Effects of two water regimes on morphological traits, nutritive value and physiology of three *Bituminaria bituminosa* varieties from the Canary Islands

Jesús Fernández-Habas[1], Mª Teresa Hidalgo-Fernández[1], José Ramón Leal-Murillo[1], Pilar Méndez[2], José L Quero[1], Tom Vanwalleghem[3], Pilar Fernández-Rebollo[1]

[1] Department of Forest Engineering, ETSIAM, University of Cordoba, Spain

[2] Department of Agronomy, ETSIAM, University of Cordoba, Spain

[3] Canary Institute of Agricultural Research, ICIA, Santa Cruz de Tenerife, Spain

Abstract:

Morphological traits, nutritive values and physiological responses to two different water regimes of three *Bituminaria bituminosa* varieties: var. *albomarginata*, var. *crassiuscula* and var. *bituminosa* were evaluated in a greenhouse experiment. Two water regimes were imposed for 63 days; well-watered (WW) plants and deficit-watered (DW) plants, both starting from a high soil water content (dripping point). The three varieties showed similar aerial biomass reduction under reduced watering, 50% for var. *albomarginata*, 51% for var. *bituminosa* and 43% for var. *crassiuscula.* Var. *Albomarginata* showed lower shoot biomass under both water regimes than var. *bituminosa* (56.2 % in WW plants and 55.2% in DW plants) and var. *crassiuscula* (52% in WW plants and 57.8% in DW plants). This lower shoot biomass could be attributed to the high initial soil water content imposed in this experiment, affecting early development. This hypothesis is supported by the lower root biomass production of var. *albomarginata* and its distribution. The DW treatment of this experiment was not sufficiently restrictive to cause morphological modifications, whilst of the forage quality variables analysed, only ash was affected. Var. *crassiuscula* and var. *albomarginata* had a lower specific leaf area (239 cm^2 g^{-1} and 235 cm^2 g^{-1}, respectively) than var. *bituminosa* (352 cm^2 g^{-1}), which might represent an important adaptation to high light intensity and temperature conditions. The values of stem mass fraction (SMF) and leaf mass fraction (LMF) for var. *crassiuscula* (SMF=0.36 and LMF=0.28) and var. *albomarginata* (SMF=0.35 and LMF=0.36) indicated better forage aptitude of these varieties than var. *bituminosa* (SMF= 0.50 and LMF=0.19). All varieties showed good values of crude protein and digestibility, although important differences were found between leaf and stem. According to the studied morphological, nutritional and physiological traits, var. *albomarginata* showed the best aptitude for being introduced as permanent grasslands in some Mediterranean farming systems. However, the possible susceptibility of var. *albomarginata* to high water content in the soil could limit its introduction. These results help to inform the potential use of these three Canarian *B. bituminosa* varieties to improve Mediterranean rainfed grasslands of extensive farming systems.

Keywords: *Roots, water-use, morphology, biomass production, net photosynthesis, crude protein.*

1. Introduction

Bituminaria bituminosa (L.) C.H. Stirton is a perennial legume widely distributed in the Mediterranean basin and Macaronesia and traditionally used as forage crop for livestock in the Canary Islands, where it is known as "*tedera*" (Méndez, Fernández, & Santos, 1990; Ventura, Castañon, & Mendez, 2009). Varieties from this archipelago are adapted to its large climatic diversity, with annual rainfall varying from 150 mm up to 800 mm. In this study the following varieties were investigated: *B. bituminosa* var. *albomarginata* (BAM), *B. bituminosa* var. *crassiuscula* (BCC) and *B. bituminosa* var. *bituminosa* (BBT). These varieties were selected based on their different morphology and distribution in the Canary Islands (Méndez et al., 1990) which may be a source of adaptation to drought stress. Although *B. bituminosa* is known for its high content of metabolites such as furanocoumarins and isoflavonoids (Pecetti, Tava, Pagnotta & Russi, 2007; Pistelli et al., 2003), previous studies have demonstrated that it is consumed by livestock in nature (Sternberg, Gishri & Mabjeesh, 2006) and safe to feed sheep maintaining liveweight in a diet of BAM and BCC (Oldham et al., 2015). Ventura et al. (2009) studied the intake of the three varieties of *"tedera"* by goats and found preference for fresh *"tedera"* versus alfalfa hay, except in summer, when alfalfa hay was preferred due to a higher concentration of secondary compounds of *"tedera"* during this season. The same authors showed that the intake of *"tedera"* in summer could be increased by hay making.

In recent years there has been a growing interest in these varieties of *B. bituminosa* because of their drought resistance and good forage aptitude, especially BAM, which has been established as the most drought- resistant variety (Martínez-Fernández, Walker, Romero, Martínez-Ballesta, & Correal, 2012; Raeside et al., 2012). Its aptitude as a fodder plant in Mediterranean-like climates has been tested in Australia and Israel, showing promising results (Oldham et al., 2013; Real, Oldham, Burgel, Dobbe & Hardy, 2017; Sternberg et al., 2006). The large genetic diversity of *B. bituminosa* is a promising source for breeding programs (Foster, Ryan, Real, Ramankutty, & Lambers, 2013; Pazos-Navarro et al., 2011). Significant advances have been made by Australian and Spanish researchers in selecting basic material for breeding programmes to produce improved lines of good forage aptitude adapted to different environmental conditions of arid lands (Pazos-Navarro et al., 2011; Raeside et al., 2012; Real & Verbyla, 2010). Indeed, a new variety, named *Lanza,* has recently been registered as a result of these breeding programmes.

The Mediterranean basin is expected to be especially vulnerable to climate change (IPCC, 2018). Lower precipitation associated with a higher uncertainty of inter-annual distribution together with increases in temperature are forecast for the 21st century (Giannakopoulos et al., 2009; Giorgi & Lionello, 2008). Gang, et al. (2015), found a decreasing trend of net primary productivity of grasslands in Europe from 1981 to 2010 and an overall decline in water use efficiency in woody savannas and non-woody grasslands in response to climate change from 2000 to 2013 (Gang et al., 2016). The expected climatic variability challenges the pasture productivity and hence the capacity to sustain livestock production. In this context, perennial legumes with drought resistance, dehydration tolerance, and consequently steady forage production, such as *B. bituminosa,* are of key importance for sustaining extensive farming systems in the Mediterranean region such as *Dehesas* (Bennett, Ryan, Colmer, & Real, 2011;; Hernández-Esteban, López-Díaz, Cáceres & Moreno, 2019; Melis, Franca, Re, & Porqueddu 2018; Porqueddu et al., 2016).

Although summer drought is a limiting factor for forage production in the Mediterranean region (Cosentino, Gresta, & Testa, 2014), soil water saturation and wet conditions frequently occur at early stages of plant establishment and development during the autumn and winter months, especially in lower topographies (Ceballos & Schnabel, 1998; Lozano-Parra, Schnabel, & Ceballos-Barbancho, 2015; Maneta, Pasternack, Wallender, Jetten & Schnabel, 2007; Maneta, Schnabel, Wallender, Panday, & Jetten, 2008). Both limiting factors are expected to increase under future climate conditions, as projections show that droughts could start earlier in the year and last longer (Beniston et al., 2007; Giannakopoulos et al., 2009), whereas wet conditions may increase in late autumn and winter due to increases in precipitation extremes (Giorgi & Lionello, 2008).

While the adaptation of *B. bituminosa* to drylands has been proven successful (Suriyagoda, Real, Renton, Lambers, & Ryan, 2013), susceptibility to wet conditions and unsuccessful development during wet winters has also been reported (Raeside et al., 2012; Real & Verbyla, 2010). This reflects the need to investigate the response of *B. bituminosa* varieties under different soil moisture conditions to ensure their successful introduction into pastures of Mediterranean farming systems in the face of climate change.

The morphological traits and nutritive value of *B. bituminosa* have been widely studied in Spain, Italy and Australia (Correal, Hoyos, Real, Snowball, & Costa, 2008; Melis et al., 2018; Méndez et al., 1990; Méndez, Santos, Correal, & Ríos, 2006; Muñoz & Correal, 2000Porqueddu, Dettori, Falqui, & Re, 2011; Raeside et al., 2012). However, to our knowledge, some traits such as leaf mass fraction or leaf area ratio and leaf and stem-nutritive value have not been investigated for Canarian varieties. These traits may have important implications since they influence forage aptitude (Abd El Moneim, Khair & Rihawi, 1990; Méndez et al., 2006).

This study aims to assess the response in terms of (i) physiology, (ii) biomass and morphological traits of shoot/root and (iii) nutritive value of leaves and stems of the three recognised Canarian varieties of *B. bituminosa* under two different water regimes in greenhouse controlled conditions: high soil water content at the beginning of the growth cycle, followed by high or low irrigation regimes (well-watered or deficit-watered).

This study could further inform the potential of these three varieties to adapt to future Mediterranean climatic conditions and elucidate the role that upcoming commercial varieties could play in improving pastures and thereby sustaining livestock production.

2. Materials and methods

2.1. Plant material

Seeds from the three varieties of *B. bituminosa* were collected from wild populations of the Canary Islands (Spain) (Table 1). Tedera is a selfpollinated diploid (2n=20) species (Pazos-Navarro et al., 2011). This together with the geographical isolation of the populations guaranteed no outcrossing pollination among varieties.

BAM is native to the island of Lanzarote, where it grows in semi-arid coastal habitats with not more than 200 mm annual rainfall, having a five to six month-long hot and dry season and high relative humidity due to maritime influence. BCC grows in the National Park of Cañadas del Teide (2200m a.s.l.) with 500 mm of annual rainfall (including snow), showing winter dormancy and the growing season during spring and mild summer (Martínez-Fernández et al., 2012). Previous research has pinpointed a biannual behaviour of this variety outside its native habitat at Mount Teide (Melis et al., 2018; Méndez, unpublished data). BBT is widespread on all the Canary Islands and the Mediterranean basin with 200-800 mm of annual rainfall during warm and dry summers (Table 1).

