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RESEARCH ARTICLE

Lotus ornithopodioides L. a potential annual pasture legume species for Mediterranean dryland farming systems

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Abstract Twenty-three populations of *Lotus ornithopodioides* L., collected from different regions of the Mediterranean basin, were investigated for their ecological and agronomic traits in Western Australia. Great variability was found between and within populations for flowering time, forage and seed yield. Flowering time ranged between 75 and 120 days, dry matter production from 2.8 to 4.3 t ha^{-1} and seed yield from 284 to 684 kg ha^{-1}. Other important traits such as non-shattering pods and hard seed were taken into account during the selection to assure an easy seed harvesting and legume persistence in the targeted environments. The high level of hard seed recorded in early winter, associated to the low seedling regeneration, indicates that *L. ornithopodioides* is best suited to ley cropping systems. Elite lines of *L. ornithopodioides* characterized by early flowering time, high seed yield and non-shattering pods were selected. Two of them, LOR02.1 and LOR03.2, showed dry matter higher than 4.0 t ha^{-1} and seed yield around 700 kg ha^{-1} resulting the lines with most potential for Mediterranean farming systems. The results encourage the exploitation of *L. ornithopodioides* germplasm to develop a new annual self-reseeding legume resource for Mediterranean farming systems for both forage production and crop rotation uses.

Keywords Dry matter · Earliness · Hard seed · Ley farming systems · *Lotus ornithopodioides* · Nutritive value · Seed yield

Introduction

The genus *Lotus* includes more than 180 species and is found worldwide with the exception of some very cold and tropical areas in southern Asia and South and Central America (Allen and Allen 1981). Four species

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of *Lotus* have been domesticated in the last century (Diaz et al. 2005) and they include *L. corniculatus* L. (birdsfoot trefoil), *L. uliginosus* Schkuhr (syn *L. pedunculatus*) (greater lotus), *L. glaber* Mill. (narrow-leaf trefoil) and *L. subbiflorus* Lagasca (hairy birdsfoot trefoil), an annual lotus with agricultural importance grown in Uruguay (Asuaga 1994).

These *Lotus* species are grown mainly in the temperate areas of Europe and Americas with 95 % of the area sown to *L. corniculatus* (Diaz et al. 2005). In Australia, *L. corniculatus* and *L. uliginosus* are commercially traded, albeit their use is relatively minor and restricted to high rainfall areas, where waterlogging is frequent and pastures are rotational grazed (Blumenthal et al. 1993; Ayres et al. 2006; Real et al. 2008). Some studies have suggested that the potential area of *L. corniculatus* may be expanded considerably to an area similar to that occupied by white clover (*Trifolium repens* L.) (Bologna et al. 1996). The relevant ecological and agronomic importance of several *Lotus* species and their great potential for adaptation to several abiotic stresses have been reviewed by Escaray et al. (2012). However, general problems related to indeterminate flowering, mature pod dehiscence and seed pod shattering that reduce harvestable seed yield are the main constraints to a wider exploitation of *Lotus* species (Fairey and Smith 1999; McGraw and Beuselink 1983; Grant 1996; Řepková and Hofbauer 2009).

Germplasm collections in Mediterranean basin have highlighted that there are other species of *Lotus* that may have potential to be domesticated and benefit Mediterranean farming systems (Howieson and Loi 1994). One of these is *Lotus ornithopodioides* L. (clustered birdsfoot trefoil) an annual species of *Lotus* which was found in many collecting sites of Mediterranean areas growing on different soil types, from sand to clay loam, and different parent rocks which included granite, limestone, schist and basalt (Loi et al. 1995; Nutt et al. 1996). According to Pignatti (1982), *L. ornithopodioides* is one of the most common and widely distributed *Lotus* species in Italy and Mediterranean basin. Other authors have indicated *L. ornithopodioides* as a common spontaneous component in Mediterranean pastures (Bullitta and Caredda 1982; Bennani et al. 2010) and in the flora of ancient olive groves of southern Italy (Perrino et al. 2014). This species has a number of desirable traits such as a deep root system, prolific seed production, tolerance to insects, excellent pod retention on stems, and minimal pod shattering compared to other species of the same genus (Loi et al. 2002). *L. ornithopodioides* has potential to be used as fodder crop (Pelikan and Zapletalova 1994), cover crop in tree orchards and as a general break crop in cereal systems (Flohrova 1998). It has been reported that a commercial variety (Junak) of *L. ornithopodioides* has been registered by the Research Institute for Fodder Crops in Troubsko (Czech Republic) from material obtained from botanic gardens (RIFC 2010). Moreover, *L. ornithopodioides* is also reported for the first time as alien species introduced to Czech Republic, growing from seed most likely originating from the above-mentioned Fodder Institute in Troubsko (Pyšek et al. 2012).

