# Maximizing genetic gains using a plant model in the Tedera (Bituminaria bituminosa var. albomarginata and var. crassiuscula) breeding program in Australia

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# Maximizing genetic gains using a "plant" model in the Tedera (*Bituminaria bituminosa* var. *albomarginata* and var. *crassiuscula*) breeding program in Australia

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**Abstract.** *Bituminaria bituminosa* var. *albomarginata* (albo tedera) and var. *crassiuscula* (teide tedera) are self-pollinated herbaceous drought tolerant perennial forage legumes. These species have the ability to remain green without shedding their leaves during summer/autumn in dry Mediterranean climates providing valuable out-of-season feed. In June 2008, three spaced plant nurseries of 1900 plants each were established in the 350 mm annual rainfall zone of the Western Australian wheat-belt at Buntine, Merredin and Newdegate. A total of 19 entries were utilized in these nurseries: 17 tedera accessions; *Medicago sativa* L. (SARDI 10) and *Dorycnium hirsutum* Ser. (TAS001). Visual scores of plant size were taken in November 2008 (three sites), before and after each grazing in May 2009 (Buntine and Newdegate) and August 2009 (three sites). Leaf retention was measured in the peak of water stress in March 2009 before the start of the rainy season. Selection of Individual plants using individual traits and using a multivariate index was based on a mixed model. The mixed model incorporated the experimental design as well as possible spatial variation. The additive and non-additive genetic effects were included by using a constructed pedigree and a factor analytic model was used to estimate the variances of the traits and correlations between traits. Non-additive effects were zero; therefore selection of best plant was based on additive genetic effects only.

**Keywords.** Breeding methods – Mixed models – Drought tolerance – Forage legumes.

***Maximisation du gain génétique en utilisant une "plante" modèle dans le programme d'amélioration de Tedera (*Bituminaria bituminosa *var.* albomarginata *et var.* crassiuscula*) en Australie***

***Résumé.*** Bituminaria bituminosa *var.* albomarginata *(albo tedera) et la var.* crassiuscula *(teide tedera) sont des légumineuses fourragères autogames, herbacées pérennes. Elles possèdent la caractéristique d'être très résistantes à la sécheresse et ont la capacité de ne pas perdre de feuilles pendant l'été/automne dans des zones à climat méditerranéen, en conservant un fourrage vert sur pied de haute qualité. En juin 2008, on a installé trois lits de semis de 1900 plants isolés, chacun dans des zones à 350 mm de pluie par an situées dans la ceinture à blé en Australie occidentale dans les sites de Buntine, Merredin et Newdegate. Un total de 19 matériels ont été évalués : 17 accessions de tedera,* Medicago sativa *L. (SARDI 10) et* Dorycnium hirsutum *Ser. (TAS001). Des scores visuels concernant la taille des plantes furent réalisés en novembre 2008 (les trois sites), pré- et post-pâturage en mai 2009 (Buntine et Newdegate) et août 2009 (les trois sites). La proportion de feuilles dans les plantes a été mesurée au moment de plus grand stress hydrique en mars 2009. Des plantes individuelles furent sélectionnées en utilisant des modèles mixtes. Ces modèles incorporent les variations dues au dispositif expérimental et modélisent également la variation spatiale. Les effets additifs et non additifs de la variance génétique ont été incorporés en utilisant les relations de parenté entre les accessions de tedera (pedigree) et un modèle de facteur analytique a été employé pour modéliser les variances de chaque caractéristique et ses corrélations. Les effets non additifs furent proches de zéro, donc la sélection des meilleures plantes a été faite sur la base des effets génétiques additifs.*

***Mots-clés.*** *Amélioration génétique – Modèles mixtes – Tolérance à la sécheresse – Légumineuses fourragères.*

# I – Introduction

*Bituminaria bituminosa* is a perennial forage legume with a broad geographical distribution along both sides of the Mediterranean Sea and the Macronesian Islands. In the Canary Islands, there are three botanical varieties: *bituminosa*, *crassiuscula* and *albomarginata*. Two of these varieties (*albomarginata* and *crassiuscula*) are allowed to be introduced into Australia by the Australian and Western Australian Quarantine and Inspection Services. The variety *albomarginata* (common name: albo tedera) originally from Lanzarote Island is the most drought tolerant of the three varieties with the ability to remain green without shedding its leaves during summer/autumn in dry Mediterranean climates (Méndez *et al.*, 2000; Correal *et al.*, 2003). The var. *crassiuscula* (common name: teide tedera) native to the Teide mountain in Tenerife Island is a high altitude species with cold tolerance as well as drought tolerance (Méndez *et al.*, 1991). Tedera will be used as the common name for both botanical varieties.