The seeds were scarified by nicking the outer seed coat using a surgical scalpel (Beard, Nichols, Loo, & Michael*,* 2014) and germinated in 90-mm Petri dishes with wet filter paper (Foster et al*.,* 2015; Pecettzi, Tava, Pagnotta, & Russi, 2007;). Once germinated, the seeds were grown in trays (17 days). Subsequently, each seedling was transplanted into individual plastic six-litre cylindrical pots 37 cm high, with a 16.80 cm and 12.5 cm diameter at the top and bottom of the pot respectively and holes at the bottom to allow free drainage of water. Ech pot was filled with a mix of commercial peat (Gramosemi GF-Anz./Verm from Gramoflor) and sand (9:1 v/v) with 2 g of 19-19-19 NPK fertiliser per pot. The soil had a pH of 7.31 (1/2.5), 2.96 g $100g^{-1}$ organic matter content, 0.647 (mmhos cm^{-1}) of electrical conductivity and cation exchange capacity of 20.69 meq 100 g^{-1}. Water was provided daily at a rate of 50 ml per pot, and the temperature set at 22°C/7°C (day/night) for 13 days in the greenhouse to ensure successful establishment and acclimatisation before the treatments were imposed.

2.2. Experimental design

The experiment was conducted at the greenhouse of the University of Cordoba, Cordoba (Spain). The experiment comprised a complete randomised design to analyse two treatments (water regimes), and three *B. bituminosa* varieties with six pots per variety and treatment, having a total of 36 pots. The water regimes imposed were: high soil water content at the beginning followed by a high irrigation regime, well-watered plants (WW); and high soil water content at the beginning followed by a reduced irrigation regime, deficit-watered plants (DW). On the first day of the experiment, all pots were watered with 2,279 ml of water to the point of dripping (~1.17 g g^{-1}) to achieve even starting conditions of high soil water content. Then, WW plants were manually watered every day with 50 ml (2.3 l m^{-2}) for the first 47 days. For the rest of the experiment (16 days), when the plants had a higher demand for water due to an increase in daily mean temperature, watering was increased to 75 ml (3.4 l m^{-2}). In the second treatment, watering was reduced by ~50%, with the DW plants being watered every second day with the same amount of water and following the same irrigation strategy as the WW plants. These watering regimes did not produce drainage in either WW or DW plants. At the end of the experiment, each WW plant received ~2,279 ml of water at initial watering, plus ~3,550 ml (263 l m^{-2}) for 63 days while DW plants received ~2,279 ml plus ~1,775 ml (183 l m^{-2}). This initial watering represents around half of the average autum rainfall of Cordoba, whilst the water supplied for 63 days is about 33% higher (WW) or 33% lower (DW) than the average spring rainfall registered in Cordoba. The greenhouse temperature was kept in the range of 30 °C /15 °C (day/night) during the experiment. The experiment started on April 1st, 2019 when all plants had approximately seven leaves (after 13 days of acclimatisation in the greenhouse as mentioned in the previous section) and the plants were harvested on June 3rd, 2019 at the beginning of flowering. The duration of the experiment was 63 days.

2.3. Water use and physiological measurements

Four pots per variety and water regime were weighed three times per week from day 30 to the end of the experiment (day 63). Pot evapotranspiration, ET (ml) was estimated as ET $=PW_i - PW_j + I_{ij}$, where PW_i is the pot weight at day *i* (g), PW_j is the pot weight at day *j* (g) and I_{ij} the irrigation water provided between days *i* and *j* (ml). During the same period, relative soil water content was estimated as RSWC= $(SW_i – DSW)/(FSW- DSW)$, where SW_i is the soil weight at day *i*, DSW is dry soil weight and FSW is the soil weight at point of dripping. DSW was obtained by weighing and averaging the oven-dried soil (105°C/24h) of four pots. For FSW, the same four pots were previously watered to point of dripping and weighed discounting the weight of an empty pot. Since at day 30 the canopy of the plant already covered the entire pot surface, transpiration can account for most of the measured ET (Or, Lehmann, Shahraeeni, & Shokri, 2013; Ritchie, 1972).

At day 46, four fully expanded leaves of each plant were removed (12:00-14:00 h) to measure the relative leaf water content (RWC). RWC was calculated as RWC = (FW – DW) / (SFW – DW) × 100, where FW is fresh leaf weight, DW is dry leaf weight and SFW is saturated fresh leaf weight. After recording FW, the leaves were immersed in 90-mm Petri dishes filled with deionised water for 24 h at 20 – 25 ºC and then weighed again to record SFW. DW was measured after oven-drying the leaves at 60ºC for 72h. Before drying, the leaves were scanned (EPSON 1640 XL) and the area was measured using the image analyser software ImageJ (https://imagej.nih.gov/ij/) for the later calculation of the specific leaf area (SLA, $cm^2\ g^{-1}$). Net photosynthesis per area (A_{area}) and stomatal conductance per area (g_{Sarea}) were also measured at day 46 (09:00-12:00 h) of mid-height, fully expanded and undamaged leaves using a portable infrared CO_2 gas analyser (LiCor Li6400XT, Li-Cor, Inc., Lincoln, NE, USA) fitted with a 2-cm^2 leaf cuvette. PAR was set at 1000 µmol photon m^{-2} s^{-1}, flow rate at 500 µmol s^{-1}, [CO2] to 400 ppm, with block temperature set at 25ºC. Water-use efficiency (WUE) was derived from the ratio A_{area}/g_{Sarea}. An example of the state of WW and DW plants at the moment of measurement of the physiological variables can be seen in Fig. S1.

2.4. Biomass and trait measurement

At the end of the experiment, plants were cut at the soil surface. Aerial plant components were separated into stem and leaves, which were also split into green/senescent leaves. Pots were cut open and the soil was separated into three layers (0-10.5, 10.5-21, 21-31.5 cm). The soil was carefully removed from the roots using a 2-mm sieve. The roots of each layer were then split into thin roots (<0.5mm) and thick roots (>0.5mm) and immediately frozen. This threshold was chosen to explicitly emphasise more absorptive roots (Wang, Liu, Fang & Shangguan, 2020). A representative subsample of thin roots from the first layer and all thick roots from the three soil layers of four WW plants per variety were split and independently scanned (EPSON 1640 XL). Root length was calculated using WinRHIZO software (http://regent.qc.ca/) and then root length to weight ratio (m g^{-1}) was also calculated. Leaves, stems and roots were oven-dried for 72 h at 60ºC and dry mass (DM) recorded.

Root to shoot ratio was calculated as the total root DM/total shoot DM. The Leaf mass fraction (LMF), Stem mass fraction (SMF) and Root mass fraction (RMF) were calculated as the proportion of the total DM corresponding to each one of these plant components. SLA, (cm^2 g^{-1}) was calculated as the ratio between the leaf area and its dry mass. Leaf area ratio (LAR, cm^2 g^{-1}), the ratio of leaf area and total plant DM, was estimated as the product of SLA and LMF (Poorter & Remkes, 1990; Villar et al., 2004). Leaf to stem ratio was measured as total leaf DM/total steam DM. Senescent to green leaf ratio was calculated as senescent leaf DM/green leaf DM. The proportion of thin roots in each layer was estimated as the thin roots DM/total roots DM.

2.5. Nutritive value

Samples of green, fully expanded leaves and stems were independently analysed for four plants of each variety and treatment at the end of the experiment. These samples were ground and passed through a 1-mm sieve. Crude protein (CP), ashes, neutral detergent fibre (NDF), acid detergent fibre (ADF) and enzyme digestibility of organic matter (EDOM) were determined by near-infrared spectroscopy (NIRS) using a portable LabSpec 5.000 spectrometer (350–2.500 nm; ASD Inc., Boulder, Colorado, USA) using IndicoPro 6.0 spectrum acquisition software (ASD Inc., Boulder, CO, USA). Spectral data of samples was recorded in the whole range of 350-2,500 nm by 1-nm step. Four replicates of each sample were scanned (each being an average of 50 internal scans). White reference scans (with a Spectralon panel) were taken between every sample scan. The final sample spectrum was obtained by averaging the four scans. The statistics of the NIRS equations used to predict nutritional values of samples in this study are presented in Supplementary material (Table S1). NIRS equations were calibrated based on 130 spectra of Mediterranean pastures and forage crops analysed by wet chemical methods at the Laboratory of Animal Nutrition of SERIDA (Villaviciosa, Spain). NIRS predictions were performed using WinISI software (Infrasoft International, Port Matilda, PA, USA).

2.6. Statistical analysis

Differences among varieties and treatments for each variable were tested by two-way ANOVAs (variety and water regimes as fixed factors). For those variables where soil layer (3 depth levels) and plant organ (stem or leaf) were included, differences were explored by three-way ANOVAs (variety, water regimes and depth/organ as fixed factors). Accumulated evapotranspiration and RSWC were tested by two-way ANOVA for each measurement date and the interactions of the factors were also considered. When differences were significant ($p<0.05$), post-hoc Tukey's test at the 0.05 probability level was carried out to test differences among means. The data was transformed, when needed, using either logarithmic or square-root transformation to meet normality and homoscedasticity assumptions. To summarise and analyse the covariation between variables, a principal component analysis (PCA) was performed with morphological and physiologic traits. Evapotranspiration and nutritive value parameters were not included in the analysis because of the different number of observations. Statistical analyses were performed using the software R v. 3.6.1 (R Development Core Team, 2019).