Additional information indicates interest on this species for land rehabilitation (Bullitta et al. 2009; Safronova et al. 2012; Porqueddu et al. 2013), as a source of secondary plant metabolites useful to improve animal health or to be used as natural herbicides (Tava et al. 2005; Piluzza and Bullitta 2010; Araniti et al. 2014).

Lotus ornithopodioides was included in the Australian Annual Pasture Legume Improvement Program (NAPLIP) and in collaboration with European research institutions, several targeted germplasm collections were conducted in Sardinia, Sicily, Spain, Cyprus and Greece in the last 20 years (Loi et al. 2002; Sulas 2005). Therefore, a domestication program of *L. ornithopodioides* germplasm for its potential fodder use in moderately acidic and alkaline fine textured soils was started within NAPLIP in Western Australia.

The evaluation of germplasm is commonly based on simultaneous examination of populations including the testing of a large number of plants for characters of agronomic and physiological interest. This paper firstly reports a study on the variability of some phenological and agronomic traits between and within natural populations of *L. ornithopodioides* collected from several regions of the Mediterranean basin. Secondly, the paper describes the selection of elite genotypes that were tested in the field for their ecological and agronomic performances.

Materials and methods

Selection framework

The selection framework for *L. ornithopodioides* started in 2000 and was based on identifying plants from the initial 100 populations that had traits of pod retention and non-shattering which would allow seed of this species to be harvested using a conventional header harvester (Loi et al. 2005). Subsequent selection was based on: (1) flowering time, which allows improved climate and plant maturity matching, (2) seed yield, an important trait for plant persistence, and (3) dry matter yield, which is an essential trait for livestock production and is usually highly correlated with nitrogen fixation potential.

Other traits of significance include the level of initial hard seededness (i.e., seed coat impermeability) and the extent to which this hard seededness softens to become available for germination. Testing for forage nutritional value was conducted as a reference check to ensure acceptable levels for livestock production.

Experiment 1 (Medina Research Station April–December 2002)

Twenty-three populations were selected for non-shattering pods types and early flowering time from the genetic resource centre at the Department of Agriculture and Food Western Australia (DAFWA). The majority of the populations originated from a targeted collection of early flowering and non-shattering pod types of *L. ornithopodioides* conducted in southern Europe by Loi and Sandral (personal communication).

Plants were cultured by placing scarified seeds (about 100 seeds for each population) into small peat pots filled with commercial potting mix and inoculated with specific root nodule bacteria WSM1293 for *L. ornithopodioides* (Howieson et al. 2011) at the beginning of April 2002. Two seeds were sown in each pot and after 1 week, only one seedling per pot was kept. The seedlings were grown in a covered plastic house for 3 weeks, hardened for 2 weeks in an open area, then transplanted into a field nursery in May 2002 at the Medina Research Station, Perth (32°13′S, 115°48′E).

Each population was represented by 40 plants where seed supply permitted, while for some populations fewer plants were grown due to limited seed supply. The populations were grown in rows (0.5 m between plants and 1.5 m between rows) overlaid with white plastic film to control weeds. The soil was fertilised with 200 kg ha^{-1} of superphosphate (0:8.8:0:11) and 100 kg ha^{-1} of muriate of potash (0:0:50:1.75) prior to the laying of the plastic film. The area was irrigated every 2nd day from September to the end of November.

Flowering time was measured as number of days from sowing to the first flower appearance. In December, following full senescence, pods were individually harvested from stems. At the time of harvest, plants that showed partial or complete pod shattering were recorded and discarded, according to the aim of selection process. Non-shattered pods were later threshed (from November to December 2002) using a Venable portable thresher, and seeds were cleaned and weighed.

Experiment 2 (Northam, May 2005–June 2006)

Background

A number of 12 populations out of the 23 grown in experiment 1 were chosen based on non-shattering pods, high seed yield, and preferred flowering time (very early to mid-maturity) combinations. Within each of the twelve populations, best single plants (out of the initial 40) were identified. For 9 out of 12 populations only one genotype was selected. For LOR03, LOR04 and LOR05 two genotypes within the populations were selected. Therefore, 15 single genotypes were obtained. Each selected single plant was considered as a distinguished single genotype thereinafter.

The seed harvested from each genotype was multiplied sowing 40 spaced plants per genotype at the Medina research station in autumn 2003. Individual plants that did not conform to the selection criteria previously described were removed from the rows.