Even though this species has been used traditionally by farmers in the Canary Islands for hundreds of years, the scientific community only began research work on this species in the last 20 years. Recently, a coordinated scientific network involving countries with Mediterranean climate began focused work on different aspects of this species (Real *et al.*, 2009).

After four years of perennial legume species evaluation in WA in areas with annual rainfall from 200 mm to 450 mm, tedera was found not only to be one of the most drought tolerant and productive herbaceous forage legumes evaluated in southern Australia, but also it has a broad adaptation to a diverse range of soil types. During two of the driest summer/autumns on record in WA (2006/2007 and 2007/2008), tedera remained green without shedding its leaves and was superior to lucerne under the same conditions. It also tolerates grazing as part of a mixed sward and importantly, competes well with annual species. Therefore, it is now regarded as a promising new herbaceous perennial legume for providing out-of-season forage production across all rainfall zones in southern Mediterranean Australia. It has become the primary focus of new plant development research.

Early generation plant breeding evaluation nurseries usually evaluate a large number of entries in several locations. Even though these trials are replicated to minimize environmental effects, in most sexually propagated breeding programs, entries are genetically different, meaning from a genetic point of view that are in fact un-replicated trials. Entries are related by ancestry and if a pedigree relationship is provided for the analysis, all entries can be grouped into families, founders, ancestors and/or genetic groups. This kind of analysis will allow the estimation of best linear unbiased predictors (BLUPs) for individual plants as well as for each heritage level. The BLUP estimation can be at an additive genetic or total genetic level, which will enable the selection of best parent plants for further crossing if based on additive genetic variance or BLUPS for total genetic effect if it is intended for seed increase and commercial release of particular plants/lines.

The purpose of this paper is to present the methodology of how to select the individual plants to be used as parents in future crossing programs according to their field performance, continuous availability of green leaves over summer/autumn, grazing tolerance and ability to recover after grazing to maximize the genetic gains by predicted individual BLUPs from individual plant data.

# II – Materials and methods

## 1. Site description

Three experimental sites representing the low-rainfall zone of the Western Australian wheatbelt were established in June 2008. These were located at Buntine (Liebe group long-term research site, 20 km west of Buntine), the Department of Agriculture and Food, Western Australia (DAFWA) Research Station at Merredin and the DAFWA Research Station at Newdegate (18

km west of Newdegate town site). The Buntine site had long been cultivated with wheat (*Triticum aestivum* L.), while the Merredin and Newdegate sites cultivated with lupins (*Lupinus angustifolius* L.) and wheat in rotation for the last 3 years.

The soils for each site are classified according to Northcote (1979) as Sandy yellow earth (Ms9, Gn2.21); Sandy yellow earth (Ms8, Gn2.21) and Loamy sand over clay (Va66, Dy3.43) for Buntine, Merredin and Newdegate respectively. Fourteen samples were taken from the top 30 cm layer of soil at the time of transplanting using a soil auger at each site. Each soil sample was analysed separately for physical and chemical characteristics [ammonium and nitrate N concentrations, available phosphorus (P), potassium (K), sulfur (S), organic carbon. Aluminium and pH] at CSBP Ltd (Bibra Lake, Australia) (Table 1). The soils of the three sites are acidic, non-saline, sandy and with low levels of organic carbon.

**Table 1. Mean values of physical and chemical soil characteristics of three sites (n = 14)**

| Site | Texture | $NO_3$-N mg/kg | $NH_4$-N mg/kg | P† mg/kg | K† mg/kg | S† mg/kg | Organic C (%) | Ph $CaCl_2$ | Al† mg/kg |
|---|---|---|---|---|---|---|---|---|---|
| Buntine | 1.5 | 12.9 | 1.0 | 33.6 | 58.6 | 8.8 | 0.65 | 4.94 | 2.4 |
| Merredin | 1.6 | 11.4 | 1.0 | 16.0 | 48.8 | 35.5 | 0.69 | 4.22 | 18.8 |
| Newdegate | 1.5 | 13.1 | 2.1 | 24.7 | 29.9 | 6.9 | 0.86 | 4.72 | 2.7 |
| $w_{\alpha=0.05}$†† | 0.1 | 1.7 | 0.3 | 4.0 | 3.4 | 2.0 | 0.06 | 0.15 | 1.5 |

†Soil available ion concentrations of P, K, S and Al are given.
††Tukey's Significant Difference.

