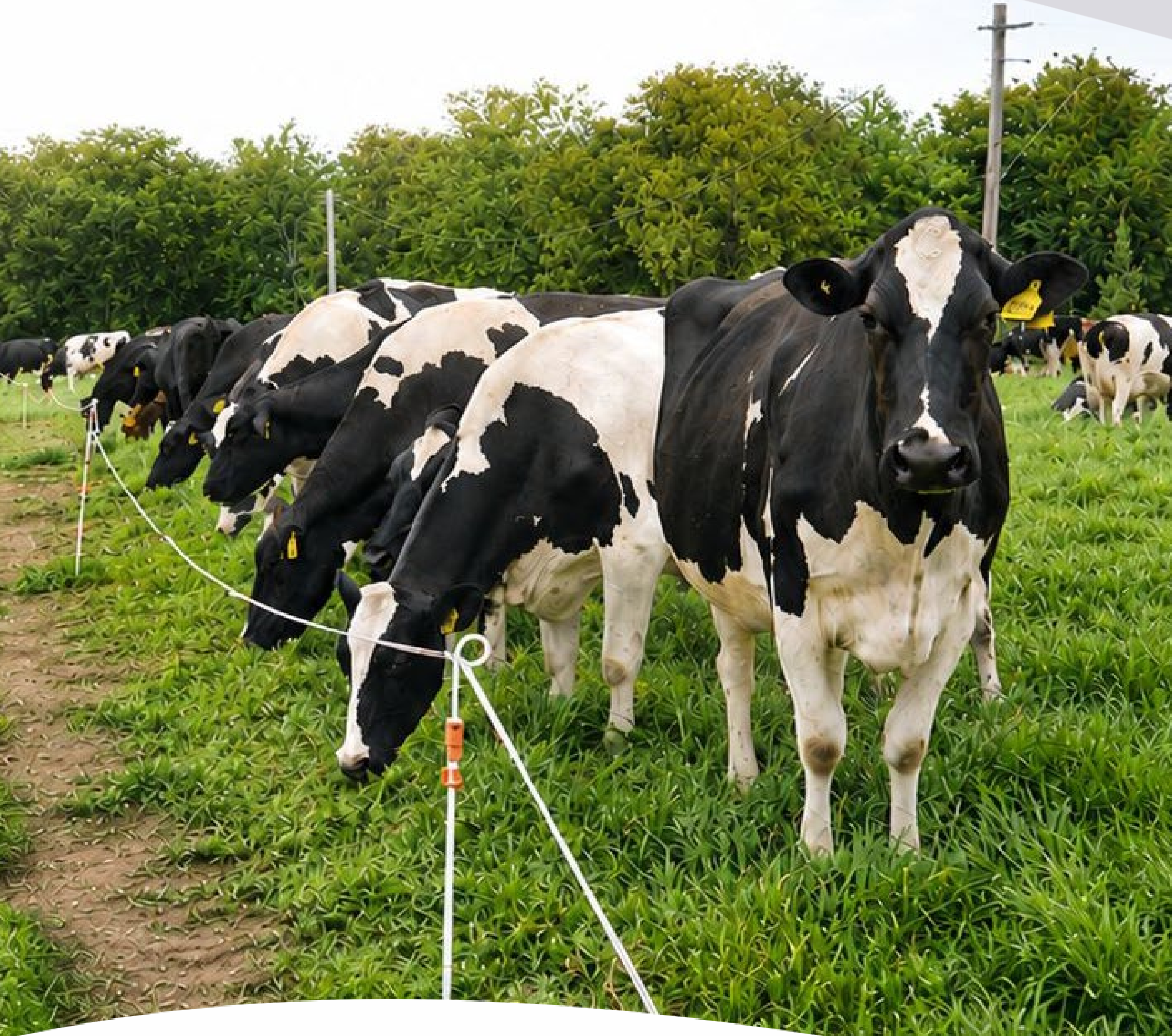
Final Report

P1c Exploring Genetic Variability of Kikuyu



THE UNIVERSITY OF SYDNEY
Dairy Research Foundation

This project was led by Richard Trethowan from the University of Sydney Dairy Research Foundation

Dairy UP (Phase 1) was a $16 million, five-year industry driven project with a portfolio of 10 research, development and adoption projects collectively aiming to realise three primary objectives:

- Increase Productivity and Profitability by unlocking the potential of milk, the cow and water,
- De-risking the industry and
- Developing new markets.

A key part of Dairy UP was a coordinated network of partner farms across New South Wales (and beyond) providing valuable insights into real world application of new practices, including the challenges and benefits of new innovative technologies.