Agronomic evaluation

In 2005, the fifteen genotypes, selected from the original 23 populations, were compared in a field investigation conducted at Northam (100 km east of Perth, 31°39′S, 116°41′E). The soil was typical of areas where annual *Medicago* spp. and rose clover (*Trifolium hirtum*) are generally grown and is described as a fine textured clay-loam, with a pH (soil water) of 5.5 in the top 10 cm. The average annual rainfall is 400 mm and the plots were sown in late autumn on the 11th of May 2005. Prima gland clover (*T. glanduliferum*) was included in the trial as a reference control for its ability to grow on a similar soil type and ease of harvesting with a conventional header harvester. Seed of each species was inoculated with the appropriate rhizobial group: WSM1293 for *Lotus* (Howieson et al. 2011) and the commercially available WSM1325 for the clover (Yates et al. 2003). The strains were applied to the seed as peat-based inoculum at recommended rates.

Plots were 10 × 2 m each in size, with a 1.2 m inter-row buffer between plots and arranged in a spatially randomised block design with three replicates for each genotype. The site was fertilised with 150 kg ha^{-1} of superphosphate (0:8.8:0:11) and 70 kg ha^{-1} of muriate of potash (0:0:50:1.75). The insecticide Talstar (Bifenthrin–Talstar 100EC 100 g l^{-1}) was applied after sowing at 120 ml ha^{-1} to protect against damage from red-legged earth mites (*Halotydeus destructor* Tucker). Plant dry matter was measured in spring in 2005, when the majority of the swards were in full flowering. Dry matter was estimated from two randomly placed quadrats (0.1 m^2) in each plot, cut to the ground level and oven dried at 60 °C for 48 h. Samples of mature pods of *L. ornithopodioides* and flower heads of gland clover were harvested from an additional two randomly placed 0.1 m^2 quadrats in late spring (late November). Then, they were threshed, cleaned and weighed to assess seed yield.

The plots were allowed to regenerate in 2006 after the break in autumn and the number of regenerating seedlings per plot was determined 2 months later in early winter by counting individuals in two squares of 0.05 m^2 placed randomly in each plot.

Nutritive analysis of aerial biomass

Organic matter samples of 5 selected genotypes, each one coming from different areas of the Mediterranean basin (Table 1), were collected in spring 2005, oven dried at 60 °C for 48 h and the nutritive value was assessed through measures of crude protein (CP%), neutral detergent fibre (NDF%), dry matter digestibility (DMD%), and metabolisable energy (ME). All samples were processed through FEEDTEST (Department of Primary Industry, Hamilton, Victoria). Analysis was based on Near Infrared spectroscopy with DMD calibrated for the pepsin cellulose enzymatic method (Jones and Hayward 1975), CP calibrated for the Kjeldahl nitrogen method and NDF calibrated for the Ankom (amylase) method (Ankom Technology Corp. 2003). Wet chemistry controls used the reference methods described in the Australian Fodder Industry Association laboratory methods manual (AFIA 2006).

Metabolisable Energy was predicted using the forage/roughage equation described in the AFIA laboratory methods manual: ME (MJ/kg DM) = 0.203 DOMD (%) (Digestible organic matter in the dry matter) − 3.001 for roughages other than silage (AFIA 2006).

Experiment 3 (Shenton Park, Summer 2006)

Samples of mature pods from the Northam site were harvested on the 1st of December 2005 and transferred to the University of Western Australia Field Station at Shenton Park in Perth (31°57′S, 115°47′E) to measure total hard seed and the rate of seed softening. Seed samples of 100 seeds from each of the fifteen genotypes were placed in fly wire mesh envelopes and laid on the soil surface at the beginning of summer (January) as described by Loi et al. (1999b). Each sample was tested for hard seed in three dates: 10 January (early summer), 15 March (mid summer), and 1 July (early winter) 2006, respectively. At each of these three dates, six replicate flywire envelopes of seed for each genotype were removed from the soil surface and seed germinated on moistened filter paper in Petri dishes for 14 days at a constant temperature of 20 °C. Germinating seed was removed every 2–3 days and the residual hard seed (un-imbibed seed) was recorded.

Statistical analysis

Analysis of variance was conducted using GenStat 16th edition (VSN International Ltd, UK) to compare the genotypes for seed yield, seedling density and hard seed in the various experiments (all randomised block designs). Where appropriate, some measurements were transformed with a $\log_{10}$ function to account for heterogeneity in the data. The variability within populations for both flowering time and seed yield was assessed through a classification regression tree analysis (RPART package in R Statistical System, http://www.R.project.org) of the coefficients of variation (CV = 100 × standard deviation of the plant data/average of the plant data).