The rainfall data for the period of study is presented in Fig. 1. The long term rainfall of Buntine, Merredin and Newdegate are 356 mm, 313 mm and 350 mm respectively. Data were obtained from the website of the Bureau of Meteorology (http://www.bom.gov.au/climate/dwo/IDCJDW0600.shtml).

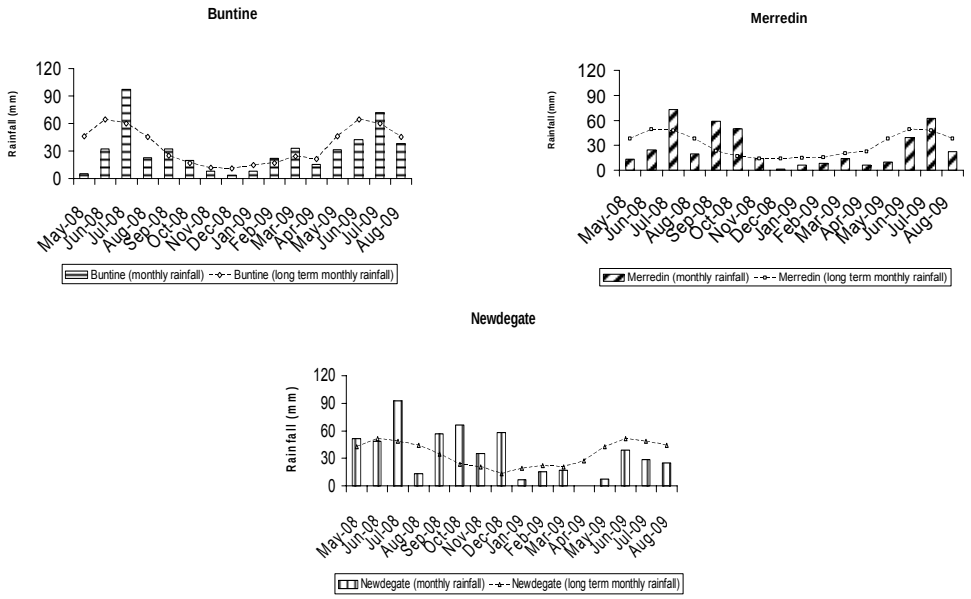


**Fig. 1. Rainfall from May 2008 until August 2009 for Buntine, Merredin and Newdegate.**

## 2. Plant material

Seventeen accessions (Lines 1 to 16 and 22) of *Bituminaria bituminosa* var. *albomarginata* and var. *crassiuscula* (tedera) were used in the study. Seeds of tedera were received from Spain starting in 2006 as part of an agreement between the Future Farm Industries Cooperative Research Centre (FFI CRC) from Australia and The University of Alicante (UA), Instituto Murciano de Investigación y Desarrollo Agrario y Alimentario (IMIDA) and Instituto Canario de Investigaciones Agrarias (ICIA). Seed for these evaluations were collected from multiplication plots established at the DAFWA Medina Research Station. The pedigree information is presented in Table 2. *Medicago sativa* L. (lucerne) cv. SARDI 10 seeds were obtained from the Genetic Resource Centre at the South Australian Research and Development Institute. *Dorycnium hirsutum* Ser. (Hairy Canary clover) accession TAS001 seeds were obtained from the Trifolium Genetic Resource Centre at DAFWA. Seeds were scarified with sand-paper, placed into Petri dishes and watered to facilitate germination. After three to four days, the seedlings were transplanted into 40-cell trays (each cell of 4 cm diameter and 9 cm deep) filled with commercial potting mix. After transplanting, seedling trays were watered with their specific rhizobia inoculant slurry. Seedlings were raised in a naturally lit glasshouse for two months at a constant temperature of 25ºC. Seedlings trays were move to an outside area to harden-up for two weeks before transplanting to the field sites in June 2008.

