

Seasonal performance of white clover in mixed-sward grazing pasture highlights genotype by environment interaction

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Abstract

White clover is an important forage crop because of its nutritional value, ability to provide plant-available nitrogen via symbiosis with *Rhizobium* soil bacteria, and year-round availability of dry matter (DM) yield. However, its performance in mixed sward-based pastures is characterised by seasonal variability and declining DM yield over time. The identification of white clover genotypes adapted for across seasonal performance is an important goal in white clover breeding. In this study, we evaluated the seasonal performance of 200 white clover half-sib families using visual growth scores and calibrated dry matter yield based on growth scores measured for three years in two locations. Results showed significant variation for growth scores across years, seasons and locations. Significant G×E was observed in the form of year, location and season interactions. Calibrated DM yield was highest in the second-year summer with clover content declining in the third year. Spring and winter were identified as potential vulnerable periods for white clover growth in pastures.

Introduction

White clover is an important forage crop because of its nutritional value, year-round availability of dry matter (DM) yield and ability to fix atmospheric nitrogen in pastures (Caradus et al., 1996). Breeding improved white clover cultivars is particularly challenging due to many factors including long breeding cycles, mixed sward-based phenotypic assessments and complex genetic architecture of important traits like seasonal dry matter (DM) yield and vegetative persistence. Significant genotype by environment (G×E) effect for these quantitative traits have been reported in white clover. The differential performance of genotypes in different locations, termed, G×E, is a familiar concept to breeders, where it is often viewed as a disadvantage or inconvenience at best. This is because it can significantly complicate traits assessment and the identification of superior genotypes, especially if there is re-ranking amongst locations (Cooper & Byth, 1996). However, this complex interaction of environmental and genotypic effects plays a vital part in eventual phenotypic expression and can allow breeders to identify material that is broadly adapted by testing breeding material in multiple locations (Falconer & Mackay, 1996).

Although, conventional forage breeding methods have been successful for increases in forage DM yield, white clover breeding is particularly complicated as it is often assessed in a mixed sward environment where inter-specific competition and specific grass-adapted management practices play key roles in clover yield and persistence (Widdup & Barrett, 2011). In many pastures, white clover DM yield tends to decline over time with the total percentage of white clover in mixed sward falling below 35% by the third year of evaluation (Chapman et al., 1996). New breeding tools like genomic selection that use molecular markers to predict the phenotypes of untested individuals require large amounts of accurate and precise phenotype data including G×E estimates. Understanding the nature and magnitude of G×E is important to breeders to increase the efficiency of breeding programs albeit at the increased cost of establishing multisite and year replicated trials. The objectives of this study were to: (i) assess the seasonal performance and magnitude of G×E for growth scores, an indirect measure of DM yield in white clover HS families evaluated over three years in two locations and; (ii) investigate the seasonal variability of calibrated DM yield across years and locations.

Methods

Plant material and field trial

Two hundred F3 half-sibling (HS) families were established in row-column, replicated field trials from 2016 to 2019 in two locations in New Zealand: AgResearch, Grasslands Research Centre in Palmerston North, Manawatu (Aorangi) and the AgResearch, Ruakura Research Farm in Hamilton, as described by Ehoche et al (2022). Briefly, fifteen plants from each HS family were transplanted into 0.5 m by 0.75 m plots in a sward of perennial ryegrass (*Lolium perenne*) cv 'Ceres One50' with AR37 endophyte. To assess seasonal growth, visual scores were taken before grazing when herbage mass was between 2500-2800 kg/ ha DM. Scoring was done on a scale of 1 (lowest) to 9 (highest) herbage production per plot, with 0.5 units increments to allow closer approximation of continuous data. Calibration cuts for each seasonal score were taken using a 0.2 m² quadrant. Three plots per score value were harvested, separated into white clover and ryegrass components and then oven dried. A linear regression analysis was performed between harvested DM and plot scores to obtain an estimate of score accuracy. The regression between plot score and clover DM content allowed an estimated clover DM yield (kg DM ha⁻¹) to be calculated for all plots.

Statistical analysis

A linear mixed model was fitted using Residual Maximum Likelihood (REML) in DeltaGen (Jahufer and Luo 2018) to estimate Best Linear Unbiased Predictors (BLUPs) for each HS family and variance components for genetic and nongenetic effects. A detailed assessment of linear mixed models employed are described in (Ehoche et al., 2022). Principal component analysis (PCA) and cluster analysis were conducted using seasonal growth score BLUPS as described in (Ehoche et al., 2022).

