Diversity of winter biomass production in the Australian arrowleaf clover collection

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Abstract
Arrowleaf clover (*Trifolium vesiculosum* Savi.) is an annual pasture legume for grazing and fodder production with deep roots, high spring biomass and readily harvestable seeds. Poor winter growth of present cultivars, however, limits early season feed availability and makes them susceptible to weed competition. Twenty seven wild accessions and 8 cultivars of arrowleaf clover were grown in a common garden to investigate winter biomass diversity and their relationships with eco-geographic variables of their collection sites. Important variation was observed for seed weight and winter biomass production. Winter biomass was significantly correlated with summer temperature. These results will assist the development of arrowleaf clover cultivars with improved winter biomass.

Keywords
Genetic diversity, pasture legumes, eco-geographic variables, biomass, seed size.

Introduction

Arrowleaf clover (*Trifolium vesiculosum* Savi.) is a dual-purpose annual pasture legume, suitable for both grazing and fodder production (Ewing et al. 2001; RIRDC 2008; Ovalle et al. 2010; Thompson et al. 2010). It has many favourable features including high dry matter yield, good nitrogen fixation ability, deep rooting, drought tolerance, late maturity with extended growth into summer (providing green feed for livestock into late spring/summer), aerial seed production for easy harvesting and high hardseededness for persistence between growing seasons (Thompson 2005; Nichols et al. 2007; 2012). However, the slow establishment and poor autumn-winter growth of current arrowleaf clover cultivars makes them susceptible to early weed competition and limits feed availability (Miller and Wells 1985; Thompson et al. 2010; Snowball and Revell 2011). One approach to increase winter biomass production is to breed more productive cultivars, but this relies on sufficient genetic diversity being present for this trait. This study reports on an investigation of variation for winter biomass production in the Australian collection of arrowleaf clover to assist the development of new cultivars with improved winter growth.

Methods

Seeds of 35 arrowleaf clovers, representing the Australian collection and comprising 27 wild accessions (coded Acc.1 - Acc.27) and eight cultivars (Amclo, Arrotas, Cefalu, Meechee, Seelu, Yuchi, Zulu and Zulumax), were obtained in 2018 from the Australian Trifolium Genetic Resource Centre (Table 1). Collection site (passport) data for the nine genotypes with available data were also obtained. Nineteen bioclimatic (BIOCLIM) variables (BIO1-BIO19) were downloaded from the ‘WorldClim’ (Version 2) website at 2.5 arc-minutes spatial resolution (Hijmans et al. 2005; Ghamkhar et al. 2015).

A row-column design with three replicates was used in a common garden experiment at The University of Western Australia Field Station at Shenton Park (31°57’S, 115°5’E). Plots consisted of 18 plants in two rows (1.8 m length), positioned along 1 m wide plastic strips (to prevent weeds). The soil at the experimental site is sandy with applied loam to the upper layers. The field was sprayed with glyphosate herbicide (2 l/ha) in early April. Super potash (3:2) was applied at a rate of 300 kg/ha in early May, prior to transplanting the seedlings. The mean maximum and minimum temperatures were 19.9°C and 11.1°C, respectively, with 556.8 mm of rainfall during the experimental period (May–August 2018) (www.bom.gov.au). Supplementary irrigation was not required during this period. Prior to germinating, the mean seed weight (SW) of each genotype was measured. Four replicates of 25 seeds per genotype were counted using a Condator seed counter (Pfeuffer, Germany) and weighed. On August 26, 118 days after sowing (DAS), winter biomass was measured in every second plant of both rows in each plot (total of eight plants). Harvested plants were cut to ground level and oven-dried at 60 °C for 72 h before recording winter biomass. Mean values for each trait are presented in Table 1.

Analyses were conducted using *R* software (version 3.6.3). Data were analysed using one-way ANOVA to compare the means of plant trait variation. One-way ANOVA was also conducted to compare differences between wild accessions and cultivars. Pearson correlation coefficients and their significance levels were calculated between plant traits, and also calculated between plant traits and eco-geographic (passport and BIOCLIM) variables. Broad sense heritability (H^2) was estimated according to Falconer (1989).