Dairy UP made a big contribution to dairy research and development rejuvenation, (attracting new researchers, PhD students and Industry investment).

Dairy UP was funded through the Australian and NSW Government's Bushfire Industry Package – Sector Development Grant (BIP-SDG) program, with cash co-contributions from Dairy Australia, The University of Sydney's Dairy Research Foundation, Local Land Services, Norco, Leppington Pastoral Co Ltd and Dairy NSW; and in kind contributions by all the above organisations plus NSW DPI (Biosecurity and Food Safety; Agriculture), Scibus, Australia Fresh Milk Holding Ltd, Dairy Connect and NSW Farmers.

This project was delivered jointly by University of Sydney's Dairy Research Foundation, Scibus, Department of Primary Industries and Regional development.

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Charles Sturt University | DairyBio | DataGene | Eagle Direct | Entegra | Macquarie University | NSW EPA | smaXtec | UC Davis | University of Technology Sydney

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1. Executive Summary

New kikuyu genotypes developed by the University of Sydney in collaboration with Hatton's Turf Research have been screened for drought and salinity tolerance. However, the pasture potential of these lines was unknown including their dry matter production, disease resistance and nutritional value. To put these materials into context, a DNA study was conducted that included the new candidate lines and kikuyu leaf samples collected from farms around NSW. The DNA fingerprints showed that the new materials were genetically distinct from the cultivars Whittet and Fulkerson, and that 35% of all pastures were related to these two cultivars. An experiment was established to screen 13 candidate lines at Lansdowne in a 2-year small plot experiment. The results identified 3 candidate lines with competitive dry matter production, suitable nutritional profiles and potentially better disease resistance. These candidates were then sown on farm in strip plots across 2 seasons and grazing was allowed on one half of each strip. Dry matter production was assessed at regular intervals across the two growing seasons and a chemical analysis conducted to determine the nutritional value of the new materials. At the same time, the disease reactions of the new candidates to black spot and kikuyu yellows disease were assessed. Results showed that the new candidate lines produced equivalent dry matter to Whittet across the season at each location. However, physical analysis of dry matter samples for nutritional traits identified two lines with better profiles. These two lines were also more resistant to black spot and kikuyu yellows disease than Whittet. Both lines offer dairy farmers valuable new genetic diversity with enhanced nutritional and disease profiles at an equivalent dry matter production per ha.

2. Project Overview

Item	Description
Project Title	Unlocking the potential of Kikuyu-based pastures
Funding Body	Dairy UP
Dairy UP Project	P1c
Project Duration	2022-2026
Lead Organisation	The University of Sydney
Project Lead	Richard Trethowan
Key Collaborators	NSW Department of Primary Industries: Elizabeth Macathur Agricultural Institute

3. Abbreviations

USYD — The University of Sydney
NDF - neutral detergent fibre
ADF - acid detergent fibre
OM - organic matter
DM – dry matter
CDM – cumulative dry matter
DOMD - dry organic matter digestibility
WSC - water soluble carbohydrates
ME - metabolizable energy
CP – crude protein
NDVI – normalized difference vegetation index

4. Project Background and Rationale

The University of Sydney in collaboration with Hatton's Turf Research has been selecting kikuyu grasses for biomass production, disease resistance and tolerance to salinity and drought for many years. These new materials represent potential valuable genetic diversity for the dairy industry. P1c aims to evaluate these promising kikuyu lines for commercially important traits in comparison with two commonly grown cultivars of kikuyu. Testing is two-tiered, with larger numbers of lines initially tested at one location across two summers, followed by a selected subset evaluated across multiple environments in strip plots under farmer practice. The results of a DNA analysis of kikuyu pastures in NSW and the evaluation of kikuyu lines, including a selected subset tested across seasons and environments, are presented in this report.

5. Project Objectives

Dairy UP's P1c project aims to unlock the potential of Kikuyu pastures used by NSW dairy farmers by exploiting genetic diversity. This project explored and evaluated promising lines of kikuyu that have been selected for tolerance to salinity and drought.

6. The genetic diversity of kikuyu pastures and the new materials

Research team: Richard Trethowan, Vivien Tan, Amit Singh, Chong Mei Dong and Laura Espinosa

6.1 Background

While the University of Sydney in collaboration with Hatton's Turf developed new kikuyu materials with potential for the dairy industry, little was known of genetic diversity among existing pastures in NSW. We hypothesised that most pastures would be related to Whittet (L15 in this report), a successful variety released more than 50 years ago and widely adopted, presenting a potential issue with genetic vulnerability in our changing climate. This study also examined the genetic diversity of existing pastures compared to the new genetic materials to better understand their potential.