3. Results

3.1. Biomass and morphological traits

The deficit-watered treatment significantly affected ($p< 0.05$, Table S2) biomass production and senescent to green leaf ratio of all varieties (Table 2). The morphological traits shown in Table 3 were modified not by the water regime but by the variety. BAM was the variety with the lowest biomass production for both water regimes, while no difference was found between BBT and BCC under each treatment (Table 2). Shoot biomass under the DW treatment as a percentage of the WW treatment was similar for the three varieties: 50% for BAM, 49% for BBT and 57% for BCC. The three varieties showed significantly different aboveground biomass allocation (Table S3). BAM had the highest leaf to stem ratio (1.06 g g^{-1}) followed by BCC (0.80 g g^{-1}) and BBT (0.38 g g^{-1}). Similarly, BAM had the highest LFM whereas BBT had significantly higher SMF than BCC and BAM (Table 3).

The SLA and LAR were not modified by the water regime although it was different for each variety (Table S3). The SLA was significantly lower for BAM (235 cm^2 g^{-1}) and BCC (239 cm^2 g^{-1}) than for BBT (352 cm^2 g^{-1}). Concerning LAR, BBT showed significantly lower LAR than BAM (Table 3).

Although root to shoot ratio did not change for either variety or treatment (Table 3), significant differences were found for root biomass and its allocation throughout the profile (Table S4). Root biomass was reduced by the DW treatment and it was always the lowest for BAM (Table 2). There was a two-way interaction among varieties and depth (p=0.017, Table S4), revealing a different pattern of root biomass distribution throughout the profile (Fig. 1: A). While BBT and BCC showed a clear stratified distribution of root biomass decreasing with depth, BAM showed similar root biomass throughout the soil profile. Root biomass in the lower layer was of similar magnitude for all three varieties and this pattern was not modified by the water regime. The proportion of thin roots (<0.5 mm) increased throughout the profile for all varieties, being 30%, 79% and 95% for upper, middle and lower levels, respectively (Fig. 1: B). BCC invested more biomass in thin roots than BAM and BBT under WW treatment (82%, 65% and

62% respectively). However, under DW treatment, BCC and BAM showed a higher proportion of thin roots than BBT (72%, 71% and 56% respectively). BCC had a significantly shorter root length to weight ratio of thin roots (33.5 m g^{-1}) than BAM (60.1 m g^{-1}) and BBT (59.1 m g^{-1}). No differences were found for thicker roots (>0.5mm) with 1.1 m g^{-1} root length to weight ratio on average.

3.2. Plant water use

The accumulated evapotranspiration over the measurement period showed a significant difference ($p<0.001$) between treatments. The total evapotranspirated water was 1,790 ± 25 ml under DW and 2,833 ± 102 ml under WW. Moreover, important differences were found when the accumulated evapotranspiration was evaluated at each measurement date. At the beginning, both treatments had similar evapotranspiration; however, from day 36 to the last measurement WW plants had significantly higher evapotranspiration than DW plants. Regarding differences by variety, BAM showed significantly lower evapotranspiration than BCC from the first measurement to day 40. Thereafter, there was an interaction that indicated lower accumulated evapotranspiration of BAM under WW treatment (Fig. 2: A).

At the beginning, relative soil water content (RSWC) was 66% on average and 45% under WW and DW respectively (Fig.2: B), showing no differences among varieties. During the measurement period, RSWC was always significantly lower under DW. From days 36 to the last measurement BAM showed significantly higher RSWC than BCC and BBT. In DW treatment, RSWC decreased to a minimum value of around 12% at day 53 when it seemed to stabilise; this value was firstly reached by BCC (Fig.2: B).

3.3. Relative water content, photosynthesis and stomatal conductance

The deficit-watered regime did not affect the RWC (Table S5), as values are similar under both water regimes. However, BAM showed higher RWC than the other two varieties (Table 4). For A_{area}, the interaction of variety and treatment (Table S5) reflected the fact that BCC was the only variety affected by DW treatment. BAM was the variety with the highest A_{area}, although it showed similar values to BCC under WW treatment (Table 4). g_{Sarea} was also higher for BAM and was reduced by DW treatment across the three varieties. WUE was not influenced by either variety or treatment.

The first two axes of the PCA accounted for 33.0% and 24.1% of total variation, respectively. The three varieties showed clear clustering (Fig. 3. A) while the treatments were more scattered on both axes (Fig. 3. B). Overall, morphological traits were more important to explain the variability of both principal components than physiological ones. LMF, leaf to stem ratio and LAR had high negative loadings and high positive loading for root biomass for the first principal component. For the second principal component, root biomass, root to shoot ratio and thin roots proportion presented high positive loadings while SMF, SLA and senescent to green leaf ratio had high negative loadings. All biomass-related variables together with water use efficiency (WUE), RMF and root to shoot ratio showed covariation. LAR, LMF and leaf/stem ratio presented covariation with A_{area}, g_{Sarea} and RWC. Finally, SLA and SMF also showed covariation.

3.4. Nutritive value

Ash content was around 10-12% (leaf and stem weighed average across varieties), showing two interactions (Table S6): variety by organ (p=0.008) and organ by treatment (p=0.019). BBT ash content was significantly lower in the stem than in the leaf while BAM and BCC showed no difference in ash content between organs (Fig. 4: A). The organ by treatment interaction reflected the effect of DW on the leaf ash content reduction whereas it had no effect on the stem (Fig. 4: B).

The other parameters analysed, EDOM, ADF, NDF and CP, were not affected by the water reduction imposed in this experiment (Table S6). The leaf EDOM was similar for all varieties, with an average value of 86.2%. Stem EDOM was significantly lower than in leaf, also being lower for BBT than for BAM and BCC, which had similar values (Fig. 5: A). CP leaf values were around 16% across all varieties. However, a two-way interaction (Table S6) reflected BAM having higher stem CP than BCC whereas BBT had the same CP value as the other varieties (Fig. 5: B). There was an interaction between variety and organ for NDF (Table S6). Although the NDF value for stem was similar for all varieties, 42.9% on average, the leaf NDF content was significantly higher for BAM than for BBT, while BCC showed no difference in leaf NDF compared to the other two varieties (Fig. 5:C). As expected, ADF was significantly higher for stem than for leaf, although values were similar across the three varieties, 19.9% for leaf and 31.4% for stem on average (Fig. 5:D).

4. Discussion

The lower shoot biomass production of BAM under both treatments differs from previous studies where BAM was highly productive (Melis et al., 2018; Real et al., 2014). These contrasting results compared to previous studies (Foster et al., 2015; Foster, Ryan, Real, Ramankutty, & Lambers, 2012; Martínez-Fernández et al., 2012) could be explained by differences in experimental conditions, in particular, the high initial soil water content and differences in the water regimes. The relatively high initial soil water content could have affected the early development of BAM leading to a lower biomass production. BAM grows naturally in a very low-rainfall environment (< 200 mm) while BCC and BBT grow in environments with higher rainfall (Table 1) where periodic wet conditions may be expected during the growing season. BCC, in particular, which grows in the National Park of Cañadas del Teide (2200m a.s.l.) and receives part of the average precipitation (500 mm) as snow (Méndez et al., 1990; Raeside et al., 2012; Real et al., 2014), may have longer wet conditions due to snow melt. Real et al. (2014) also suggested that variability in wet conditions tolerance might be expected based on the high natural habitat variability of *B. bituminosa.*

This hypothesis is supported by the lower root biomass production of BAM and its distribution throughout the profile compared to BCC and BBT (Fig. 1: A). In the same line, BAM was the only variety for which the WW treatment reduced the proportion of thin roots, as these are expected to be more susceptible to root rot. This poor root development limited the soil water use, as shown by the high RSWC maintained over the last growing period even for DW plants (Fig. 2: B). Similarly, the evapotranspiration of BAM could be affected by the lower shoot biomass and by a root system that was unable to use the soil water content as much as BCC and BBT which developed larger root systems. *B. bituminosa* susceptibility to stem and root rot has been pointed out in previous studies (Martínez-Fernández et al., 2012; Real et al., 2014). Real et al. (2014) found *B. bituminosa* accessions were generally sensitive to wet conditions although four accessions showed tolerance. Raeside et al. (2012) also found BAM failed to persist and produce biomass during wet winters. These results have important agronomic implications since BAM susceptibility to wet conditions may limit its potential as a novel forage legume (Raeside et al., 2012) in areas with contrasting rainfall during the plant growing season. In the Mediterranean basin, clay/silty soils and high-rainfall periods could often contribute to periodic wet conditions that might limit BAM root system development and therefore its resistance to subsequent drought periods. This could also mean a competitive disadvantage of BAM in grassland mixtures at initial stages, since more tolerant species to initial high soil water content could dominate and outcompete BAM. For example, as shown in Fig. 2: A, BAM under WW conditions showed lower accumulated evapotranspiration, while no differences between varieties were found under DW. Breeding programmes have used BCC to improve BAM cold-resistance (Real et al., 2014; Walker, Romero & Correal 2010); these crossed lines with BCC could also improve BAM susceptibility to wet conditions (Raeside et al., 2012). The efforts of the breeding programs to deliver wet conditions- and drought-resistant ideotypes (Real et al., 2014) will be crucial to overcoming this potential limitation of BAM in permanent grasslands of the Mediterranean basin.

