque Natural de Sierra Nevada. Paisaje, Fauna, Flora, Itinerarios*. Ed. Rueda. Madrid; 1992.

Moll, M. *Medicina popular Menorquina. Segles XVI-XXI*. Ed. Documenta Balear. Mallorca; 2003.

Morales, J; Rodríguez, A; Alberto, V; Machado, C; Criado, C; Rando, JC. The impact of human activities on the natural environment of the Canary Islands (Spain) during the pre-Hispanic stage (3rd–2ndCentury BC to 15th Century AD): an overview. *Environmental Archaeology*, 2009, 14(1), 27-36.

Mulet, P. *Estudio etnobotánico de la provincia de Castellón.* Excma. Diput. de Castelló; 1991.

Muñoz, A; Correal, E. *Bituminaria bituminosa* (L.) Stirton, leguminosa de interés forrajero en la Cuenca Mediterránea: I. Situación taxonómica, distribución y autoecología. In: *Actas XXXVIII Reunión Científica de la SEEP* Soria (Spain); 1998, 87-91.

Muñoz, A; Correal, E. *Bituminaria bituminosa* (L) Stirton, leguminosa de interés forrajero en la Cuenca Mediterránea: II. Comportamiento frente a frío y selección de material tolerante. In: *Actas XXXIX Reunión Científica de la SEEP* Almería (Spain); 1999, 257-262.

Muñoz, A; Ortiz-Dorda, J; Correal, E. Morphological and molecular characterization of *Bituminaria bituminosa* accessions from south-east Spain and the Canary Islands. In: CIHEAM, *Cahiers Options Méditerranéenne,* 2000, 45, 103-107.

Obón, C. & Rivera, D. *Las plantas medicinales de nuestra Región*. Ed. Regional de Murcia; 1991.

Pagnotta, MA; Bennet, PS; Cocks, PS; Ewing, M; Howieson, J; Moore, G; Norman, H; Nutt, B; Porqueddu, C; Russi, L; Snowball, R. Evaluation of perennial legumes collected in Italy: First year data. In: Bennett SJ editor. *New perennial legumes for sustainable agriculture*. University of Western Australia Press, 2003; 224.

Parada, M; Selga, A; Bonet, Mª; Valles, J. *Etnobotànica de les Terres Gironines.* Dip. de Girona, 2002.

Pascual, H. *Leguminosas de la Península Ibérica y Baleares.* INIA/Ministerio de Agricultura. Madrid; 1978.

Pascual, MJ; Correal, E. *Especies leguminosas de interés económico*. Pisa Ediciones, Valencia. 1991, 94 pp.

Pazos-Navarro, M; Dabauza, M; Correal, E; Hanson, K; Teakle, N; Real, D; Nelson, M. Next generation DNA sequencing technology delivers valuable genetic markers for the genomic orphan legume species, *Bituminaria bituminosa*. *BMC Genetics*, 2011, 12, 104.

Pazos-Navarro, M; Del Río, JA; Ortuño, A; Correal, E; Dabauza, M. Micropropagation from apical and nodal segments of *Bituminaria bituminosa* and the furanocoumarin content of propagated plants. *Journal of Horticultural Science & Biotechnology*, 2012, 87(1), 29-35.

Pazos-Navarro, M; Del Río, JA; Ortuño, A; Romero-Espinar, P; Correal, E; Dabauza, M. Plant regeneration from different explant types of *Bituminaria bituminosa* and furanocoumarin content along plant regeneration stages. *Plant Growth Regulation*, 2013 (in press) DOI 10.1007/s10725-013-9784-9.

Pellicer, J. *Costumari Botànic. (2)*. Ed. del Bullent. Valencia, 2000.

Pérez, J. La ganadería canaria. Notas Histórico- Etnográficas. *Anuario de Estudios Atlánticos*, 1963, 9, 237.

Pérez, V; Sagot, P. *De la végétation aux îles Canaries. Des plantes des pays tempérés et des plantes des régions intertropicales et Physionomie générale de leur agricultura*. París, 1867.

Pistelli, L; Noccioli, C; Appendino, G; Bianchi, F; Sterner, O; Ballero, M. Pterocarpans from *Bituminaria bituminosa* and *Bituminaria morisiana*. *Phytochemistry*, 2003, 64, 595-598.

Ramos, G; Frutos, P; Giráldez, FC; Mantecón, AR. Los compuestos secundarios de las plantas en la nutrición de los herbívoros. *Archivos de Zootecnia*, 1998, 47, 597-620.