Results and Discussion

There was significant ($P < 0.05$) additive genetic variation (σ^2_A) for growth scores among the HS families across locations for each of the four seasons except for 2017 summer and autumn and 2018 summer (Table 1). Narrow-sense heritability was low to moderate and tended to increase with successive years. The significant additive genetic variance and heritability estimated for growth scores across years, locations and seasons indicates substantial genetic gain to be made with selection in this population. Family \times location ($\sigma^2_{A,L}$) (G \times E) interaction was significant ($P < 0.05$) for individual years and across years and was always larger than the additive genetic variance component. Significant G \times E interaction has been reported for many yield traits in white clover (Caradus et al., 1993; Jahufer et al., 2009). As expected, for a polygenic trait like yield, the magnitude of G \times E was often greater than the family additive genetic variance component, demonstrating a change in relative performance of HS families across environments. The presence of G \times E implies that DM yield at Aorangi would be a poor predictor of DM yield at Ruakura and shows the need to run trials across multiple environments and years to assess the true breeding potential of selection candidates, especially if the goal is to breed for broad adaptation (Brown & Caligari, 2008). Furthermore, these multi-location and year trials enable reduction of the confounding effects of G \times E interactions (Cooper & Byth, 1996) and increase the efficiency with which genotypes can be identified for superior performance across or within specific environments.

Year and season had significant ($P < 0.001$) effects on calibrated DM yield. There were significant ($P < 0.001$) differences among seasons within years, across years, as well as significant year-by-season interactions (Figure 1A). In the second year of trial establishment (2018), DM yield averaged over seasons and locations was 80% higher than 2017 and 78% higher than 2019, respectively. The decrease in DM yield in the third year could be explained by the plants becoming more susceptible to biotic and abiotic stresses like adverse weather conditions (Wachendorf et al., 2001), pests and diseases and reliance on a weak fibrous root system (Brock & Caradus, 1996), as well as the intraspecies competition in the pasture (Chapman et al., 1996).

Table 1: Estimated additive genetic (σ^2_A), family-by-location interaction ($\sigma^2_{A,L}$), family-by-year interaction ($\sigma^2_{A,Y}$), pooled error (σ_e) variance components, their associated standard errors (\pm SE) and family mean narrow-sense heritability (h^2_n) for seasonal growth scores from combined analyses for 200 half-sib white clover families across two locations, Aorangi and Ruakura.

Variance components	Summer	Autumn	Winter	Spring
	2017			
σ^2_A	0.11 ± 0.08	0.06 ± 0.07	0.09 ± 0.04*	0.20 ± 0.07*
$\sigma^2_{A.L}$	0.72 ± 0.11*	0.18 ± 0.08*	0.29 ± 0.05*	0.45 ± 0.07*
σ^2_ϵ	2.16 ± 0.06	1.91 ± 0.09	1.57 ± 0.04	1.65 ± 0.05
h^2_n	n.s	n.s	0.17 ± 0.08	0.29 ± 0.08
	2018			
σ^2_A	0.14 ± 0.08	0.21 ± 0.07*	0.24 ± 0.09*	0.33 ± 0.10*
$\sigma^2_{A.L}$	0.57 ± 0.10*	0.44 ± 0.08*	0.32 ± 0.12*	0.71 ± 0.11*
σ^2_ϵ	1.7 ± 0.06	1.21 ± 0.05	2.06 ± 0.10	2.01 ± 0.06
h^2_n	n.s	0.31 ± 0.10	0.33 ± 0.10	0.32 ± 0.08
	2019			
σ^2_A	0.28 ± 0.13*	0.23 ± 0.13	0.78 ± 0.27*†	0.29 ± 0.12*
$\sigma^2_{A.L}$	1.21 ± 0.16*	0.57 ± 0.16*	–	0.78 ± 0.15*
σ^2_ϵ	1.74 ± 0.05	2.06 ± 0.12	1.49 ± 0.45†	1.96 ± 0.08
h^2_n	0.28 ± 0.11	0.27 ± 0.12	0.38 ± 0.10†	0.29 ± 0.10
	Across all years			
σ^2_A	0.11 ± 0.05*	0.13 ± 0.05*	0.13 ± 0.06*	0.14 ± 0.05*
$\sigma^2_{A.L}$	0.36 ± 0.07*	0.30 ± 0.03*	0.23 ± 0.06*	0.23 ± 0.06*
$\sigma^2_{A.Y}$	0.07 ± 0.04*	0.03 ± 0.03	0.04 ± 0.08	0.07 ± 0.05
$\sigma^2_{A.Y.L}$	0.46 ± 0.06*	0.04 ± 0.04	0.01 ± 0.09	0.04 ± 0.07
σ^2_ϵ	1.98 ± 0.04	1.76 ± 0.05	1.81 ± 0.06	1.80 ± 0.06
h^2_n	0.27 ± 0.10	0.41 ± 0.11	0.46 ± 0.14	0.44 ± 0.14