The DW treatment of this experiment was not restrictive enough to cause SLA or other morphological modifications seen in previous studies (Foster et al., 2012; Foster et al., 2015; Martínez-Fernández et al., 2012). Only biomass-related variables and senescent to green leaf ratio contributed to differentiating between both treatments (Fig. 3. B). However, BAM and BCC had a significantly lower SLA than BBT, which could indicate a thicker and/or denser leaf for these varieties. The higher SLA of BBT could also be related to the lower NDF content in leaf (which indicates the cell-wall material) of this variety (Fig.5:C). Khaled, Duru, Decruyenaere, Jouany and Cruz, (2006) also found a negative correlation between SLA and fibre content in grassland species. SLA values for the three varieties were higher than those reported by Martínez-Fernández et al. (2012) for pot-grown plants and lower than SLA values of plants grown in the field in the same study. As these authors state, these differences might be due to different temperature and light intensity exposure. Foster et al. (2013) and Martínez-Fernández et al. (2012) suggested that lower SLA in BAM might play an important role in its protection against light intensity and water loss. Since BCC and BAM showed lower SLA (i.e., thicker and/or denser leaves), these two varieties could be better adapted than BBT to high light intensity and temperature. A_{area} and g_{Sarea} values of BAM measured at day 46 (that was not the point of maximum drought stress) were associated with higher leaf RWC (Foster et al., 2015) which confirms the drought adaptation of this variety (Foster et al., 2013). The differences in A_{area} of BCC by treatment could also be influenced by its RSWC at measurement time (day 46) under DW treatment, which was the lowest of the three varieties (12% vs 15% and 23% for BBT and BAM, respectively) (Fig.2: B). The significant lower A_{area} recorded for BBT under WW treatment might indicate higher responsiveness to high temperature leading to earlier A_{area} reduction. The A_{area} and g_{Sarea} response of the different *B. bituminosa* varieties to increasing temperature and also light intensity could be further investigated in future research as it might play an important role in their adaptation to Mediterranean permanent grasslands.

In agreement with the results of this study, previous research has found little or no effect of moderate drought stress on the quality parameters of forage legumes

(Komainda, et al., 2019; Kuchenmeister, Kuchenmeister, Kayser, Wrage & Isselstein, 2013). As Kuchenmeister et al. (2013) stated, selection of legume species and cultivation in mixture or monoculture may have more influence on quality parameters than drought stress. These studies reported values of CP for alfalfa between 19% and 27% and informed of minor effect of drought stress on CP content (Kuchenmeister et al. 2013; Staniak & Harasim, 2018). In this study, just green leaves were sampled for nutritive analysis. Although green leaves might have no differences in quality, it is worth noting that drought stressed plants with senescent leaves may have lower global quality (lower digestibility and CP content and higher NDF and ADF) than those with no senescent leaves.

Overall, BAM and BCC showed better forage aptitude than BBT due to the higher leaf proportion (Table 3). The leaf proportion is an important morphological value to assess the nutritive value of forage legumes (Abd El Moneim et al., 1990). Although BBT had similar leaf nutritive value to the other two varieties (Fig.4), the lower leaf to stem ratio of this variety (Table 3) makes it less suitable as forage since it reduces its palatability compared to BAM and BCC. As the PCA showed, the LMF, leaf to stem ratio (both higher in BAM) and the SMF clearly contributed to differentiating the three varieties, especially between BAM and BBT. The weighed average value of CP between stem and leaf of BAM (15%), BBT (12.5 %) and BCC (12.4%) were lower than concentrations found for Canarian varieties with values ranging from 15 to 17.7% for samples taken in summer (Ventura et al., 2009; Ventura, Méndez, Flores, Rodriguez & Castañon, 2000), although similar to CP values from 9.4 to 16.1% reported for Italian accessions (Pecetti et al., 2007). As Ventura et al. (2009) informed, these varieties have higher forage quality than alfalfa hay, which showed values of 11.8% CP, 57.4% NDF and 43.7% digestible organic matter. The weighed average concentrations of NDF (BAM 34.8%, BBT 38.4% and BCC 32.1%) and ADF (BAM 26.7%, BBT 28.1% and BCC 25.5%) were lower than previously evaluated concentrations of Canarian varieties and other for Italian accessions that reported values of NDF from 41.1 to 53.7% (Pecetti et al., 2007; Ventura et al., 2000; Ventura et al., 2009). These differences in NDF may be explained by the age of the plants sampled, three-year-old plants in the case of the Italian accessions (Pecetti et al., 2007). Evaluations of BAM nutritive value have shown CP to be highly variable depending on the accession analysed, with values ranging from 12.8% to 24.2% (Oldham et al., 2013; Raeside et al., 2012). The same studies presented lower values of NDF (25.4 - 32.8 %) and ADF (19.4 - 22.3 %) (Oldham et al., 2013; Raeside et al., 2012) although these differences may be accounted for by the earlier phenological stage. Mismatches in reproductive development of the three studied varieties could be observed due to the different meteorology in their native environment (Méndez et al., 1990). Although phenology was not the subject of study here, the three varieties showed signs of being at the beginning of the reproductive stage without considerable differences between them. Melis et al. (2018) found some differences in the date of the first flower appearance between *B. bituminosa* Spanish accessions, Sardinian accessions and *Bituminaria morisiana* all grown in Sardinia (Italy). However, no significant differences were found for accessions of the three varieties from the Canary Islands. The intrinsic morphological traits such as leaf and stem mass fraction seem to have stronger effects on the forage aptitude than possible mismatches of the phenology between the three varieties. CP (15.0%), NDF (34.8%) and ADF (26.6%) stem and leaf average concentration of BAM were very similar to the concentrations also evaluated by near infrared analysis for the same variety by Adriansz, Hardy, Milton, Oldham, & Real, (2017), CP (15.0%), NDF (37.6%) and ADF (26.6%). However, it would be worth investigating the drought stress effect on phenology of the studied varieties since it would directly affect their nutritive value.

Leaf ash content increased with WW treatment. This finding is consistent with other research that reported an increase in most leaf nutrient under well irrigated condition (Olivera-Viciedo et al., 2020), especially those nutrients that are passively uptaken (Wu, Liu, Wang, Zhang & Xu, 2012). Furthermore, ash content has been positively correlated with transpiration ratio (ratio of water transpired to carbon fixed) (Masle, Farquhar & Wong, 1992; Merah, Deleens, Souyris & Monneveux, 2001), and an increase in the later can occur under well irrigated condition. However, Masle et al. (1992) stated, that changes in the transpiration ratio induced by environmental factors (as atmospheric humidity or carbon dioxide concentration) do not cause a noticeable change in the mineral content. Leaf ash content can affect the response of the plant to water stress due to some nutrient are involved in water flow regulation. For example, moderate potassium starvation inhibits the mechanism of stomatal closure and can cause tissue dehydration in water-stressed plants (Benlloch-González, Arquero, Fournier, Barranco & Benlloch, 2008). In fact, we found a positive correlation between leaf ash content and RWC (result not shown). The ash content may seem higher compared to previous studies Italian accessions (6.0-7.7%) (Pecetti et al., 2007); however, similar values in Spanish accessions (9.18-12.2%) are reported by Oldham et al., (2013) and SIA (2019). These Canarian varieties show higher CP and lower NDF and ADF content than Mediterranean rainfed grasslands, at a similar phenological stage (Henkin et al., 2011; Vázquez De Aldana, García-Ciudad, & García-Criado, 2008; Zarovali, Yiakoulaki, & Papanastasis, 2007). This higher nutritive value together with its drought and grazing tolerances make this species a promising fodder plant for Mediterranean grasslands (Oldham et al., 2013; Real et al., 2017; Sternberg et al., 2006).

5. Conclusion

Water stress reduced biomass production equally for the three varieties. However, the initial soil water content affected the early development and biomass of BAM, which was reflected by the poor root system development. This denotes susceptibility of BAM to high soil water content which may limit its introduction to some Mediterranean farming systems, where periodic soil water saturation can be expected, especially at the beginning of the growing season. According to the morphological traits studied, BAM showed the best forage aptitude. The lower SLA of BAM and BCC is an important adaptation against high light intensity and temperature in arid environments. The three *B. bituminosa* native to the Canary Islands showed good nutritive value for their use to improve the quality of Mediterranean rainfed pastures.

The wide diversity of existing varieties and the progress of the breeding programmes developed in Spain and Australia based on Canarian *B. bituminosa* varieties bring forward the opportunity for the incorporation of these lines for the improvement of Mediterranean pastures. However, additional research through continuous monitoring of its use and long-term field experiments will be needed to confirm the proper adaptation of the improved lines and selected ideotypes to the wide range of Mediterranean environmental conditions.

6. Acknowledgements

This study is funded by the European Union's Horizon 2020 research and innovation programme, grant agreement 774124, project SUPER-G (Sustainable Permanent Grassland). The study was developed thanks to a PhD fellowship FPU (code FPU18/02876) of the Spanish Ministry of Education awarded to J. Fernández-Habas.

The authors would like to thank the anonymous reviewers for providing insightful suggestions that have allowed to significantly improve the paper.

7. References

Abd El Moneim, A. M., Khair, M. A., & Rihawi, S. (1990). Effect of genotypes and plant maturity on forage quality of certain forage legume species under rainfed conditions. *Journal of Agronomy and Crop Science*, 164, 85-92. https://doi.org/10.1111/j.1439-037X.1990.tb00790.x

Adriansz, T. D., Hardy, J. L. M., Milton, J. T. B., Oldham, C. M., & Real, D. (2017). Near infrared analysis for nutritive attributes of tedera (*Bituminaria bituminosa* var. *albomarginata*). *Journal of Near Infrared Spectroscopy*, 25, 215–218. https://doi.org/10.1177/0967033517712133

Beard, C., Nichols, P.G.H., Loo, C., Michael, P. (2014). *Establishment of Tedera (Bituminaria Bituminosa var. albomarginata)* (Technical Report nº 13). Retrieved from Future Farm Industries CRC: Crawley, UK website: http://www.futurefarmonline.com.au/publications/other-publications.htm.