Table 1 Origin and collection sites characteristics of 23 *L. ornithopodioides* populations used in experiment 1

Population code	Origin country	Site	Latitude (N)	Longitude (E)	Altitude (m a.s.l.)	Parent Rock	pH ($CaCl_2$)
LOR01	Italy	Sicily	30°01′	14°12′	18	Limestone	8.5
LOR02	Italy	Sicily	36°43′	14°45′	32	Granite	7.0
LOR03	Italy	Sicily	36°42′	14°47′	24	Calcareous	8.0
LOR04	Italy	Sicily	36°42′	14°47′	24	Calcareous	8.0
LOR05	Italy	Sicily	36°43′	14°43′	26	Schist	7.5
LOR06	Italy	Sicily	36°43′	14°43′	26	Schist	7.5
LOR07	Italy	Sicily	36°46′	14°34′	10	Alluvial	8.5
LOR08	Italy	Sicily	37°04′	14°15′	18	Calcareous	7.0
LOR09	Italy	Sicily	37°35′	12°49′	45	Calcareous	8.0
LOR10	Italy	Sicily	38°06′	13°18′	40	Basalt	8.0
LOR11	Italy	Sardinia	39°07′	08°36′	75	Granite	7.0
LOR12	Italy	Sardinia	39°10′	09°21′	70	Granite	6.5
LOR13	Greece	Naxos	37°07′	25°24′	70	Schist	7.0
LOR14	Greece	Paros	37°04′	25°08′	10	Granite	6.0
LOR15	Greece	Mykonos	37°27′	25°20′	20	Granite	6.0
LOR16	Greece	Samos	37°41′	26°56′	20	Calcareous	8.5
LOR17	Greece	Patmos	37°21′	26°34′	30	Basalt	7.5
LOR18	Greece	Patmos	37°18′	26°32′	160	Basalt	8.5
LOR19	Medit. basin	Unknown	n/a	n/a	n/a	n/a	n/a
LOR20	Medit. basin	Unknown	n/a	n/a	n/a	n/a	n/a
LOR21	Medit. basin	Unknown	n/a	n/a	n/a	n/a	n/a
LOR22	Tunisia	Unknown	n/a	n/a	n/a	n/a	n/a
LOR23	Tunisia	Unknown	n/a	n/a	n/a	n/a	n/a

n/a not available

Results

Experiment 1

The populations of *L. ornithopodioides* originated from different regions of the Mediterranean basin (Table 1), with soils from granitic and slightly acidic (pH 6.0) to calcareous alkaline (pH 8.5). The averages and the range of the population means for flowering time and single plant seed yield are shown in Table 2. Large variation was found between and within populations for the measured traits. Flowering time ranged from 84 to 122 days in LOR05 and LOR10, respectively. Populations LOR01, LOR03, LOR05, LOR17 and LOR18 showed the highest coefficient of variation (CV) for flowering time ranging from 8 to 12.9, with differences between individuals up to 52 days (LOR03). In contrast, populations LOR20, LOR21, LOR08, LOR10 and LOR14 showed the lowest CV for flowering time (ranging from 1.8 to 3.3) with differences within populations ranging from 5 to 20 days.

Greater variation was found in the seed yield trait between and within populations. Average seed yield in single plants ranged from 4.7 to 73 g plant^{-1} for LOR13 and LOR11, respectively. The populations LOR01, LOR03, LOR09, LOR10, LOR17, LOR19, LOR22 and LOR23 showed the highest CV (33.8–44.7 %) and seed yield between single plants ranged from 7 to 110 g plant^{-1}. The populations LOR04, LOR02, LOR11, LOR14, LOR15, LOR16 and LOR21 were less variable and the CV for seed yield per plant was low, ranging between 10.6 and 13.6 %.

The variability between populations for flowering time and seed yield is also represented in Fig. 1. The approximate cut-off point (dashed line) is presented

Table 2 Experiment 1: average, standard deviation (SD), coefficient of variation (CV) and range of flowering time and single plant seed yield of 23 populations of *L. ornithopodioides* grown at the Medina Research Station (Perth, WA)