Table 1. List of 35 genotypes (27 accessions and 8 cultivars) used in this study with the mean values of measured traits: mean seed weight (SW, n=4) and winter biomass (n=24). Country of origin and 'passport data', consisting of latitude, longitude and altitude of their sites of collection (where available) are shown. Maximum temperature of the warmest month (BIO5) at their sites of origin is also shown. Blanks indicate missing data. Significance level of one-way ANOVA and broad-sense heritability (H^2) across all 35 genotypes are estimated for both traits.

Genotype	Country of origin	Latitude (°N)	Longitude (°E)	Altitude (m)	BIO5 (°C)	SW (mg)	Biomass (g)
Accessions							
Acc. 1						0.86	4.3 ±0.4
Acc. 2	Bulgaria	42.06	26.47	50	30.0	0.65	4.4 ±0.4
Acc. 3	Greece	39.00	22.24	590	32.5	0.93	6.5 ±0.5
Acc. 4	Greece	39.00	22.24	590	32.5	0.92	6.6 ±0.9
Acc. 5						1.31	11.5 ±1.2
Acc. 6						1.27	11.9 ±1.5
Acc. 7						1.51	12.6 ±1.8
Acc. 8						1.31	13.3 ±0.9
Acc. 9						1.21	13.5 ±1.3
Acc. 10						1.30	13.8 ±1.9
Acc. 11						1.27	14.0 ±1.4
Acc. 12						1.29	14.0 ±0.8
Acc. 13	Italy	43.72	10.40	5	28.7	1.28	15.6 ±2.6
Acc. 14						1.61	15.8 ±1.4
Acc. 15						1.40	17.7 ±2.1
Acc. 16						1.64	17.8 ±2.9
Acc. 17	Spain	37.33	–6.95	3	29.9	0.75	18.0 ±1.3
Acc. 18						1.30	18.2 ±1.7
Acc. 19						1.20	19.1 ±1.4
Acc. 20						1.35	19.3 ±1.9
Acc. 21						1.19	19.3 ±2.2
Acc. 22						1.39	19.8 ±2.0
Acc. 23	Italy	38.53	14.35	169	29.2	0.87	21.5 ±1.4
Acc. 24	Italy	38.06	15.40	510	26.3	1.04	21.5 ±2.7
Acc. 25						1.31	21.9 ±1.6
Acc. 26						1.35	22.0 ±1.7
Acc. 27	Turkey	38.49	43.39	1700	27.9	1.58	23.8 ±2.0
Cultivars							
Yuchi						1.22	11.1 ±1.7
Meechee						1.32	15.3 ±2.1
Seelu						1.33	15.5 ±1.5
Amclo	Italy	41.88	12.50	30	30.7	1.42	16.1 ±1.9
Zulu						1.56	16.3 ±1.6
Arrotas						1.53	17.5 ±1.4
Zulumax						1.31	22.6 ±2.3
Cefalu						1.57	24.1 ±2.1
			Significance level			P<0.001	P<0.001
				H^2		97%	87%

Results

High variation for winter biomass and seed weight (Table 1) was observed among genotypes. Heritability was high (≥87%) for both traits and cultivars had significantly higher means than wild accessions (Table 2).

The mean seed weight and winter biomass had a significant positive correlation ($P \leq 0.01$; $r = 0.50$). Winter biomass had no correlations with any passport data or BIOCLIM variables, while the only significant correlation for winter biomass was a negative correlation with Maximum temperature of the warmest month (BIO5) ($P \leq 0.05$; r= -0.7).

Table 2. Mean and range for the mean seed weight (SW) and winter biomass for 27 wild accessions and 8 cultivars of arrowleaf clover. Different letters indicate significant differences for each trait, comparing wild accessions and cultivars (*P*<0.05; Fisher LSD test).

Trait	Wild accessions			Cultivars		
	Minimum	Mean	Maximum	Minimum	Mean	Maximum
SW (mg)	0.5	1.2b	1.7	1.2	1.4a	1.6
Biomass (g)	0.7	15.6b	56.8	2.02	17.5a	57.5

Discussion

This study confirmed that broad variation exists among arrowleaf clover genotypes for winter biomass production. Several accessions also had individual plants with high biomass production, indicating the potential to breed arrowleaf clovers with improved winter performance. The results indicate that the larger the seed, the greater the winter biomass. Therefore, selection for seed size is likely to translate to increased winter biomass production. Seedlings from large seeds also have an advantage for light interception, giving them a stronger competitive ability (Donald 1951; Black 1958).

