Examining perennial ryegrass (*Lolium perenne L.*) persistence through identifying genetic shifts within two cultivars after nine years in the field.

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ABSTRACT

Perennial ryegrass (*Lolium perenne L.*) is a commercially important forage species in New Zealand agriculture. Ryegrass persistence is important for farmers as it substantially decreases the costs associated with reseeding pastures. Breeding for ryegrass persistence is difficult because of the complex interaction between genotype and environment; and the short time of field trial assessment compared to the expected longevity of pasture. A nine year old cultivar comparison trial at Poukawa, Hawkes Bay, New Zealand was identified and plants surviving in the cultivar plots of 'Grasslands Samson' and 'Commando' were retrieved. These populations that had survived were termed Persistent. A sample of commercially sourced seed of these cultivars were also grown to represent the 'Original' genetic pool of the cultivars sown in the field. Persistent populations were compared to Original cultivar seed to characterise morphology and underlying genetics associated with persistence. Results were interpreted to determine if a genetic shift had occurred in Persistent populations due to advantageous phenotypes surviving.

Three methodologies were used to compare populations: 1) In a glasshouse, eight morphological traits were measured after 10 weeks growth for Original and Persistent populations of 'Grasslands Samson'; 2) Half-sibling families were generated from Persistent and Original populations for both cultivars and were assessed for additive genetic variation of seven traits as one metre rows in the field over 13 months; 3) Simple sequence repeat (SSR) markers were used to explore the genetic composition of Original and Persistent populations of each cultivar. Analysis and interpretation of data showed genetic shifts were cultivar specific. The greatest differences were identified between populations of 'Grasslands Samson'. Compared to the Samson Original population, Samson Persistent plants had significantly greater means for four traits in the glasshouse

and half-sibling families showed evidence of shifting population means of traits associated with animal grazing avoidance. SSR marker results were confounded by late detection of contamination in samples. Analysis of a reduced sample size showed no significant differences between any of the four populations using F statistics and genetic structure analysis.

These results suggest future studies could reduce risk of contamination by collecting single tillers from the field of Persistent populations. Further investigation of the genetics of persistence should focus on the role of lamina sheath lengths in tiller production, and using the half-sibling families identified in this study for germplasm development and quantification of genotype-by-environment interactions.

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ABBREVIATIONS

%amp	Success rate of marker amplification
%P	Percentage of polymorphic loci
μ	Population mean
AFLP	Amplified Fragment Length Polymorphism
AMH	Aftermath heading score
AMOVA	Analysis of molecular variance
AR1	Commercially available endophyte strain in perennial ryegrass cultivars
BLUE	Best linear unbiased estimator
BLUP	Best linear unbiased predictor
bp	Base pairs
CASS	Cheaply amplified size standard
CV_A	Co-efficient of additive variation
df	Degrees of freedom
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleotide triphosphate
DW	Dry weight (per plant)
DWT	Dry weight (per one metre row)
E	Environment
EST-SSRs	Expressed sequence tag simple sequence repeats
Est. Var.	Estimated variance components
F_{IS}	Component of Wright's (1921) fixation index, used to define within population structure by calculating the average observed heterozygosity of an individual relative to the expected heterozygosity of individuals in the population it belongs to.
F_{ST}	Component of Wright's (1921) fixation index, used to define between population structure by comparing the expected heterozygosity of individuals within a subpopulation to the total expected heterozygosity of individuals across all populations
G	Genotype
G_C	Genetic gain per cycle
h^2_n	Narrow-sense heritability
H_E	Expected heterozygosity

HGs	Herbage growth score
H_O	Observed heterozygosity
kg ha^{-1}	Kilograms per hectare
LL	Leaf length
LnP(D)	Mean posterior probability
LT	Leaf thickness
LW	Leaf width
LWs	Leaf width score
MAF QT	Ministry of Agriculture and Forestry quick test, a soil testing metric
mL ha^{-1}	Millilitres per hectare
MS	Mean of squares
N	Number of samples
Na	Number of alleles
Na_v	Total number of allele variants (within a cultivar)
$N(0,\sigma 2\varepsilon)$	Normally and independently distributed
N-P-K-S	Units of nitrogen, phosphorus, potassium, and sulphur. Used to express fertiliser contents.
n_r	Number of replications
n_s	Number of seasons
P	Phenotype
PC	Principal components
PCA	Principal components analysis
PCR	Polymerase chain reaction
PHs	Plant habit score
RAPD	Random amplified polymorphic DNA
REML	Residual maximum likelihood
RP	Percentage of reproductive tillers
RT	Number of reproductive tillers.
RTD	Reproductive tiller development score
Ru	Rust score
S	Selection differential (average superiority of the selected parents), used to predict genetic gain per cycle S

SA_P	Proportion of shared alleles
SL	Leaf sheath length
SS	Sum of squares
SSRs	Simple sequence repeats, a type of microsatellite marker.
TN	Tiller number
Var. %	Percentage of variation
σ^2_ε	Experimental error variance
σ^2_a	Additive genetic variance
σ^2_{as}	Additive additive-by-season interaction variance
σ^2_D	Dominance variance
σ^2_g	Genetic variance
σ^2_P	Phenotypic variance
$\bar{x}$	Population mean

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rows (r) and columns (c); M is the overall mean; f_i is the random effect of HS family i, N(0,σ^2_f); s_j is the fixed effect of season j; $(fs)_{ij}$ is the effect of the interaction between HS family i and season j, N(0,σ^2_{fs}); b_{jk} is the random effect of replicate k within season j N(0,σ^2_b); r_{jkl} is the random effect of row l within replicate k within season j, N(0,σ^2_r); c_{jklm} is the random effect of column m within replicate k within season j, N(0,σ^2_c); ε_{ijklm} is the residual effect of HS family i in row l and column m of replicate k in season j.: $Y_{ijklm} = M + f_i + s_j + (fs)_{ij} + b_{jk} + r_{jkl} + c_{jklm} + \varepsilon_{ijklm}$, ……..…58

3.3; Narrow-sense heritability (h^2_n) = additive genetic variation (σ^2_a) / additive genetic variation (σ^2_a) + additive by season interaction variance (σ^2_{as}) / number of seasons (n_s) + experimental error (σ^2_ε) / number of replications (n_r) x number of seasons (n_s): $h^2_n = \frac{\sigma^2_a}{\sigma^2_a + \frac{\sigma^2_{as}}{n_s} + \frac{\sigma^2_\varepsilon}{n_r n_s}}$……..……..…59

3.4; The co-efficient of additive variation (CV_A(%))= (square root of phenotypic (σ^2_a) variance component / trait population mean ($\bar{x}$)) × 100: $CV_A\,(\%) = \frac{\sqrt{\sigma^2_a}}{\bar{x}} \times 100$……59