Beniston, M., Stephenson, D. B., Christensen, O. B., Ferro, C. A. T., Frei, C., Goyette, S., … Woth, K. (2007). Future extreme events in European climate:

An exploration of regional climate model projections. *Climatic Change*, 81, 71–95. https://doi.org/10.1007/s10584-006-9226-z

Benlloch-González, M., Arquero, O., Fournier, J. M., Barranco, D., & Benlloch, M. (2008). K+ starvation inhibits water-stress-induced stomatal closure. *Journal of plant physiology*, 165, 623-630. https://doi.org/10.1016/j.jplph.2007.05.010

Bennett, R. G., Ryan, M. H., Colmer, T. D., & Real, D. (2011). Prioritisation of novel pasture species for use in water-limited agriculture: A case study of Cullen in the Western Australian wheatbelt. *Genetic Resources and Crop Evolution*, 58, 83–100. https://doi.org/10.1007/s10722-010-9567-3

Ceballos, A., & Schnabel, S. (1998). Hydrological behaviour of a small catchment in the dehesa landuse system (Extremadura, SW Spain). *Journal of Hydrology*, 210, 146–160. https://doi.org/10.1016/S0022-1694(98)00180-2

Correal E., Hoyos A., Ríos S., Méndez P., Real D., Snowball R., & Costa J. (2008). Seed production of *Bituminaria bituminosa*: size, production, retention and germination capacity of the legumes. *Options Méditerranéennes*, 79, 379-383.

Cosentino, S. L., Gresta, F., & Testa, G. (2014). Forage chain arrangement for sustainable livestock systems in a Mediterranean area. *Grass and Forage Science*, 69, 625–634. https://doi.org/10.1111/gfs.12085

Foster, K., Lambers, H., Real, D., Ramankutty, P., Cawthray, G. R., & Ryan, M. H. (2015). Drought resistance and recovery in mature *Bituminaria bituminosa* var. *albomarginata*. *Annals of Applied Biology*, 166, 154–169.

https://doi.org/10.1111/aab.12171

Foster, K., Ryan, M. H., Real, D., Ramankutty, P., & Lambers, H. (2012). Drought resistance at the seedling stage in the promising fodder plant tedera (*Bituminaria bituminosa* var. *albomarginata*). *Crop and Pasture Science*, 63, 1034–1042. https://doi.org/10.1071/CP12216

Foster, K., Ryan, M. H., Real, D., Ramankutty, P., & Lambers, H. (2013). Seasonal and diurnal variation in the stomatal conductance and paraheliotropism of tedera (*Bituminaria bituminosa* var. *albomarginata*) in the field. *Functional Plant Biology*, 40, 719–729. https://doi.org/10.1071/FP12228

Gang, C., Wang, Z., Zhou, W., Chen, Y., Li, J., Chen, J., Qi, I., Odeh & Groisman, P. Y. (2016). Assessing the spatiotemporal dynamic of global grassland water use efficiency in response to climate change from 2000 to 2013. *Journal of Agronomy and Crop Science*, 202, 343-354. https://doi:10.1111/jac.12137

Gang, C., Zhou, W., Wang, Z., Chen, Y., Li, J., Chen, J., Qi, I., Odeh & Groisman, P. Y. (2015). Comparative assessment of grassland NPP dynamics in response to climate change in China, North America, Europe and Australia from 1981 to 2010. *Journal of Agronomy and Crop Science*, 201, 57-68. https://doi:10.1111/jac.12088

Giannakopoulos, C., Le Sager, P., Bindi, M., Moriondo, M., Kostopoulou, E., & Goodess, C. M. (2009). Climatic changes and associated impacts in the Mediterranean resulting from a 2 °C global warming. *Global and Planetary*

Change, 68, 209–224. https://doi.org/10.1016/j.gloplacha.2009.06.001

Giorgi, F., & Lionello, P. (2008). Climate change projections for the Mediterranean region. *Global and Planetary Change*, 63, 90–104. https://doi.org/10.1016/j.gloplacha.2007.09.005

Henkin, Z., Ungar, E. D., Dvash, L., Perevolotsky, A., Yehuda, Y., Sternberg, M., … Landau, S. Y. (2011). Effects of cattle grazing on herbage quality in a herbaceous Mediterranean rangeland. *Grass and Forage Science*, 66, 516–525. https://doi.org/10.1111/j.1365-2494.2011.00808.x

Hernández-Esteban, A., López-Díaz, M. L., Cáceres, Y., & Moreno, G. (2019). Are sown legume-rich pastures effective allies for the profitability and sustainability of Mediterranean dehesas?. *Agroforestry Systems*, 93, 2047-2065. https://doi.org/10.1007/s10457-018-0307-6

IPCC. (2018). *Global Warming of 1.5°C.An IPCC Special Report on the impacts of global warming of 1.5°C above pre-industrial levels and related global greenhouse gas emission pathways, in the context of strengthening the global response to the threat of climate change, sustainable development, and efforts to eradicate poverty* [Masson-Delmotte, V., P. Zhai, H.-O. Pörtner, D. Roberts, J. Skea, P.R. Shukla, A. Pirani, W. Moufouma-Okia, C. Péan, R. Pidcock, S. Connors, J.B.R. Matthews, Y. Chen, X. Zhou, M.I. Gomis, E. Lonnoy, T. Maycock, M. Tignor, and T. Waterfield (eds.)]. In Press.

Khaled, R. A. H., Duru, M., Decruyenaere, V., Jouany, C., & Cruz, P. (2006). Using leaf traits to rank native grasses according to their nutritive value. *Rangeland Ecology and Management*, 59, 648–654.

https://doi.org/10.2111/05-031R2.1

Komainda, M., Küchenmeister, K., Küchenmeister, F., Breitsameter, L., Wrage-Mönnig, N., Kayser, M., & Isselstein, J. (2019). Forage legumes for future dry climates: Lower relative biomass losses of minor forage legumes compared to *Trifolium repens* under conditions of periodic drought stress. *Journal of Agronomy and Crop Science*, 205, 460-469. https://doi.org/10.1111/jac.12337

Kuchenmeister, K., Kuchenmeister, F., Kayser, M., Wrange-Mönning, N., & Isselstein, J. (2013). Influence of drought stress on nutritive value of perennial forage legumes. *International Journal of Plant Production*, 7, 1735–8043.

Lozano-Parra, J., Schnabel, S., & Ceballos-Barbancho, A. (2015). The role of vegetation covers on soil wetting processes at rainfall event scale in scattered tree woodland of Mediterranean climate. *Journal of Hydrology*, 529, 951–961. https://doi.org/10.1016/j.jhydrol.2015.09.018

Maneta, M.P., Schnabel, S., Wallender, W. W., Panday, S., & Jetten, V. (2008). Calibration of an evapotranspiration model to simulate soil water dynamics in a semiarid rangeland. *Hydrological Processes*, 22, 4655–4669. https://doi.org/10.1002/hyp

Maneta, M.P., Pasternack, G. B., Wallender, W. W., Jetten, V., & Schnabel, S. (2007). Temporal instability of parameters in an event-based distributed hydrologic model applied to a small semiarid catchment. *Journal of Hydrology*, 341, 207–221. https://doi.org/10.1016/j.jhydrol.2007.05.010

Martínez-Fernández, D., Walker, D. J., Romero, P., Martínez-Ballesta, M. C., & Correal, E. (2012). The Response of the Leguminous Fodder Plant *Bituminaria bituminosa* to Water Stress. *Journal of Agronomy and Crop Science*, 198, 442–451. https://doi.org/10.1111/j.1439-037X.2012.00515.x

Melis, R. A. M., Franca, A., Re, G. A., & Porqueddu, C. (2018). Bio-agronomic characterization and implications on the potential use as forage of *Bituminaria bituminosa* and *B. morisiana* accessions. *Grass and Forage Science*, 7, 284–296. https://doi.org/10.1111/gfs.12342

Méndez, P., Fernández, M., & Santos, A. (1991). Variedades de "*Bituminaria bituminosa*" (L.) Stirton (Leguminosae) en el archipiélago canario. *Pastos: Revista de La Sociedad Española Para El Estudio de Los Pastos*, 2021, 157–166.

Méndez, P., Santos, A., Correal, E., & Ríos, S. (2006). Agronomic traits as forage crops of nineteen population of *Bituminaria bituminosa*. In : *Sustainable grassland productivity: Proceedings of the 21st General Meeting of the European Grassland Federation, Badajoz, Spain, 3-6 April, 2006* (pp. 300–302). Sociedad Española para el Estudio de los Pastos (SEEP).

Muñoz, A., Ortiz-Dorda, J., & Correal, E. (2000). Morphological and molecular characterization of *Biturninaria bituminosa* accessions from South-East Spain and the Canary Islands. *Cahiers Options Méditerranéennes,* 45, 103–107.

Oldham, C., Real, D., Bailey, H. J., Thomas, D., Van Burgel, A., Vercoe, P., … Rios, S. (2013). Australian and Spanish scientists are collaborating in the domestication of tedera: Young Merino sheep grazing a monoculture of tedera in autumn showed preference for certain accessions but no signs of ill health. *Crop and Pasture Science*, 64, 399–408. https://doi.org/10.1071/CP13059

Oldham, C. M., Wood, D., Milton, J., Real, D., Vercoe, P., & Van Burgel, A. J. (2015). An animal house study on utilisation of fresh tedera (*Bituminaria bituminosa* var. *albomarginata* and *crassiuscula*) by Merino wethers. *Animal Production Science*, 55, 617-624. https://doi.org/10.1071/AN13068

Olivera-Viciedo, D., de Mello Prado, R., Martinez, C. A., Habermann, E., Branco, R. B. F., de Cássia Piccolo, M., ... & Lata Tenesaca, L. F. (2020). Water stress and warming impact nutrient use efficiency of Mombasa grass (*Megathyrsus maximus*) in tropical conditions. *Journal of Agronomy and Crop Science*. https://doi.org/10.1111/jac.12452

Or, D., Lehmann, P., Shahraeeni, E., & Shokri, N. (2013). Advances in soil evaporation physics—A review. *Vadose Zone Journal*, 12(4)*, 1-16.*.