6.2 Objectives

To compare the DNA fingerprints of kikuyu materials collected on farm with the new genetic materials developed at the University of Sydney

6.3 Materials & Methods

Tissue samples were collected from dairy farms on the mid north coast (MC), north coast (NC) and south coast (SC) of NSW. These samples included the varieties Whittet and the Bill Fulkerson line and the 13 candidate kikuyu lines. Kikuyu DNA was extracted from dried leaves using the CTAB method and the samples subsequently genotypes using DArTseq. A total of 91 kikuyu samples were genotyped, and the locations of these samples are given in Table 1. Genetic distance and the dendrogram were generated by computing pairwise genetic distance (Hamming-style distance) between samples using their SNP genotypes. Distances between two samples were calculated per SNP as follows:

- 0 if both samples have the same homozygote or the same heterozygote
- 1 if the samples have different homozygotes (e.g., 0/0 vs 1/1)
- 0.5 if one sample is homozygous and the other is heterozygous
- SNPs with missing values in either sample are excluded

The final distance between two samples was the average per-SNP distance across all markers with valid genotypes. All analysis were conducted using R.

Table 1. Location of samples collected for DNA analysis on the mid-north coast (MC), north coast (NC) and south coast (SC) of NSW. New genotypes are marked TC. Toxicity is indicated if reported in the pasture at time of sampling.

Sample code	Sample information	Toxicity reported
MC-1	Neals Dairy, Patrick and Louise Neal, 31.53138 S, 152.33593 E, 40 Neals Ln, Oxley Island NSW 2430 (close to Taree).	
MC-2	Tocal LLS, Tocal, Paddock 9, 32.37492 S, 151.35288 E, Tocal College 815 Tocal Road PATERSON NSW 2421 (The Hunter)	
MC-3	Nicholson Dairy and Silage Contracting, Sam Nicholson, House, 31.51433 S, 152.35534 E,NSW. (20km North of Taree)	

Sample code	Sample information	Toxicity reported
MC-4	Bale's dairy, Tim Bale, House, 31.43344 S, 152.38344 (between Taree and Port Macquarie ,Stewarts River NSW 2443	
MC-5	Neilson dairy, Emily and Matt Neilson, 31.43527 S, 152.41440 E, farm at Bendolba, north of Dungog.	
MC-6	Glen Martin farm, Hunter Valley (Doug Cowan) mid level Kikuyu paddock, site of original toxicity outbreak.	Yes
MC-7	Glen Martin farm, Hunter Valley (Doug Cowan) mid level Kikuyu paddock, site of original toxicity outbreak.	Yes
MC-8	Glen Martin farm, Hunter Valley (Doug Cowan) low level Kikuyu paddock, near water hole where animals died.	Yes
MC-10	421 Marshdale Rd, Hunter Valley, main site of Kikuyu toxicity (2018) 16/40 cows dead.	Yes
MC-11	421 Marshdale Rd, Hunter Valley, secondary site of Kikuyu toxicity (2018) 2/3 cows dead. Lower elevation, wetter field than main site, other grasses present	Yes
MC-12	Stuart and Lorraine, Hunter Valley, Main site of cattle death (2018), low site, dense Kikuyu.	Yes
MC-13	Stuart and Lorraine, Hunter Valley, Main site of cattle death (2018), low site, dense Kikuyu.	Yes
MC-14	Geoff and Narelle, Hunter Valley, 6/15 cattle lost (2018), affected paddock, short Kikuyu mixed with rye and clover	Yes
MC-15	Geoff and Narelle, Hunter Valley, 6/15 cattle lost (2018), upper paddock unaffected mid length Kikuyu mixed with rye	Yes
MC-16	Glen Martin, Hunter Valley 2019 outbreak, 9/250 cattle (all young), paddock on hill down to water, mixed grasses and clover with Kikuyu	Yes
MC-17	Glen Martin, Hunter Valley 2019 outbreak, 9/250 cattle (all young), paddock on hill down to water, mixed grasses and clover with Kikuyu, mid hill	Yes
MC-18	James Neal, 75 Templetons Ln Oaxley Island, -31.886972079332907, 152.58513364202872 sample 1	
MC-19	James Neal, 75 Templetons Ln Oaxley Island, -31.886972079332907, 152.58513364202872 sample 2	
MC-20	James Neal, 75 Templetons Ln Oaxley Island, -31.886972079332907, 152.58513364202872 sample 3	
MC-21	Simon Scowen, Manning Valley Dairy, Warriwillah, 700 Bungay Rd, Whingham, Mid Coast DAGs, -31.906336037683786, 152.34403923104526, sample 1	