	Days to flower (no.)						Seed yield (g plant^{-1})					
Populations	Plants	Avg	Min	Max	SD	CV	Plants	Avg	Min	Max	SD	CV
LOR01	40	110.1	101.0	141.0	8.8	8.0	40	34.0	7.0	77.0	11.5	33.8
LOR02	40	106.4	97.0	123.0	7.3	6.8	37	54.1	40.0	68.0	6.9	12.7
LOR03	40	105.5	89.0	141.0	9.1	8.6	40	39.7	25.0	110.0	16.5	41.5
LOR04	40	99.8	89.0	119.0	6.4	6.4	40	59.9	48.0	90.0	8.1	13.6
LOR05	40	84.6	66.0	108.0	7.7	9.1	37	39.6	20.0	55.0	10.5	26.6
LOR06	40	99.5	89.0	107.0	4.1	4.1	39	40.3	24.0	55.0	9.0	22.4
LOR07	40	106.0	99.0	118.0	5.0	4.7	39	56.9	40.0	78.0	10.6	18.6
LOR08	40	107.2	101.0	121.0	3.7	3.4	37	57.1	28.0	80.0	15.6	27.2
LOR09	40	112.9	86.0	121.0	6.4	5.7	40	35.3	14.0	60.0	12.2	34.7
LOR10	40	122.4	116.0	131.0	4.0	3.2	39	46.3	28.0	98.0	18.6	40.2
LOR11	40	110.3	85.0	131.0	7.1	6.5	37	73.6	45.0	88.0	8.5	11.5
LOR12	40	113.3	86.0	121.0	6.0	5.3	37	48.4	30.0	95.0	14.3	29.6
LOR13	10	103.9	99.0	112.0	4.3	4.1	10	4.7	3.0	7.0	1.3	28.5
LOR14	10	108.0	105.0	110.0	1.8	1.7	10	51.7	39.0	61.0	6.7	12.9
LOR15	10	112.2	107.0	121.0	5.5	4.9	10	46.9	39.0	57.0	5.6	12.0
LOR16	10	119.8	110.0	131.0	6.2	5.2	10	45.7	39.0	53.0	5.0	11.0
LOR17	10	108.1	85.0	117.0	9.3	8.6	10	11.1	7.0	19.0	3.9	35.4
LOR18	3	101.0	86.0	110.0	13.1	12.9	0	n/a	n/a	n/a	n/a	n/a
LOR19	40	116.2	86.0	124.0	6.1	5.2	39	38.8	12.0	80.0	17.3	44.7
LOR20	40	120.8	117.0	125.0	3.0	2.4	36	81.9	52.0	115.0	14.3	17.5
LOR21	40	117.9	110.0	129.0	3.3	2.8	36	75.6	51.0	87.0	8.0	10.6
LOR22	31	115.3	107.0	126.0	4.8	4.2	31	44.0	26.0	80.0	16.9	38.3
LOR23	40	108.0	95.0	139.0	8.3	7.6	35	53.1	15.0	77.0	21.0	39.6

for each of the classification regression tree analysis for flowering time and seed yield. The populations in the bottom left corner of the graph are those that are more uniform with a very low level of variability within populations for both flowering time and seed yield. In contrast, those populations on the top right corner of the graph are the most diverse for flowering time and seed yield traits, although the CV for flowering time is much smaller than that for seed yield.

Single plants selections were made within *L. ornithopodioides* populations based on observations of pod retention and non-shattering type and measurements that identified plants with a high seed yield ranking and early flowering time. A number of 11 out of 23 original populations were discarded (Table 4). Among the remaining populations, single plants were selected from LOR02, LOR06, LOR07, LOR08, LOR11, LOR12, LOR14, LOR19 and LOR23 (Table 4). Two separate plants were selected from LOR03, LOR04 and LOR05. In particular, LOR14 was selected as it showed very low coefficients of variation for seed yield and flowering time and met the other criteria for seed yield and flowering time. All single plant selections were subsequently maintained as unique genotype.

Experiment 2

In autumn 2005 the site received an optimal starting rain (87 mm May and 124 mm in June) which allowed a good establishment of the trial sown on May 11 (Table 3).

Table 4 shows the fifteen *L. ornithopodioides* genotypes varied in their dry matter production at

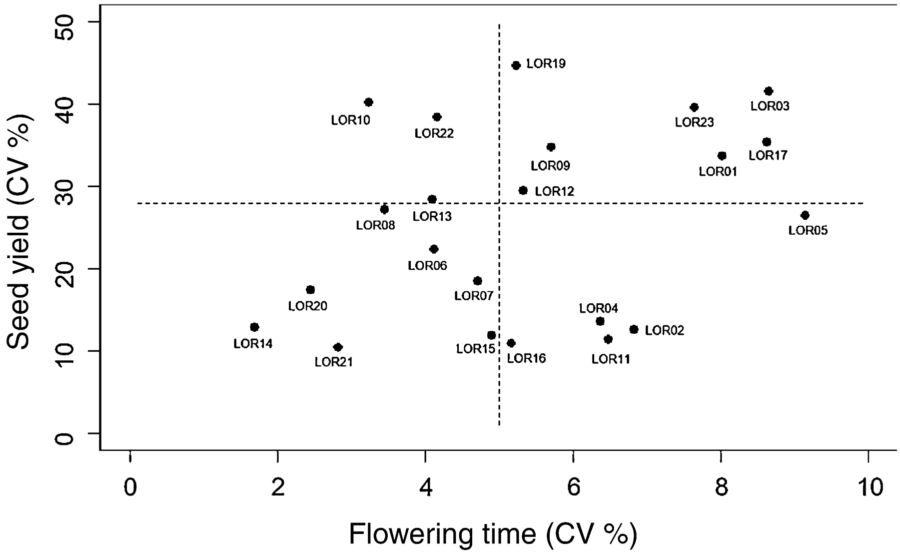

Fig. 1 Plot of the coefficients of variation (CV %) for each population for the two traits of flowering time and seed yield. The *dashed lines* were derived by a classification tree (RPART package in R) for each of the two summary variables. The partition found (the *dashed line*) maximised the populations into two groups for each of the two summary statistics (The population LOR18 has not been included in the figure because the seed yield data was not available)