Pazos-Navarro, M., Dabauza, M., Correal, E., Hanson, K., Teakle, N., Real, D., & Nelson, M. N. (2011). Next generation DNA sequencing technology delivers valuable genetic markers for the genomic orphan legume species, *Bituminaria bituminosa*. *BMC Genetics*, 12, 140. https://doi.org/10.1186/1471-2156-12-104

Pistelli, L., Noccioli, C., Appendino, G., Bianchi, F., Sterner, O., & Ballero, M. (2003). Pterocarpans from *Bituminaria morisiana* and *Bituminaria bituminosa*. *Phytochemistry*, 64, 595-598. https://doi.org/10.1016/S0031-

9422(03)00190-0

Pecetti, L., Tava, A., Pagnotta, M., & Russi, L. (2007). Variation in forage quality and chemical composition among Italian accessions of *Bituminaria bituminosa* (L.) Stirt. *Journal of the Science of Food and Agriculture*, 87, 985–991. https://doi.org/10.1002/jsfa.2792

Poorter, H., & Remkes, C. (1990). Leaf area ratio and net assimilation rate of 24 wild species differing in relative growth rate. *Oecologia*, *83*, 553–559.

Porqueddu, C., Ates, S., Louhaichi, M., Kyriazopoulos, A. P., Moreno, G., del Pozo, A., … Nichols, P. G. H. (2016). Grasslands in "Old World" and "New World" Mediterranean-climate zones: Past trends, current status and future research priorities. *Grass and Forage Science*, 71, 1–35. https://doi.org/10.1111/gfs.12212

Porqueddu, C., Dettori, G., Falqui, A., & Re, G. A. (2011). Bio-agronomic evaluation of fifteen accessions within Psoralea complex. In *Grassland farming and land management systems in mountainous regions. Proceedings of the 16th Symposium of the European Grassland Federation, Gumpenstein, Austria, 29th-31st August, 2011* (pp. 374–376). Agricultural Research and Education Center (AREC) Raumberg-Gumpenstein.

R Development Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Raeside, M. C., Nie, Z. N., Clark, S. G., Partington, D. L., Behrendt, R., & Real, D. (2012). Evaluation of tedera [(*Bituminaria bituminosa* (L.) C.H. Stirton var. *albomarginata*)] as a forage alternative for sheep in temperate southern Australia. *Crop and Pasture Science*, 63, 1135–1144. https://doi.org/10.1071/CP12293

Real, D., Oldham, C. M., Nelson, M. N., Croser, J., Castello, M., Verbyla, A., … Ewing, M. A. (2014). Evaluation and breeding of tedera for Mediterranean climates in southern Australia. *Crop and Pasture Science*, 65, 1114–1131. https://doi.org/10.1071/CP13313

Real, D. & Verbyla, A. (2010). Maximizing genetic gains using a plant model in the Tedera (*Bituminaria bituminosa* var. *albomarginata* and var. *crassiuscula*) breeding program in Australia. *Options Méditerranéennes*, 92, 87-95.

Real, D, Oldham, C., Burgel, A., Dobbe, E., & Hardy, J. (2017). Tedera proves its value as a summer and autumn feed for sheep in Mediterranean-like climates, *Animal Production Science* 58, 2269–2279. https://doi.org/10.1071/AN16432

Ritchie, J. T. (1972). Model for predicting evaporation from a row crop with incomplete cover. *Water Resources Research*, 8, 1204–1213. https://doi.org/10.1029/WR008i005p01204

SIA. (2019, December 4). *Servicio de Información sobre Alimentos: Base de datos pastos españoles (SEEP)/alimentos para animales*. Retrieved from http://www.uco.es/sia/banco_de_datos/index.php?banco=8

Staniak, M., & Harasim, E. (2018). Changes in nutritive value of alfalfa (medicago× varia t. martyn) and festulolium (festulolium braunii (k. richt) a.

camus) under drought stress. *Journal of Agronomy and Crop Science*, 204(5), 456-466.

Sternberg, M., Gishri, N., & Mabjeesh, S. J. (2006). Effects of grazing on *Bituminaria bituminosa* (L) Stirton: A potential forage crop in Mediterranean grasslands. *Journal of Agronomy and Crop Science*, 192, 399–407. https://doi.org/10.1111/j.1439-037X.2006.00227.x

Suriyagoda, L. D. B., Real, D., Renton, M., Lambers, H., & Ryan, M. H. (2013). Establishment, survival, and herbage production of novel, summer-active perennial pasture legumes in the low-rainfall cropping zone of Western Australia as affected by plant density and cutting frequency. *Crop and Pasture Science*, 64, 71–85. https://doi.org/10.1071/CP12398

Vázquez De Aldana, B. R., García-Ciudad, A., & García-Criado, B. (2008). Interannual variations of above-ground biomass and nutritional quality of Mediterranean grasslands in Western Spain over a 20-year period. *Australian Journal of Agricultural Research*, 59, 769–779. https://doi.org/10.1071/AR07359

Ventura, M. R., Castañon, J. I. R., & Mendez, P. (2009). Effect of season on tedera (*Bituminaria bituminosa*) intake by goats. *Animal Feed Science and Technology*, 153, 314–319. https://doi.org/10.1016/j.anifeedsci.2009.06.018

Ventura, M. R., Méndez, P., Flores, M. P., Rodriguez, R., & Castañon, J. I. R. (2000). Energy and protein content of Tedera (*Bituminaria bituminosa*). *Cahiers Options Méditerranéennes*, 45, 219-221.

Villar, R., Ruiz-Robleto, J., Quero, J. L., Poorter, H., Valladares, F., & Marañón,

T. (2004). Tasas de crecimiento en especies leñosas: aspectos funcionales e implicaciones ecológicas. In Valladares, F. (Ed). *Ecología del bosque mediterráneo en un mundo cambiante.* (pp. 191-227). Ministerio de Medio Ambiente, EGRAF, S. A., Madrid.

Walker, D. J., Romero, P., & Correal, E. (2010). Cold tolerance, water relations and accumulation of osmolytes in *Bituminaria bituminosa*. *Biologia Plantarum*, 54, 293–298. https://doi.org/10.1007/s10535-010-0051-x

Wang, G., Liu, S., Fang, Y., & Shangguan, Z. (2020). Adaptive changes in root morphological traits of Gramineae and Leguminosae seedlings in the ecological restoration of the semiarid region of northwest China. *Land Degradation & Development,* 1-13. https://doi.org/10.1002/ldr.3616

Wu, Y., Liu, X., Wang, W., Zhang, S., & Xu, B. (2012). Calcium regulates the cell-to-cell water flow pathway in maize roots during variable water conditions. *Plant Physiology and Biochemistry*, 58, 212–219. https://doi.org/10.1016/j.plaphy.2012.07.005

Zarovali, M. P., Yiakoulaki, M. D., & Papanastasis, V. P. (2007). Effects of shrub encroachment on herbage production and nutritive value in semi-arid Mediterranean grasslands. *Grass and Forage Science*, 62, 355–363. https://doi.org/10.1111/j.1365-2494.2007.00590.x

Table 1. Population and descriptive location data for the three varieties used. *B. bituminosa* var. *albomarginata* (BAM); *B. bituminosa* var. *bituminosa* (BBT); *B. bituminosa* var. *crassiuscula* (BCC).

Variety	Population	Location	Average Rainfall (mm)	Altitude (m)	Mean temperature (ºC) hottest/coldest months	Average annual evapotranspiration (mm)
BAM	Malpaso	Lanzarote	150-250	250-280	24/16	1293
BCC	Chavao-Cañadas	Tenerife	500	1900-2200	22/8	1585
BBT	Tamarco	Tenerife	400	400	22/15	1208

Table 2. Dry mass production and senescent leaves to green leaves ratio at the end of the experiment (63 days) for *B. bituminosa* varieties under two water regimes.

Variety	Treatment	Shoot dry mass (g)	Root dry mass (g)	Total dry mass (g)	Senescent leaves: Green leaves (g g^{-1})
BAM	WW	6.0 ± 1.0 c	2.7 ± 0.4 c	8.7 ± 1.4 c	0.38 ± 0.10 b
	DW	3.0 ± 0.7 d	1.2 ± 0.4 d	4.2 ± 1.1 d	0.52 ± 0.03 a
BBT	WW	13.7 ± 1.4 a	5.9 ± 0.9 a	19.6 ± 1.9 a	0.33 ± 0.06 b
	DW	6.7 ± 1.4 b	3.1 ± 0.5 b	9.8 ± 1.8 b	0.35 ± 0.03 a
BCC	WW	12.5 ± 1.7 a	8.0 ± 1.3 a	20.5 ± 2.2 a	0.18 ± 0.04 b
	DW	7.1 ± 0.4 b	3.5 ± 0.3 b	10.6 ± 0.5 b	0.32 ± 0.04 a

Mean values (n = 6) not sharing a common letter differ significantly *($P < 0.05$)* according to Tukey's test. Standard error is showed. BAM: *B. bituminosa* var. *albomarginata*; BBT: *B. bituminosa* var. *bituminosa*; BCC: *B. bituminosa* var. *crassiuscula*.