Ramos-Rodríguez, E; de la Puente-Jiménez, JL. *Posibilidades de desarrollo ganadero con base territorial en las islas Canarias*. Informe de la Agencia de Desarrollo Ganadero de la Consejería de Agricultura, Ganadería y Pesca de la Junta de Canarias. (inédito). 1983.

Real, D; Albersten, T; Snowball, R; Howieson, J; Revell, C; Ewing, M; Correal, E; Méndez, P; Ríos, S. *Bituminaria bituminosa* var. *albomarginata* (Lancelot trefoil), a novel perennial forage legume for low-rainfall Mediterranean environments in Western Australia. In: *XXI International Grassland Congress and VIII International Rangeland Congress*, Hohhot, China, 2008, Vol II, 452.

Real, D; Correal, E; Méndez, P; Santos, A.; Ríos, S; Sternberg, M; Dini-Papanastasi, O; Pecetti, L; Tava, A. *Bituminaria bituminosa* C.H. Stirton. Grassland Species Profiles, CIAT/FAO. www.fao.org/ag/AGP/AGPC/doc/GBASE/new_species/tedera/bitbit. htm. 2009.

Real, D; Foster, K; Correal, E; Kidd, D; Méndez, P. Techniques for artificial hybridization in the self-pollinated forage legume *Bituminaria bituminosa* and the cross-pollinated forage legume *Hedysarum coronarium*. In: XXI *International Grassland Congress and VIII International Rangeland Congress*, Hohhot, China, 2008, Vol II, 347.

Real, D; Li, GD; Clark, S; Allbersten, TO; Hayes, RC; Denton, MD; D‘antuono, MF; Dear, BS. Evaluation of perennial forage legumes and herbs in six Mediterranean environments. *Chilean Journal of Agricultural Research*, 2011, 71(3), 357-369.

Reyes Prosper, E. *Las estepas de España y su vegetación*. Madrid, 1915.

Ríos, S; Correal, E; Robledo, A. First screening of the more interesting pasture legumes present in matorral areas of south-east Spain. In: *Proceedings IVth. Int. Rangeland Congress*. Montpellier, Francia Edit. AFP, 1993. Vol. 1, 417-421.

Ríos, S; Robledo, A; Correal, E. Perennial pasture legumes of south-east Spain. FAO Bull. *Herba*, 1992, 5, 68-76.

Rivera, D; Alcaraz, F; Verde, A; Fajardo, J; Obón, C. *Las plantas en la cultura popular. Vol. 9*. Ed. Soc. Medit. de Historia Natural/CAM. Murcia, 2008.

Rivera, D; Matilla, G; Obón, C; Alcaraz, F. *Plants and humans in the Near East and Caucasus. Vol. 2. The Plants: Angiosperms*. Plants and humans/Edit.um. Murcia, 2012.

Rivera, D; Obon, C; Cano, F; Robledo, A. *Introducción al mundo de las plantas medicinales en Murcia*. Ayuntamiento de Murcia, 1994.

Sánchez, MA; Soriano, MC; Pérez, O; Correal, E. Determination of condensed tannins in perennial forage legumes and its effect on their palatability for sheep. In: *18th International Conference on Polyphenols*. Bourdeaux (France); 1996, Vol.1, 243-244.

Sandral, GA; Dear, BS; Viragona, JM; Swan, AD; Orchard, BA. Changes in soil water content under annual- and perennial-based pasture systems in the wheatbelt of southern New South Wales. *Australian Journal of Agricultural Research*, 2006, 57, 321-333.

Scarisbrick, JJ; Taylor, P; Holtick, U; Makar, Y; Douglas, K; Berlin, G; Juvonen, E; Marshall, S. U.K. consensus statement on the use of extracorporeal photopheresis for treatment of cutaneous T-cell lymphoma and chronic graft-versus-host disease. *British Journal of Dermatology*, 2008, 158 (4), 659-78.

Singh, RJ. Landmark research in grain legumes. In: Singh RJ and Jauhar PP editors. *Genetic Resources, Chromosome Engineering, and Crop Improvement Series: Grain legumes*, CRC Press, Inc., Boca Ratón, Fla., 2005, Vol 1 1-9.

Sing, RJ. Landmark research in grain legumes. In: Singh RJ and Jauhar PP editors. *Genetic Resources, Chromosome Engineering, and Crop Improvement Series: Oilseed crops*. CRC Press, Inc., Boca Ratón, Fla., 2007, Vol 4, 1-12.