the Northam site, with the lowest yielding genotypes, including LOR05.2 and LOR07.1, being significantly lower (less than 3 t ha^{-1}) than 7 out of 15 others. The genotypes LOR02.1, LOR03.1, LOR03.2, LOR04.1, LOR04.2 and LOR14.1 all produced dry matter yields equal or greater than 4.0 t ha^{-1} while the reference control, *T. glanduliferum* Prima, produced 5.5 t ha^{-1}.

Five genotypes LOR02.1, LOR03.2, LOR05.1, LOR06.1, and LOR23.1 had the highest seed yields (672–720 kg ha^{-1}), which were significantly higher than LOR12.1 (284 kg ha^{-1}) and LOR08.1 (296 kg ha^{-1}), which had the lowest seed yields. No significant differences were found between *T. glanduliferum* Prima and the *L. ornithopodioides* genotypes for seed yield.

As a whole, seed yield ranged from 284 to 704 kg ha^{-1} for genotypes with flowering on or later than 115 days, while seed yields varied from 504 to 720 kg ha^{-1} for genotypes that flowered on or earlier than 100 days. The late flowering and high yielding genotypes included LOR03.2 and LOR23.1 (Table 4).

Among the *L. ornithopodioides* genotypes, initial seedling density ranged from 1 to about 2 plant m^{-2} and in LOR07.1 it was significantly different from LOR05.1, LOR11.1 and LOR12.1 (Table 4). *L. ornithopodioides* seedling numbers regenerating in early winter 2006 were significantly lower (raw mean 22–156 plants m^{-2}) than *T. glanduliferum* Prima (5114 plants m^{-2}).

The forage quality measurements of 5 selected genotypes collected from different areas of the Mediterranean basin (Table 5) showed that *L. ornithopodioides* has high quality forage in terms of dry matter digestibility (73–76 %), metabolisable energy (11 %) and crude protein (17–19 %) compared with *T. glanduliferum* Prima (71, 10.6 and 17.7 % respectively).

Experiment 3

The variation in hard seed breakdown over the 2006 summer amongst the selected genotypes is presented in Table 6. The level of initial hard seed was generally high, and no significant differences were found between genotypes at any of the sampling times. The hard seed of *T. glanduliferum* Prima however decreased significantly from its initial level of 95 %, down to 75 and 54 % for mid-summer and early winter, respectively. In contrast, the hard seed of *L. ornithopodioides* genotypes did not soften significantly between mid-summer and the early winter with the exception of LOR14.1, which had an initial hard seed level of 85 % and softened to 70 %. The genotype LOR11.1 recorded the lowest level of hard seed in early winter but this was not significantly different from LOR14.1. This may be compared to the *T. glanduliferum* Prima gland clover that recorded 28 % of its hard seed softening between the last two sampling dates.

Table 3 Experiment 2: monthly rainfall (mm) for Northam (Western Australia) during the experimental period (data from Australian Government, Bureau of Meteorology)

Year	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
2005	0.6	1.6	22.3	13.8	87.0	123.8	32.5	50.6	39.3	26.1	8.6	2.0	408.2
2006	135.4	33.8	1.0	18.9	11.0	21.3	33.8	65.5	67.1	10.2	11.0	8.6	417.6

Table 4 Experiment 2: mean dry matter yield (t ha^{-1}) of 15 genotypes of *L. ornithopodioides* and *Trifolium glanduliferum* Prima sown at Northam (Western Australia), measured at peak biomass in spring and with resulting seed yield in 2005

Genotype	Dry matter yield (t ha^{-1})	Seed yield (Kg ha^{-1})	Seedlings density (plant m^{-2})
LOR02.1	4.3	692	2.03 (156)
LOR03.1	4.1	384	1.58 (39)
LOR03.2	4.0	704	1.69 (58)
LOR04.1	4.3	516	1.40 (25)
LOR04.2	4.0	561	1.25 (58)
LOR05.1	3.5	720	1.01 (34)
LOR05.2	2.9	504	1.31 (22)
LOR06.1	3.3	684	1.50 (33)
LOR07.1	2.8	525	2.10 (142)
LOR08.1	3.7	296	1.68 (47)
LOR11.1	3.9	348	1.20 (111)
LOR12.1	3.5	284	1.08 (31)
LOR14.1	4.0	616	1.85 (75)
LOR19.1	3.8	526	1.62 (78)
LOR23.1	3.5	672	1.92 (97)
T. glanduliferum Prima	5.5	519	3.71 (5114)
l.s.d. ($P = 0.05$)	1.0	338	0.86