Table 3. Morphological traits at the end of the experiment (63 days) for the *B. bituminosa* varieties. Mean of the two water regime treatments is presented. Leaf to stem ratio, root to shoot ratio, Leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), specific leaf area (SLA) and leaf area ratio (LAR)

Variety	Leaf:Stem ratio ($g\ g^{-1}$)	Root: Shoot ratio ($g\ g^{-1}$)	LMF ($g\ g^{-1}$)	SMF ($g\ g^{-1}$)	RMF ($g\ g^{-1}$)	SLA ($cm^2\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)
BAM	1.06 ± 0.10 a	0.46 ± 0.05 n.s.	0.36 ± 0.02 a	0.35 ± 0.02 b	0.29 ± 0.02 b	235 ± 10.0 b	86.9 ± 7.3 a
BBT	0.38 ± 0.04 c	0.47 ± 0.04 n.s.	0.19 ± 0.02 c	0.50 ± 0.02 a	0.31 ± 0.02 ab	352 ± 17.0 a	64.1 ± 4.8 b
BCC	0.80 ± 0.03 b	0.59 ± 0.06 n.s.	0.28 ± 0.01 b	0.36 ± 0.01 b	0.36 ± 0.02 a	239 ± 9.3 b	67.6 ± 3.7 ab

Mean values (n = 6) not sharing a common letter differ significantly *($P < 0.05$)* according to Tukey's test. Standard error is showed. BAM: *B. bituminosa* var. *albomarginata*; BBT: *B. bituminosa* var. *bituminosa*; BCC: *B. bituminosa* var. *crassiuscula.* n.s.: no significant.

Table 4. Relative water content (RWC) of the leaf, net photosynthesis per area (A_{area}), stomatal conductance per area (g_{Sarea}) and water use efficiency (WUE) for *B. bituminosa* varieties under two water regimes measured at day 46.

Variety	Treatment	RWC (%)	A_{area} (µmol CO_2 m^{-2} s^{-1})	g_{Sarea} (mmol H_2O m^{-2} s^{-1})	WUE (µmol CO_2 $(molH_2O)^{-1}$))
BAM	WW	85.5 ± 1.5 a	19.9 ± 1.6 a	192.5 ± 8.5 a	105.6 ± 11.3 n.s.
	DW	92.1 ± 1.0 a	15.5 ± 2.1 a	164.9 ± 20.4 b	104.9 ± 21.4 n.s.
BBT	WW	83.5 ± 2.5 b	6.0 ± 2.0 bc	54.7 ± 11.8 c	117.2 ± 18.0 n.s.
	DW	77.3 ± 4.7 b	4.7 ± 1.1 c	35.2 ± 4.3 d	124.7 ± 21.6 n.s.
BCC	WW	81.9 ± 3.4 b	13.3 ± 2.7 ab	75.5 ± 29.9 c	138.9 ± 28.4 n.s.
	DW	74.8 ± 4.2 b	2.5 ± 0.6 c	23.2 ± 5.3 d	86.3 ± 13.6 n.s.

Mean values (n = 6) not sharing a common letter differ significantly *($P < 0.05$)* according to Tukey’s test. Standard error is showed. BAM: *B. bituminosa* var. *albomarginata*; BBT: *B. bituminosa* var. *bituminosa*; BCC: *B. bituminosa* var. *crassiuscula.* n.s.: no significant.

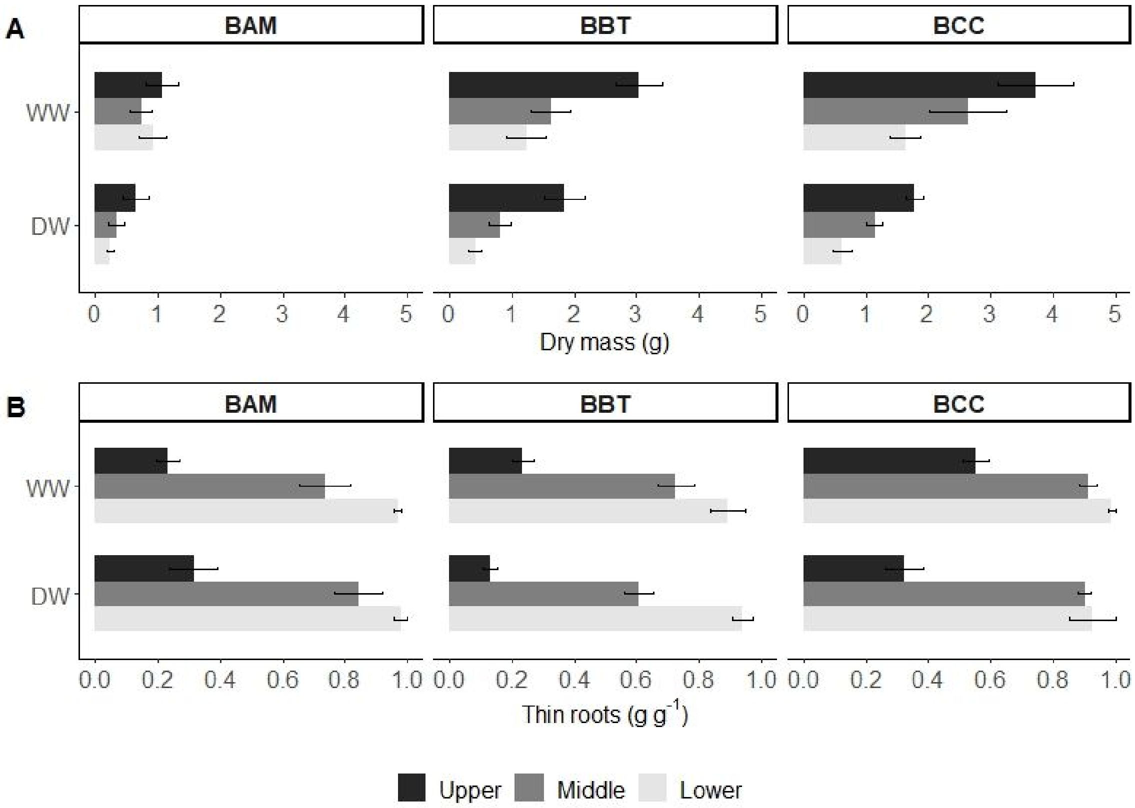

Fig.1. **A**: Root dry mass distribution along the three soil layers at the end of the experiment (63 days) for *B. bituminosa* varieties under two water regimes and **B**: Proportion of thin roots. Thin roots proportion down the three soil layers for *Bituminaria bituminosa* varieties. BAM=*B. bituminosa* var. *albomarginata*; BBT=*B. bituminosa* var. *bituminosa*; BCC= *B. bituminosa* var. *crassiuscula*; WW= well-watered; DW=deficit-watered. Mean values and standard errors (N=6).

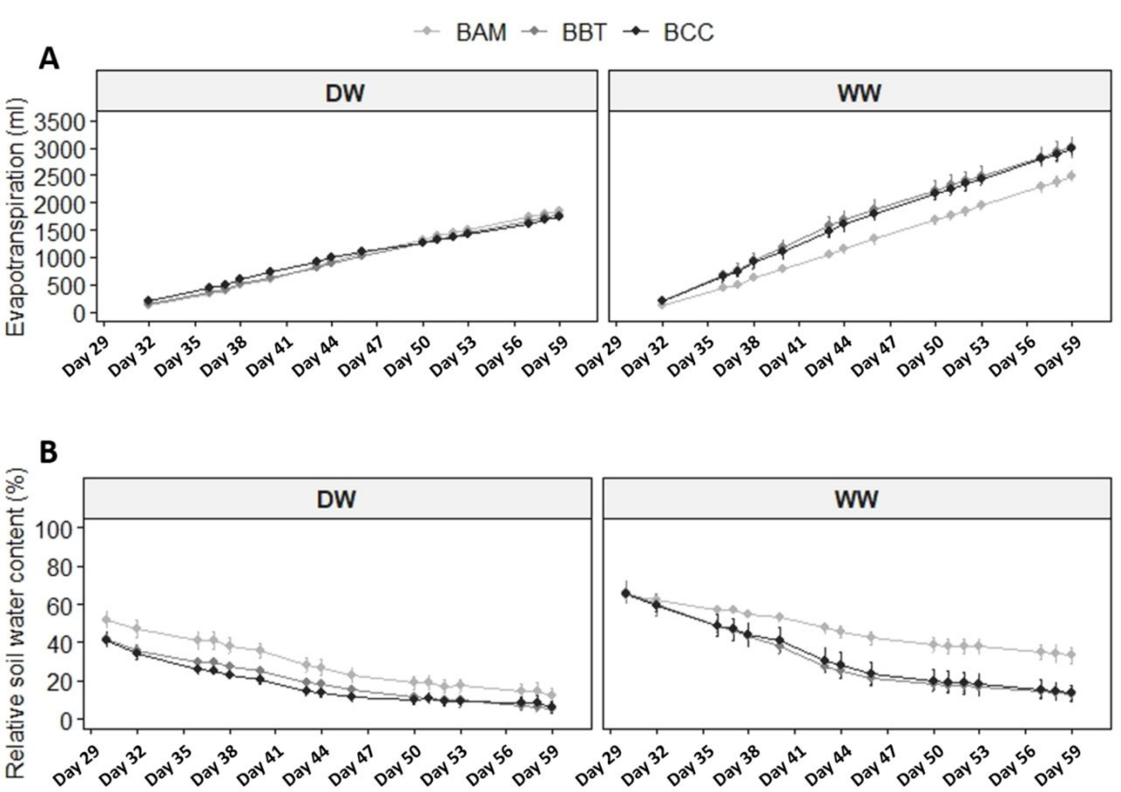

Fig.2. Evapotranspiration (ml) (**A**) and relative soil water content (RSWC) (%) (**B**) of *B. bituminosa* varieties under two water regimes WW= well-watered and DW=deficit-watered. BAM=*B. bituminosa* var. *albomarginata*; BBT=*B. bituminosa* var. *bituminosa*; BCC= *B. bituminosa* var. *crassiuscula.* Mean values and standard errors (N=4).