Singh, RJ; Chung, GH; Nelson, RL. Landmark research in legumes. *Genome*, 2007, 50, 525-537

Stenberg, M; Gishri, N; Mabjeesh, SJ. Effects of grazing on *Bituminaria bituminosa* (L.) Stirton: a potential forage crop in Mediterranean grasslands. *Journal of Agronomy and Crop Science*, 2006, 192, 399-407.

Stern, RS. Psoralen and ultraviolet A light therapy for psoriasis. *The New England Journal of Medicine*, 2007, 357, 682-690.

Stevenson, PC; Simmonds, MSJ; Yule, MA; Veitch, NC; Kite, GC; Irwin, D; Legg, M. Insect antifeedant furanocoumarins from *Tetradium daniellii*. *Phytochemistry*, 2003, 63, 41-46.

Tava, A; Pecetti, L; Ricci, M; Pagnotta, MA; Russi, L. Volatile compounds from leaves and flowers of *Bituminaria bituminosa* (L.) Stirt. (Fabaceae) from Italy. *Flavour and Fragance Journal*, 2007, 22 (5), 363-370.

Tournefort, J.P. *Institutiones rei Herbariae.* T.I. Paris, 1700.

Vasconsuelo, A; Boland, R. Molecular aspects of the early stages of elicitacion of secondary metabolites in plants. *Plant Science*, 2007, 172, 861-875.

Ventura, MR; Bastianelli, D; Hassoun, P; Flores, MP; Bonnal, L; González-García, E. Nutrient and tannins content of browsing shrub legumes informally used for small ruminant feeding in Canary *Island*, Spain. In: Acar Z, López-Francos A, Porqueddu C. editors. *New approaches for grassland research in a context of climate and socio-economic changes*. Zaragoza: Centre International de Hautes Etudes Agronomiques Méditerranéennes (CIHEAM) / Ondokuz Mayis University. (Options Méditerranénnes, Series A: Mediterranean Seminars) 2012, 102, 391-394.

Ventura, MR; Castañón, JIR; Méndez, P. Effect of season on tedera (*Bituminaria bituminosa*) intake by goats. *Animal Feed Science and Technology*, 2009, 153, 314-319.

Ventura, MR; Castañón, JIR; Pieltain, MC; Flores, MP. Nutritive value of forage shrubs: *Bituminaria bituminosa*, *Rumex lunaria*, *Acacia salicina*, *Cassia surtii* and *Adenocarpus foliolosus*. *Small Ruminant Research*, 2004, 52, 13-18.

Ventura, MR; Méndez, P; Flores, MP, Rodríguez and Castañón, JIR. Energy and protein content of tedera (*Bituminaria bituminosa*). In: *Cahiers Options Mediterranéennes*, 2000, 45, 219-221.

Verde, A; Rivera, D; Fajardo, J; Obón, C; Cebrián, F. *Guía de las plantas medicinales de Castilla la Mancha*. Altaban Ediciones. Albacete, 2008.

Villar, L; Palacín, JMª; Calvo, C; Gómez García, D; Montserrat Martí, G. *Plantas Medicinales del Pirineo Aragonés y demás tierras oscenses*. CSIC/Dip. Huesca, 1987.

Walker, DJ; Bernal, MP; Correal, E. The influence of heavy metals and mineral nutrient supply on *Bituminaria bituminosa*. *Water, Air & Soil Pollution*, 2007, 184, 335-345.

Walker, DJ; Martínez-Fernández, D; Correal, E; Romero-Espinar, P; Del Río, JA. Accumulation of furanocoumarins by *Bituminaria bituminosa* in relation to plant development and environmental stress. *Plant Physiology and Biochemistry*, 2012, 54, 133-139.

Walker, DJ; Moñino, I; Correal, E. Genome size in *Bituminaria bituminosa* (L.) C.H. Stirton (*Fabaceae*) populations: separation of –true‖ differences from environmental effects on DNA determination. *Environmental and Experimental Botany*, 2006, 55, 258-265.

Walker, DJ; Romero, P; Correal, E. Cold tolerance, water relations and accumulation of osmolytes in *Bituminaria bituminosa*. *Biologia Plantarum*, 2010, 54 (2), 293-298.

Wölfel, DJ. *Monumenta Linguae Canariae. Die kanarischen sprachdenkmäler. Eine Studie zur Vor- und Frühgeschichte Weißafrikas*. Akademische Druck-u. Verlagsanstalt. Graz (Austria); 1965.

Xin, Z; Browse, J. Cold comfort farm: the acclimation of plants to freezing temperatures. *Plant, Cell and Environment*, 2000, 23, 893-902.