**Table 2. Pedigree relationships (Genetic groups, ancestors and founders)**

| Me | Mother | Father | Me | Mother | Father |
|---|---|---|---|---|---|
| G1 | 0 | 0 | Line 1 - E7PF3A14 | Albo1 | Albo1 |
| G2 | 0 | 0 | Line 2 - E7PF24A13 | Albo2 | Albo2 |
| G3 | 0 | 0 | Line 3 - E53NPF33A4 | E7PF17A9 | E7PF17A9 |
| G5 | 0 | 0 | Line 4 - E53NPF33A2 | E7PF17A9 | E7PF17A9 |
| G6 | 0 | 0 | Line 5 - E7PF31A3 | Albo3 | Albo3 |
| Albo1 | G1 | G1 | Line 6 - E53NPF23A15 | Teno3 | Teno3 |
| Albo2 | G1 | G1 | Line 7 - E53NPF23A11 | Teno3 | Teno3 |
| Albo3 | G1 | G1 | Line 8 - E53NPF34A7 | E7PF24A4 | E7PF24A4 |
| Albo8 | G1 | G1 | Line 9 - E53NPF41A4 | E7PF31A10 | E7PF31A10 |
| Teno3 | G2 | G2 | Line 10 - E53NPF34A5 | E7PF24A4 | E7PF24A4 |
| Teno4 | G2 | G2 | Line 11 - E7PF31A2 | Albo3 | Albo3 |
| Teide3 | G3 | G3 | Line 12 - E53NPF22A15 | Teno4 | Teno4 |
| Famara1 | G5 | G5 | Line 13 - E53NPF22A13 | Teno4 | Teno4 |
| Malpaso1 | G6 | G6 | Line 14 - Malpaso1 | Malpaso1 | Malpaso1 |
| E7PF24A4 | Albo2 | Albo2 | Line 15 - Famara1 | Famara1 | Famara1 |
| E7PF31A10 | Albo3 | Albo3 | Line 16 - OMV33EF31A9 | Teide3 | Teide3 |
| E7PF17A9 | Albo8 | Albo8 | Line 22 - E7PF3A8 | Albo1 | Albo1 |

## 3. Field design, site preparation and management

Glyphosate (Roundup) was applied in May 2009 at a rate of 540 g of a.i./ha before site preparation to ensure a weed-free seedbed. Two days before transplanting, SpraySeed was sprayed to the area at a rate of 135 g Paraquat/115 g Diquat of a.i./ha to control recently germinated weeds. All sites were surrounded by high mesh fences to avoid disturbance from wild animals and livestock. The field layout was a latinized block design of 18 plots x 6 replicates (16 plots of tedera + one of lucerne + one of hairy canary clover). Each replicate consisted of 3 rows x 6 columns. This produced a latinized design of 9 rows by 12 columns of experimental units. Each experimental unit consisted of 16 spaced plants in a grid of 1 m x 1 m

(4 rows x 4 columns). One plot per replicate had 16 plants each belonging to a different accession of tedera, instead of having the 16 plants from the same tedera accession. The inclusion of this "mixed" plot allowed the evaluation of 17 accessions of Tedera. The spaced plant nursery had 36 rows (9 rows x 4 spaced plants per row) x 48 columns (12 columns x 4 plants per column) making a total of 1728 plants. The experiment was surrounded by one row of spaced plants (172 plants) to minimize the edge effect in the trial, adding to a total of 1900 spaced plants per site. The spaced plant nurseries were kept weed free by hand-weeding for the first 4 to 6 months and then competition with background species was allowed. No fertilisers were added.

## 4. Data collection

(i) Plant size (PS): visual score (0 to 10 = largest plants) before grazing (bg) and after grazing (ag) taken in November 2008 (three sites), May 2009 (Buntine and Newdegate) and August 2009 (three sites).

(ii) Leaf retention (LR): proportion of leaves in the plants measured in March 2009, in the peak of water stress, before the start of the rainy season and with high temperatures.