Sample code	Sample information	Toxicity reported
MC-22	Simon Scowen, Manning Valley Dairy, Warriwillah, 700 Bungay Rd, Whingham, Mid Coast DAGs, -31.906336037683786, 152.34403923104526, sample 2	
MC-23	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, 4 Paddock 1 (affected) yellow (looks like the grass from before the rain), confirmed kikuyu poisoning	Yes
MC-24	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, 3.1 paddock 1 (affected) (shoot), confirmed kikuyu poisoning	Yes
MC-25	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, 3.2 paddock 1 (affected) (long grass), confirmed kikuyu poisoning	Yes
MC-26	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, #2 Paddock 1 (affected), confirmed kikuyu poisoning	Yes
MC-27	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, #1 1404 76C Bucketts Way Paddock 1 (affected), confirmed kikuyu poisoning	Yes
MC-28	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, 5.1 Paddock 2 (less affected) Grazed (shoot) patch, confirmed kikuyu poisoning	Yes
MC-29	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, #6 Paddock 2 (less affected) Yellow, long, ungrazed, confirmed kikuyu poisoning	Yes
MC-30	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, 5.2 Paddock #2 Long not-grazed, confirmed kikuyu poisoning	Yes
MC-31	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, 8 Paddock 3, confirmed kikuyu poisoning	Yes
MC-32	CW & JI Edwards, 1404 The Bucketts Way, Stroud Road, Hunter Valley, 3 cows dead, 4 sick, 7 Paddock 3 Unaffected Grazed okay, confirmed kikuyu poisoning	Yes
MC-33	Midgy Gharret Rd, -31.9227848, 152.5283997	
NC-1	Merryvale Jerseys, Jo and Lee Behrens, 28.40285 S, 152.54458 E, 431 Knights Road 2474 Ettrick NSW	
NC-2	Kupidabin farm, Fleur and Sam Tonge, 28.48341 S, 153.04110 E, Kupidabin, NSW	
NC-3	Loongana Park, Paul and Wayne Clarke, 28.48492 S, 152.57323 E,	

Sample code	Sample information	Toxicity reported
	Loongana Park,Dobies Bight NSW	
NC-4	Riverside, Krystie and Adam Gould, Paddock 15, 28.54299 S, 153.06529 E, Greenridge NSW 2471	
NC-5	220 Elfords Rd, Dobies Bight NSW 2470	
NC-6	Near Brombin, -31.4721884, 152.6406919	
NC-7	Comboyne, -31.6174141, 152.4422854	
NC-8	Near Brombin, -31.4538014, 152.6426120	
NC-9	634-600 Bowman Farm Rd, -31.9327622, 151.9312421	
NC-10	Comboyne, -31.5919996, 152.4978439	
NC-11	Near Wingham, -31.9024723, 152.3411391	
NC-12	Bowman Farm, 31.55592 S, 151.55540 E,Bowman Farm NSW 2422	
NC-13	Dobies Bight, Fleur and Sam Tonge, 28.48197 S, 152.59120 E,220 Elfords Rd Dobies bight 2470, Suspected case of toxicity in Feb 2019	Yes
NC-14	Lamont terra B, 829 Barrington west rd Darringlton	
SC-1	Old Bemboka, Kev and Brodie Game, L Paddock, 36.65758 S, 149.60966 E,Bemboka NSW 2550	
SC-2	Hillgrove dairy, Phil Ryan, Paddock C2, 36.76422 S, 149.75675 E,Toothdale NSW 2550	
SC-3	Jelgowry, Will Russell, Paddock 'Center Pivot 3', 36.71750 S, 149.86821 E,Jellat Jellat NSW 2550	
SC-4	Yarranung, Ryan Apps, Paddock Rd 12, 36.64427 S, 149.83831 E,Angledale NSW 2550	
SC-5	Ferncliff dairy, Sam Holmes, Paddock 32, 36.81076 S, 149.71243 E,Candelo NSW 2550	
SC-6	Hillgrove Dairy, Phil Ryan, Little Irrigation Paddock, 36.76241 S, 149.75510 E,Whittet variety sowed 3 years ago, Toothdale NSW 2550	
SC-7	Ferncliff Dairy, Sam Holmes, 24a Paddock, 36.81358 S, 149.70649 E Candelo NSW 2550	
SC-8	36.37093628600237 S, 149.95213265366874 E, 5 Narira Park Ln, Cobargo NSW 2550	
SC-9	Phil Ryan, 36.76241 S, 149.75510 E, Trial at Bega	
SC-10	Phil Ryan, Hillgrove Dairy - Front Irrigation Paddock, Toothdale NSW 2550, 3 mature aged cows (7-10 years), kikuyu based with Italian ryegrass and legume mix	Yes