Summer temperature influenced winter growth diversity in arrowleaf clover, showing that winter biomass decreases when climate of origin has hot summers. Notably, wild accession Acc.27 had the highest winter biomass and had the lowest annual mean temperature (8.6°C), coldest winter temperature (-8.4°C) and highest altitude (1700 m a.s.l.). The high winter biomass of this genotype supports Ghamkhar et al. (2015) that mean winter temperature is associated with winter growth productivity.

Conclusion

This research suggests the winter productivity of arrowleaf clover can be improved by plant breeding and utilising germplasm from the most productive wild accessions. This study will enhance knowledge on adaptation of arrowleaf clover and potential distribution extent through comparison eco-geographic variables of existing collection sites. Unfortunately, only nine genotypes in the Australian collection have detailed passport data, which prevents conclusive analysis of relationships between traits. The genetic resources of arrowleaf clover could be diversified by collecting in areas where it is reported as being distributed, but not yet collected from, such as Albania, Hungary, Romania, Corsica, Crimea and south-western Russia (Zohary and Heller 1984). Future collections could focus on ecological and climatic features of sites. Landscapes in Albania and Corsica are highly diverse and could be useful for targeting highlands and cooler areas for collection to improve our understanding of winter biomass production of arrowleaf clover.

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References

Black J (1958). Competition between plants of different initial seed sizes in swards of subterranean clover (Trifolium subterraneum L.) with particular reference to leaf area and the light microclimate. Australian Journal of Agricultural Research 9, 299-318.

Donald C (1951). Competition among pasture plants. I. Intraspecific competition among annual pasture plants. *A*ustralian Journal of Agricultural Research 2, 355-376.

Ewing MA, Poole C, Skinner P, et al. (2001). Sulla and other forage species for southern Australia. Rural Industries Research and Development Corporation

Falconer DS (1989). Introduction to quantitative genetics, Longman, Harlow, England.

Ghamkhar K, Nichols PGH, Erskine W, et al. (2015). Hotspots and gaps in the world collection of subterranean clover (Trifolium subterraneum L.). Journal of Agricultural Science 153, 1069-1083.

Hijmans RJ, Cameron SE, Parra JL, et al. (2005). Very high resolution interpolated climate surfaces for global land areas. International Journal of Climatology 25, 1965-1978.

Miller JD and Wells HD (1985). Arrowleaf clover. In: Clover science and technology. NL Taylor Eds. pp. 503-514, Madison, Wisconsin, USA.

Nichols PGH, Loi A, Nutt BJ, et al. (2007). New annual and short-lived perennial pasture legumes for Australian agriculture -15 years of revolution. Field Crops Research 104, 10-23.
Nichols PGH, Revell CK, Humphries AW, et al. (2012). Temperate pasture legumes in Australia - their history, current use, and future prospects. Crop and Pasture Science 63, 691-725.
Ovalle CM, Pozo A, Fernandez F, et al. (2010). Arrowleaf clover (Trifolium vesiculosum Savi): A new species of annual legumes for high rainfall areas of the Mediterranean climate zone of Chile. Chilean Journal of Agricultural Research 70, 170-177.
RIRDC (2008). RIRDC completed projects in 2007-2008 and research in progress as at June 2008 : pasture seeds. Barton, Rural Industries Research and Development Corporation.
Snowball R and Revell C (2011). Farmnote: Cefalu arrowleaf clover. Government of Western Australia, Department of Agriculture and Food.
Thompson AN, Kennedy AJ, Holmes J, et al. (2010). Arrowleaf clover improves lamb growth rates in late spring and early summer compared with subterranean clover pastures in south-west Victoria. Animal Production Science 50, 807-816.
Thompson RB (2005). Arrowleaf clover. Primefacts 102.
Zohary M and Heller D (1984). The genus Trifolium. The Isreal Academy of Science and Humanities. Jerusalem.