## 5. Statistical analysis

Mixed models were developed for the analysis. These models incorporate the experimental design outlined above and included effects to incorporate possible spatial variation along the lines of Gilmour *et al.* (1997). These effects were small because the data are scores. A multi-trait analysis was performed to enable genetic variation for each trait to be incorporated together with genetic correlations between traits. This allows selection on an individual trait basis and also across traits if desired. The model fitted had the general (symbolic) form:

$$y = \text{Trait.Site.Type.DeadAlive} + \textit{Trait.ped(Plant)} + \textit{Trait.ide(Plant)} + \textit{Trait.Rowrep} + \textit{Trait.Colrep} + \textit{Trait.MRow.MCol} + \textit{error}$$

where "y" represents the data across all Traits, "Site" refers to a factor of 3 levels (the experimental locations), "Type" separates Tedera from *Dorycnium* and Lucerne (a factor with 3 levels) and allows the genetic components to reflect only the Tedera lines, "DeadAlive" is a factor that details the state of the plant, "Plant" is a factor for all the plants in the experiment, "Rowrep" and "Colrep" are row and column factors refecting the two-way blocking as specified by Reps 1 to 6 and Columns A to F, while "Mrow" and "Mcol" reflect the 9 rows and 12 columns in the Latinized design. Terms joined by a dot are interactions and terms in italics are random effects. The expressions "ped(Plant)" and "ide(Plant)" are respectively the pedigree or additive effects and non-additive effects for plants; see Oakey *et al.* (2006, 2007) for a discussion on using pedigrees in plant studies. The two terms involving plants effects allow for different variances for traits and correlation between traits. A Factor analytic model (Smith *et al.*, 2005) was used to model the variances and correlations. The other random effects involving "Trait" allowed for different variances for each effect.

The error was initially modelled using an AR1 x AR1 structure as in Smith *et al.* (2005), in conjunction with Site by Trait combinations. This allows for possible spatial dependence of data on each trait at each site. It was found that spatial correlation was very low and hence a general multi-trait variance matrix was fitted for each site. This incorporates possible correlation between traits at the error or residual level. A multivariate selection index was generated using four traits (weights within brackets): PS Nov08 (0.1); PS bgMay09 (0.4); PS bgAug09 (0.2) and LR Mar09 (0.3).

## III – Results and discussion

The model was fitted using ASReml (Butler *et al.*, 2009) in R (R_Development_Core_Team, 2009) using a factor analytic model with two factors (17 parameters) for the additive genetic effects. The estimated variances for non-genetic variation were zero. The spatial effects as modelled in the *error* were quite small, a consequence of the measurement scale being scores. This also led to a multi-trait (correlated) model for each site.

The estimated genetic correlations between the 6 traits are presented in Table 3. The genetic correlations were very high (>0.8) among all plant size score measurements taken in Nov 08, May 09 and August 09. Leaf retention was moderately correlated to the plant size measurements, being the best correlation with plant size before grazing in May 09.

**Table 3. Genetic correlations among all six traits**

| | PS (Nov08) | PS bg (May09) | PS ag (May09) | PS bg (Aug09) | PS ag (Aug09) | LR (Mar09) |
|---|---|---|---|---|---|---|
| PS (Nov08) | 1 | 0.845 | 0.998 | 0.985 | 0.998 | 0.376 |
| PS bg (May09) | 0.845 | 1 | 0.854 | 0.923 | 0.878 | 0.813 |
| PS ag (May09) | 0.998 | 0.854 | 1 | 0.987 | 0.997 | 0.393 |
| PS bg (Aug09) | 0.985 | 0.923 | 0.987 | 1 | 0.994 | 0.527 |
| PS ag (Aug09) | 0.998 | 0.878 | 0.997 | 0.994 | 1 | 0.436 |
| LR (Mar09) | 0.376 | 0.813 | 0.393 | 0.527 | 0.436 | 1 |

Selection of individual plants was the aim of the trials. Table 4 lists the top 10 plants (in order from best to 10$^{th}$ best) out of 4608 Tedera plants for each individual trait but based on the full analysis of all traits.