The raw means for the seedlings densities in 2006 are presented in parentheses to indicate approximate differences on the raw scale

Table 5 Experiment 2: mean quality of herbage mass of a selected group of *L. ornithopodioides* genotypes and *T. glanduliferum* Prima

Genotype	Crude protein (% DM)	Neutral detergent fibre (% DM)	Metabolisable energy (MJ kg^{-1} DM)	Dry matter digestibility (%)
LOR02.1	17.7	33.5	11.2	74.6
LOR05.2	18.5	32.9	11.4	76.0
LOR11.1	18.4	35.9	11.0	73.6
LOR12.1	19.8	33.9	11.2	74.3
LOR14.1	17.6	34.9	11.0	73.2
T. glanduliferum Prima	17.7	38.3	10.6	71.2

Discussion

The aim of this study was to explore opportunities to select genotypes of *L. ornithopodioides* adapted to low and medium rainfall farming environments (<400 mm annual rainfall) and that featured characters that provided ease of harvesting, as previously summarised (Loi et al. 2005).

Table 6 Experiment 3: seed size and level of hard seed (%) in 15 genotypes of *L. ornithopodioides* and *T. glanduliferum* Prima tested on the soil surface at Shenton Park (Perth) during summer in 2006

Genotype	Seed size (g)	Early summer (%)	Mid summer (%)	Early winter (%)
LOR02.1	1.68	87	84	86
LOR03.1	1.62	85	85	83
LOR03.2	1.54	87	79	82
LOR04.1	1.52	90	85	77
LOR04.2	1.60	85	85	85
LOR05.1	1.64	84	85	84
LOR05.2	1.80	83	85	81
LOR06.1	1.66	88	85	79
LOR07.1	1.48	82	85	80
LOR08.1	1.66	87	86	84
LOR11.1	1.70	82	71	67
LOR12.1	1.54	86	85	78
LOR14.1	1.86	85	80	70
LOR19.1	1.78	86	82	79
LOR23.1	1.96	86	84	83
T. glanduliferum Prima	0.70	95	75	54

l.s.d. ($P = 0.05$) = genotype*time = 9.0

A significant focus in the plant exploration phase was collection of non-shattering populations to redress the lack of suitable *L. ornithopodioides* germplasm already preserved in genetic resource centres. This was achieved by means of targeted collections, where seed was taken only from plants that presented entire mature pods in the early summer. This pre-screening allowed us to work on a more restricted group of substantially non-shattering populations of *L. ornithopodioides* and to subsequently focus more attention on other traits such as flowering time and seed yield.

An important consideration in plant development is to match the growing season length to flowering time, to ensure the reproduction of the species within the farming system and climate (Craufurd and Wheeler 2009). The germplasm of *L. ornithopodioides* evaluated in this study provided a wide genetic base to explore the variability in flowering time within and between populations. Most of the populations observed in experiment 1 presented a wide range of flowering time between individuals (differences up to 40 days); however, some populations such as LOR14 and LOR20 were very uniform with a very narrow range of flowering time (5–7 days difference). The selection of individual genotypes from different populations in experiment 1 generated a cohort of genotypes with ideal flowering times that match the targeted area of our work: low rainfall areas (250–300 mm) with flowering times of 75–85 days and medium rainfall areas (350–400 mm) with flowering times of 100–120 days.

Similar results were found by Bennani et al. (2010) in a study on morphological and agronomic traits of several Moroccan populations of *L. ornithopodioides*, suggesting that populations with high dry matter and seed yields could be used as forage crop.

It has been argued that a population should be kept as genetically broad as possible throughout a selection process to maintain the genetic variation developed at the site of collection (Piano et al. 1982). The presence of high variability within the population is considered an ideal strategy to survive the harsh Mediterranean climatic conditions, as highlighted by Loi et al. (1999a) in a genetic study conducted on populations of biserrula (*Biserrula pelecinus* L.) and yellow serradella (*Ornithopus compressus* L.). However, this variability can be a constraint to the registration process of new cultivars, which need to pass tests based on uniformity, stability and distinctiveness (PBR 2015).