*Significant at $P < 0.05$, n.s = not significant, † = estimated only at Ruakura

Significantly higher DM yield was observed in summer 2018 than other seasons-year combinations while winter and spring had the lowest DM yield. Low herbage production in winter is explained primarily by a reduced leaf area index due to fewer number of leaves and lower leaf surface area to intercept radiation thus resulting in restricted photosynthetic ability (Woledge et al., 1990). Low yield in spring is due to several reasons, outlined by Brock et al. (1988) such as drought conditions, lax defoliation regimes and excessive nitrogen fertilizer applications. Another primary reason was the temperatures being around 15.5°C (Ehoche et al. 2022), which is optimum for ryegrass growth, thereby tilting the balance in favour of the actively growing ryegrass (Woodfield & Caradus, 1996; Wachendorf et al., 2001). At the onset of summer, however, due to warmer temperatures, now in the optimum range for white clover, ryegrass growth rate decreases and white clover is able to contribute more to the total herbage yield during this period. Overall, even though the white clovers' contribution to yield in a mixed sward is characterised by variability, a major benefit of growing clover in a mixed sward with ryegrass is this seasonal complementarity which ensures available feed for livestock all year round (Caradus et al., 1996).

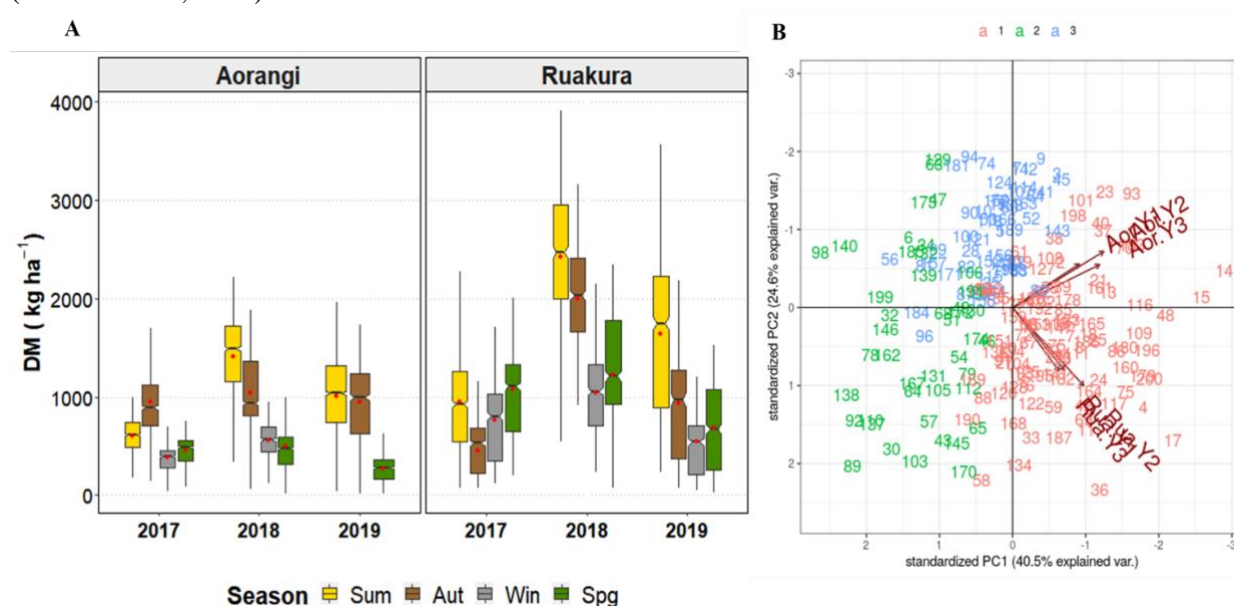


Figure1: A) Notched boxplots showing the variation in dry matter yield in white clover due to year and season effects. Data taken from seasonal calibration cuts. LSD 86 kg/ha. B) Biplot generated from pattern analysis using standardised Best Linear Unbiased Predictor (BLUP) values of 200 HS families assessed in two locations, Aorangi (Aor) and Ruakura (Rua) over three years for seasonal herbage growth scores.

The directional vectors on the biplot generated from the PCA of seasonal growth scores in years and locations shows a weak (0.3) but positive correlation between both locations (Figure 1B). Three groups were generated by cluster analysis with Group 1 showing the highest mean expression for growth in all seasons across the two sites. Group 3 contained families that performed better predominantly at Aorangi while Group 2, on average, consisted of the lowest performing families. These results provide insight into the patterns of adaptation of the HS families for these locations. For instance, HS 48 can be selected for superior performance across both locations, while HS families 14 and 15, might be selected for specific adaptation to Aorangi.

Conclusion

Significant G×E for growth scores was observed in the form of year, location and season interactions validating the need for multi-site trials across different years and seasons. Herbage yield showed great plasticity in response to year and season changes. DM yield was highest in summer and clover content started to decline in the third year. Identification of key periods of susceptibility is important to tailor management practises for optimum sward performance.

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