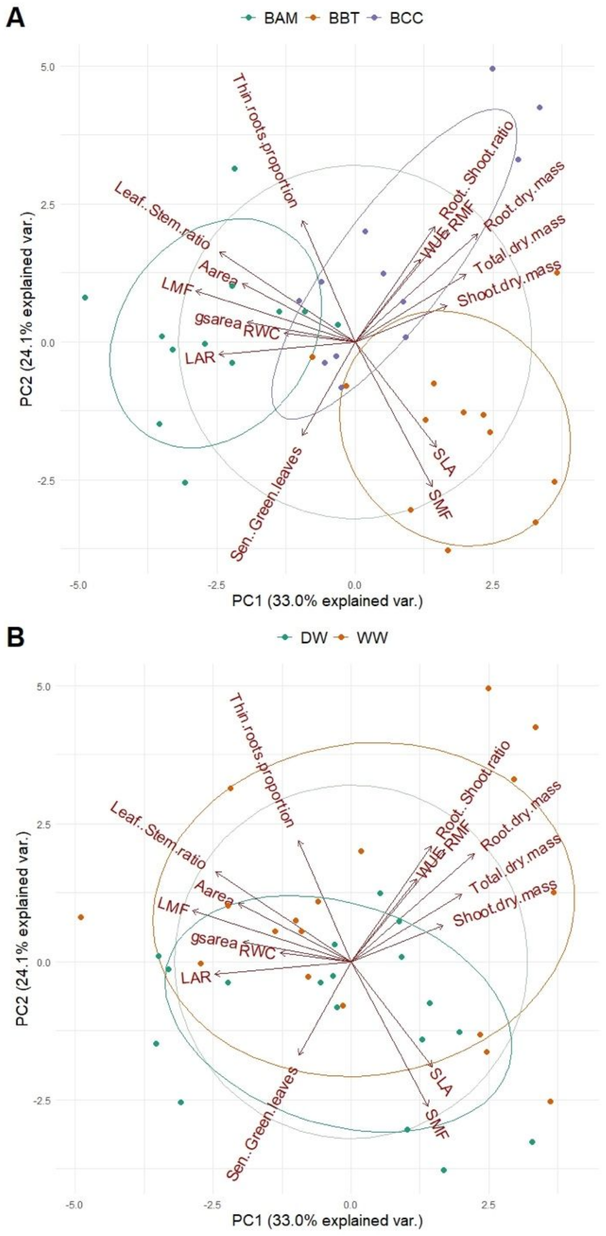

Fig. 3. Principal component analysis (PCA) showing the two main axes of variability in morphological and physiological variables. Normal ellipse grouping observations by *B. bituminosa* variety: BAM=*B. bituminosa var. albomarginata;* BBT=*B. bituminosa var. bituminosa;* BCC= *B. bituminosa var. crassiuscula* (**A**) and water regime: WW= well-watered and DW=deficit-watered (**B**). Abbreviations: net photosynthesis per area (Aarea), stomatal conductance per area (gsarea), water use efficiency (WUE), Leaf mass fraction (LMF), stem mass fraction (SMF), root mass fraction (RMF), specific leaf area (SLA) and leaf area ratio (LAR), Senescent to green leaves ratio (Sen.green,leaves).

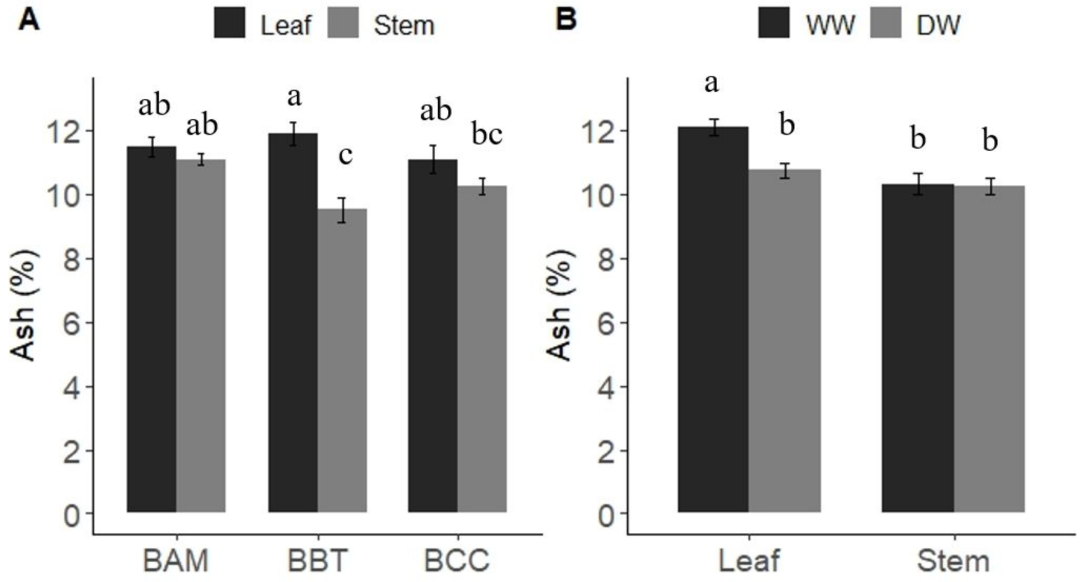

Fig. 4. Ash content (%) in leaf and stem of *B. bituminosa* varieties (means of two water regimes) (**A**), and in leaf and stem under two water regimes WW= well-watered and DW=deficit-watered (means of three *B. bituminosa* varieties) (**B**) at the end of the experiment (63 days). BAM=*B. bituminosa var. albomarginata*; BBT=*B. bituminosa var. bituminosa*; BCC= *B. bituminosa var. crassiuscula*. Mean values and standard errors (N=4). Means not sharing a common letter differ significantly (P < 0.05) according to Tukey's test.

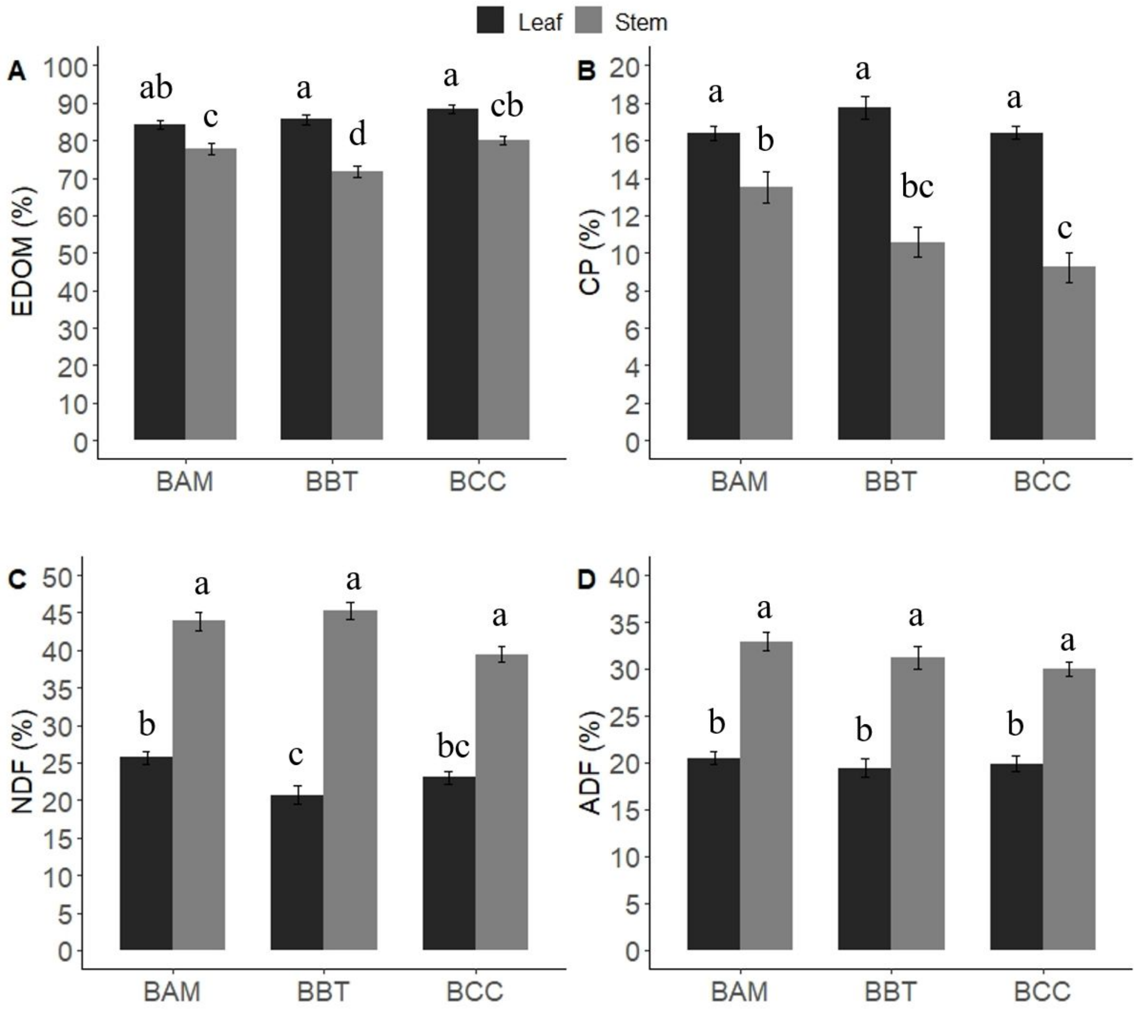

Fig. 5. Enzyme digestibility of organic matter (EDOM) (**A**), crude protein (CP) (**B**), neutral detergent fibre (NDF) (**C**) and acid detergent fibre (ADF) (**D**) of leaf and stem of *B. bituminosa* varieties at the end of the experiment (63 days). BAM=*B. bituminosa* var. *albomarginata*; BBT=*B. bituminosa* var. *bituminosa*; BCC= *B. bituminosa* var. *crassiuscula*. Mean values and standard errors (N=4). Means not sharing a common letter differ significantly ($P < 0.05$) according to Tukey's test.

8. Supplementary material