The second most important trait targeted in the selection process reported here was seed yield. This is an extremely important trait for an annual species to ensure plant persistence through drought, false breaks (unseasonal rainfall) and intensive cropping rotations, after which adequate seedling regeneration is required (Harper 1977; Fenner 1985). In our study, the seed yield from single spaced plants varied greatly within and between populations. Seed yield of the studied genotypes was well maintained during the agronomic evaluation phase and most of the high yielding genotypes selected compared favorably to *T. glanduliferum* Prima, with a range of 284–684 kg ha^{-1} of seed (Table 4). In Mediterranean climate, seed yield is often correlated with flowering time, however it is interesting to note that for these test conditions the *L. ornithopodioides* genotypes had no significant correlations between flowering time, seed yield and dry matter (data not shown). The classification regression tree analysis reports a clear partition between the populations on the base of the variability in seed yield and flowering time. These results may be used in future for other breeding programs.

The ability of annual legumes to survive, reproduce and regenerate is mainly related to the seed production and level of ‘seed protection’ (hard seededness and softening pattern) (Cocks 1994). Moreover, the small seed size of several annual legumes combined with hard seededness is an important trait to survive ingestion by sheep during summer grazing. A previous study from Russi et al. (1992) revealed that over 23 % of 2 mg legume seeds passed through the digestive track unharmed, while 36 % of 0.5 mg seed survived. A further study conducted by Edward et al. (1998) showed that almost 50 % of the seeds of biserrula (average 1.2 mg) were able to pass undigested, indicating that seed shape (flat and heart shaped) may play an important role in the seed survival through the digestive system. The seed of *L. ornithopodioides* closely resembles the seed of biserrula in size and in shape and similar survival percentages may be envisaged under summer grazing.

When seed size data (seed size ranged from 1.5–2 mg in weight) is combined with seed yield data, *L. ornithopodioides* produced from 20,000 to 30,000 seed m^{-2}, which is 10–20 times more seed than annual medics or subterranean clover (average seed size 2.5–8 mg, respectively) (Loi et al. 1999a).

The high level of initial hard seed combined with low seedlings density in the second year indicates that *L. ornithopodioides* could be considered a hard seeded species. For the same species similar findings were reported by Do Canto et al. (2013) in Uruguay, who found high levels of hard seededness (96–100 %) and absence of softening, even under temperate and subtropical conditions. Another study conducted by Gresta et al. (2011) reported that initial level of hard seededness in mature seed of *L. ornithopodioides* were near to 100 %. The authors also showed that higher germination capability (up to 90 %) was found in immature seed of *L. ornithopodioides,* condition in which the seeds are already able to germinate but coat-dormancy has not yet been imposed.

Our study has shown that two genotypes (LOR11.1 and LOR14.1) had a lower level of hard seeded (67 and 70 % respectively) compared to the other genotypes. These levels of softening were not matched by high seedling establishment in autumn 2006, which suggests perhaps high seed losses during the summer. On the basis of these general high levels of hard seed it is likely that *L. ornithopodioides* will be best suited to ley cropping systems where, after the first seed set occurs, cropping may follow for the next one or 2 years before returning to pasture. This practise is recommended for biserrula when included in rotations with cereals (Loi et al. 2014). Results from Vanstone et al. (2008) support the use of *L. ornithopodioides* in cereal cropping systems where it has been shown to be resistant to parasitic nematodes (*Pratylenchus neglectus*) that can reduce yields of crops (Taylor et al. 1999).

The availability of very early maturing genotypes, combined with the ability to grow in the range of soil types, suggest also potential alternative uses for *L. ornithopodioides*. For example, early genotypes may be used successfully as a cover crop in fruit tree plantations, or as a catch crop in temperate regions due to the short growing season usually required by both systems. The good quality of the dry matter and the comparable yield to clovers indicate this species may be an excellent hay producer and a potential nitrogen provider to the following cereal or canola crop.

Conclusions

This study has analysed the variability within and between Mediterranean populations of *L. ornithopodioides* and has highlighted the importance of working with a broad range of germplasm. The high variability found within and between populations has allowed the selection of elite lines in *L. ornithopodioides,* characterized by important traits such as adequate flowering time and seed yield and non-shattering pods.

The variability found in the investigated traits is very useful in order to enhance the species persistence in targeted environments, allow an easier and less expensive seed harvesting with conventional header equipment and contribute to its potential exploitation for multiple uses.

Matching the dry matter and seed yield, among the fifteen selected genotypes, two of these, LOR02.1 and LOR03.2, showed dry matter higher than 4.0 t ha^{-1} and seed yield around 700 kg ha^{-1} resulting the most interesting lines for Mediterranean farming systems.

Further studies are currently underway for examining the field performance of elite lines of this species in order to select and release commercial varieties of *L. ornithopodioides* specifically suited for the conditions of Mediterranean farming systems, where this species could represent an important and additional option to the annual self-reseeding legumes already available in the seed market.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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