**Table 4. Ranking of 10 best plants for each trait**

| Ranking | PS Nov08 | | PS bg May09 | | PS ag May09 | | PS bg Aug09 | | PS ag Aug09 | | LR Mar09 | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Plant Code | BLUP | Plant Code | BLUP | Plant Code | BLUP | Plant Code | BLUP | Plant Code | BLUP | Plant Code | BLUP |
| 1 | 2489 | 3.28 | 4129 | 3.84 | 617 | 4.01 | 2489 | 5.49 | 2489 | 4.91 | 4129 | 2.82 |
| 2 | 2487 | 3.18 | 411 | 3.78 | 973 | 3.96 | 3522 | 5.18 | 2487 | 4.70 | 4231 | 1.90 |
| 3 | 617 | 3.07 | 2489 | 3.67 | 626 | 3.95 | 2487 | 5.18 | 3487 | 4.56 | 330 | 1.89 |
| 4 | 973 | 3.03 | 541 | 3.47 | 974 | 3.93 | 3487 | 5.13 | 3522 | 4.56 | 2803 | 1.82 |
| 5 | 626 | 3.02 | 974 | 3.35 | 2489 | 3.85 | 1539 | 5.07 | 1539 | 4.54 | 541 | 1.81 |
| 6 | 974 | 3.00 | 4318 | 3.30 | 695 | 3.83 | 2463 | 4.85 | 617 | 4.45 | 37 | 1.79 |
| 7 | 952 | 2.92 | 2467 | 3.30 | 952 | 3.83 | 974 | 4.73 | 973 | 4.44 | 2231 | 1.79 |
| 8 | 695 | 2.92 | 494 | 3.29 | 1002 | 3.73 | 973 | 4.73 | 974 | 4.41 | 1420 | 1.78 |
| 9 | 2463 | 2.91 | 973 | 3.25 | 2487 | 3.73 | 2467 | 4.72 | 2463 | 4.33 | 3141 | 1.70 |
| 10 | 1539 | 2.88 | 1539 | 3.23 | 719 | 3.68 | 617 | 4.67 | 626 | 4.32 | 86 | 1.66 |

A total of 28 plants were ranked best in one or more of the traits. The frequency varied from 1 to 5 times. For example, plant codes 973, 974 and 2489 were ranked in the top 10 plants in five of the six measurements. The pedigree of these best plants is presented in Table 5.

Out of the 28 plants, seven plants had a genetic heritage of Albo2, another seven plants of

Albo3, seven of Albo8 and the remaining 7 plants had diverse heritages from Albo1, Teno3, Teno4, Malpaso1 and Teide3. Albo2, Albo3 and Albo8 are providing the best source of genetic background material for these traits.

**Table 5. Pedigree of best ranked plants for each of the 6 traits**

| Plant code | Mother | Father | Heritage | Plant code | Mother | Father | Heritage |
|---|---|---|---|---|---|---|---|
| 37 | EP7F3A14 | E7PF3A14 | Albo1 | 1002 | E7PF31A3 | E7PF31A3 | Albo3 |
| 86 | E7PF3A14 | E7PF3A14 | Albo2 | 1420 | E7PF31A3 | E7PF31A3 | Albo3 |
| 231 | E7PF3A14 | E7PF3A14 | Albo2 | 1539 | E53NPF23A15 | E53NPF23A15 | Teno3 |
| 330 | E7PF24A13 | E7PF24A13 | Albo2 | 2463 | E53NPF41A4 | E53NPF41A4 | Albo3 |
| 411 | E7PF24A13 | E7PF24A13 | Albo2 | 2467 | E53NPF41A4 | E53NPF41A4 | Albo3 |
| 494 | E7PF24A13 | E7PF24A13 | Albo2 | 2487 | E53NPF41A4 | E53NPF41A4 | Albo3 |
| 541 | E7PF24A13 | E7PF24A13 | Albo2 | 2489 | E53NPF41A4 | E53NPF41A4 | Albo3 |
| 617 | E53NPF33A4 | E53NPF33A4 | Albo8 | 2803 | E53NPF34A5 | E53NPF34A5 | Albo2 |
| 626 | E53NPF33A4 | E53NPF33A4 | Albo8 | 3141 | E7PF31A2 | E7PF31A2 | Albo3 |
| 695 | E53NPF33A4 | E53NPF33A4 | Albo8 | 3487 | E53NPF22A15 | E53NPF22A15 | Teno4 |
| 719 | E53NPF33A4 | E53NPF33A4 | Albo8 | 3522 | E53NPF22A15 | E53NPF22A15 | Teno4 |
| 952 | E53NPF33A2 | E53NPF33A2 | Albo8 | 4129 | Malpaso1 | Malpaso1 | Malpaso1 |
| 973 | E53NPF33A2 | E53NPF33A2 | Albo8 | 4231 | Malpaso1 | Malpaso1 | Malpaso1 |
| 974 | E53NPF33A2 | E53NPF33A2 | Albo8 | 4318 | OMV33EF31A9 | OMV33EF31A9 | Teide3 |

In addition, the best overall plants from best to 10$^{th}$ best which was found by using an example of a multivariate selection index generated with three plant size traits (PS Nov08; PS bg May09; PS bg Aug09) and the leaf retention trait (LR Mar09) with weights of 0.1, 0.4, 0.2 and 0.3 respectively were: 2489, 541, 974, 2467, 1539, 4318, 973, 2463, 411 and 2487. Other traits and weights can be used that will provide a different set of best 10 ranked plants. There are no economic weights available to be used in a tedera selection index for Australian conditions. The 10 best ranked plants in the selection index were included in the best 28 plants ranked best in the individual traits. Plant such as 541 that was ranked fourth and fifth only in traits PS bg May 09 and LR Mar 09, was second in the selection index ranking.

The data analysed using the statistical model described above not only allowed us to generate BLUPs for the 4608 tedera plants (note: results for the 576 lucerne and Hairy Canary clover plants were not presented in this analysis), but also for the different generations of ancestors provided in the pedigree of the plants (Table 2). The rankings of genetic groups, ancestors and founders for plant size scores taken in November 08, May 09, August 09 and leaf retention percentage in March 09 are presented in Table 6.

The genetic group G1, which has all the Albo heritage, was ranked first and second for all plant size traits and leaf retention, thus being the most valuable genetic group. The best genetic group for leaf retention was G6, with Malpaso heritage. The identification of the best genetic groups provides information of most valuable genetic resources that could be possible target regions in future collecting missions. Within the 12 ancestors, E7PF17A9 and E7PF31A10 were the best ones for plant size, but ninth and sixth respectively for leaf retention. The best one for leaf retention was Malpaso1. The best founder lines were: "Line 3" for PS Nov08; "Line 9" for PS bg May09 and PS bg Aug09; and "Line 5" for LR Mar09. These founder lines as well as the ancestors can be the target of further crossing or selfing depending on the objectives of the breeding program.

**Table 6. Ranking of genetic groups, previous generation and founders for plant size scores taken in November 08, May 09, August 09 and leaf retention percentage in March 09**

| | PS Nov08 | PS bg May09 | PS bg Aug09 | LR Mar09 | | PS Nov08 | PS bg May09 | PS bg Aug09 | LR Mar09 |
|---|---|---|---|---|---|---|---|---|---|
| **Genetic groups** | | | | | **Founders** | | | | |
| G1 | 1 | 1 | 2 | 2 | Line 1 - E7PF3A14 | 7 | 3 | 8 | 4 |
| G2 | 2 | 3 | 1 | 5 | Line 2 - E7PF24A13 | 5 | 2 | 4 | 3 |
| G3 | 3 | 2 | 3 | 3 | Line 3 - E53NPF33A4 | 1 | 9 | 3 | 14 |
| G5 | 4 | 5 | 4 | 4 | Line 4 - E53NPF33A2 | 4 | 7 | 11 | 8 |
| G6 | 5 | 4 | 5 | 1 | Line 5 - E7PF31A3 | 14 | 6 | 15 | 1 |
| **Ancestors** | | | | | Line 6 - E53NPF23A15 | 8 | 8 | 11 | 5 |
| Albo1 | 5 | 2 | 7 | 2 | Line 7 - E53NPF23A11 | 10 | 13 | 6 | 16 |
| Albo2 | 4 | 4 | 8 | 4 | Line 8 - E53NPF34A7 | 11 | 12 | 14 | 11 |
| Albo3 | 7 | 3 | 9 | 3 | Line 9 - E53NPF41A4 | 2 | 1 | 1 | 9 |
| Albo8 | 3 | 5 | 4 | 8 | Line 10 - E53NPF34A5 | 9 | 8 | 12 | 5 |
| Teno3 | 9 | 9 | 3 | 11 | Line 11 - E7PF31A2 | 12 | 10 | 13 | 7 |
| Teno4 | 10 | 11 | 6 | 12 | Line 12 - E53NPF22A15 | 13 | 16 | 10 | 17 |
| Teide3 | 6 | 6 | 5 | 7 | Line 13 - E53NPF22A13 | 15 | 14 | 7 | 15 |
| Famara1 | 11 | 12 | 11 | 10 | Line 14 - Malpaso1 | 17 | 15 | 17 | 2 |
| Malpaso1 | 12 | 10 | 12 | 1 | Line 15 - Famara1 | 16 | 17 | 16 | 13 |
| E7PF24A4 | 8 | 8 | 10 | 5 | Line 16 - OMV33EF31A9 | 3 | 4 | 2 | 10 |
| E7PF31A10 | 2 | 1 | 1 | 6 | Line 22 - E7PF3A8 | 6 | 5 | 9 | 6 |
| E7PF17A9 | 1 | 7 | 2 | 9 | | | | | |

## IV – Conclusions

The "plant" model utilized in this analysis provided BLUPs at an additive genetic level for individual plants, based on individual traits and/or a selection index. It also provided BLUP estimates at founder (line or family) level, at an ancestor level and at a genetic group level. Best individual plants for further crossing work in the breeding program were identified according to a selection index (2489, 541, 974, 2467, 1539, 4318, 973, 2463, 411 and 2487), best founders (Line 3 – E53NPF33A4, Line 9 – E53NPF41A4, Line 5 – E7PF31A3), best ancestors (E7PF17A9, E7PF31A10 and Malpaso1) and best genetic groups (G1, G2 and G6).

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## References

**Butler D., Cullis B.R., Gilmour A.R. and Gogel B.J., 2009.** ASREML-R, reference manual. Technical report. Queensland Department of Primary Industries.

**Correal E., Monino I., Méndez P. and Ríos S., 2003.** Valoración agronómica de siete poblaciones de *Bituminaria bituminosa*: Efecto de la sequía estival y siega sobre su persistencia y producción de hoja. In: *Pastos, Desarrollo y Conservación*, p. 34-40.

**Gilmour A.R., Cullis B.R. and Verbyla A.P., 1997.** Accounting for natural and extraneous variation in the analysis of field experiments. In: *Journal of Agricultural, Biological, and Environmental Statistics*, 2, p. 269-293.

**Méndez P., Fernández M. and Santos A., 1991.** Variedades de *Bituminaria bituminosa* (L.) Stirton (Leguminosae) en el archipélago canario. In: *Pastos*, 20-21, p. 157-166.

**Méndez P., Peters J., Morales D. and Jiménez M.S., 2000.** Características del intercambio gaseoso de las tederas *(Bituminaria bituminosa)* de Canarias. In: *3ra Reunión Ibérica de Pastos y Forraxes*, p. 91-95.

**Northcote K.H., 1979.** *A Factual Key for the Recognition of Australian Soils*, 4th edn. Glenside, SA: Rellim Technical Publishers.

**Oakey H., Verbyla A.P., Cullis B.R., Wei X. and Pitchford W.S., 2007.** Joint modeling of additive and non-additive (genetic line) effects in multi-environment trials. In: *TAG Theoretical and Applied Genetics*, 114, p. 1319-1332.

**Oakey H., Verbyla A.P., Pitchford W.S., Cullis B.R. and Kuchel H., 2006.** Joint modeling of additive and non-additive genetic line effects in single field trials. In: *Theoretical and Applied Genetics*, 113, p. 809-819.

**R_Development_Core_Team, 2009.** *R: A Language and Environment for Statistical Computing.* Vienna (Austria): R. Foundation for Statistical Computing.

**Real D., Correal E., Méndez P., Santos A., Ríos Ruiz S., Sternberg M., Dini-Papanastasi O., Pecetti L. and Tava A., 2009.** *Bituminaria bituminosa* C.H. Stirton (synonym: *Psoralea bituminosa* L.). In: *Grassland Species*. FAO. http://www.fao.org/ag/AGP/AGPC/doc/GBASE/new_species/tedera/bitbit.htm.

**Smith A.B., Cullis B.R. and Thompson R., 2005.** The analysis of crop cultivar breeding and evaluation trials: An overview of current mixed model approaches. In: *Journal of Agricultural Science*, 143, p. 449-462.