Sample code	Sample information	Toxicity reported
SC-11	Ferncliff/Sam Holmes, Bega, 5 cows, pure kikuyu pasture, suspected kikuyu bloating	Yes
SC-12	Ken Kimber, Ottonville Rd, Angledate, -36.64167, 149.879134	
SC-13	Ken Kimber, Ottonville Rd, Angledate, -36.64167, 149.890167	
SC-14	Brodie Game, "Blackjack Holsteins", 2367 Snowy Mountains Hwy, Bemboka, -36.6523647, 149.6064037	
SC-15	Andrew Irvin, "Aljo", Snowy Mountains Hwy, Bemboka, -36.6583577, 149.6350883	
SC-16	Brad Smith, "Oakleigh", 69 Jews Creek Rd, Brogo, -36.5949042, 149.8483728	
SC-17	Sam Holmes, "Ferncliff", Towridgee Lane, Candelo, -36.8072846, 149.7073084	
SC-18	Sam Holmes, "Ferncliff", Towridgee Lane, Candelo, -36.8126446, 149.712964	
SC-19	Hayden Parbery, "Willowdene", Tantawangalo Mtn Rd, Candelo, -36.811284, 149.712964	
SC-20	Hayden Parbery, "Willowdene", Tantawangalo Mtn Rd, Candelo, -36.782057, 149.644978	
SC-21	Phil Ryan, "Hillgrove", Wyndham Lane, Kanooma, -36.7627400, 149.7561343	
SC-22	Ryan Apps, "Yarranung", 180 Angledale Rd, Bega, -36.6571331, 149.8338638	
SC-23	Darren Wallis, Kameruka Lane, Candelo, -36.7513585, 149.7240622	
SC-24	Will Russell, "Jelgowry", 1159 Tathra Rd, Bega, -36.718967, 149.873522	
SC-25	Wal Jasper	
TC-1	Line 43, small plot experiment in Cobbitty	
TC-2	Line 51`, small plot experiment in Cobbitty	
TC-3	Line 133, small plot experiment in Cobbitty	
TC-4	Line 134, small plot experiment in Cobbitty	
TC-5	Line 6, small plot experiment in Cobbitty	
TC-6	Line 119, small plot experiment in Cobbitty	
TC-7	Line 96, small plot experiment in Cobbitty	
TC-8	Line 90, small plot experiment in Cobbitty	

Sample code	Sample information	Toxicity reported
TC-9	Line 7, small plot experiment in Cobbitty	
TC-10	Line 143, small plot experiment in Cobbitty	
TC-11	Line 44, small plot experiment in Cobbitty	
TC-12	Line 73, small plot experiment in Cobbitty	
TC-13	Line 59, small plot experiment in Cobbitty	
TC14	Bill Fulk from runners	
TC15	Whittet	
Bill S	Seedling source	
CRF	Corstorphine Dairy Farm, University of Sydney, Mid of Paddock 1 & Paddock 2, 34.024116 S, 150.654738 E, 335 C07BP, Brownlow Hill NSW 2570, Australia	
GD	Gallpen Dairy, 35.31417 S, 144.57237 E,Deniliquin NSW 2710	
RT	Galston farm, 33.38135 S, 151.04012 E, Galston NSW 2159	
UN	Brown pot, Unknown	

6.4 Key Findings

A dendrogram was constructed based on DNA diversity and this is presented in Figure 1. Four main groups of materials were identified. The cultivars Whittet and Fulkerson fell into one group (Group 1), whereas the new lines were distributed in Groups 2, 3 and 4 with a higher number in Group 4. None (apart for the two check cultivars, TC-14 (Fulkerson) and TC-15 (Whittet)) appeared in Group 1. Results indicate that there is significant diversity in kikuyu pastures across NSW with just 35% of locations sampled similar to Whittet and Fulkerson. No distinct grouping based on region (MC, NC, SC) was evident with sites spread across the groups. Interestingly, these appears to be a genetic relationship with reported toxicity with most instances appearing in Group 1 along with Whittet and Fulkerson.

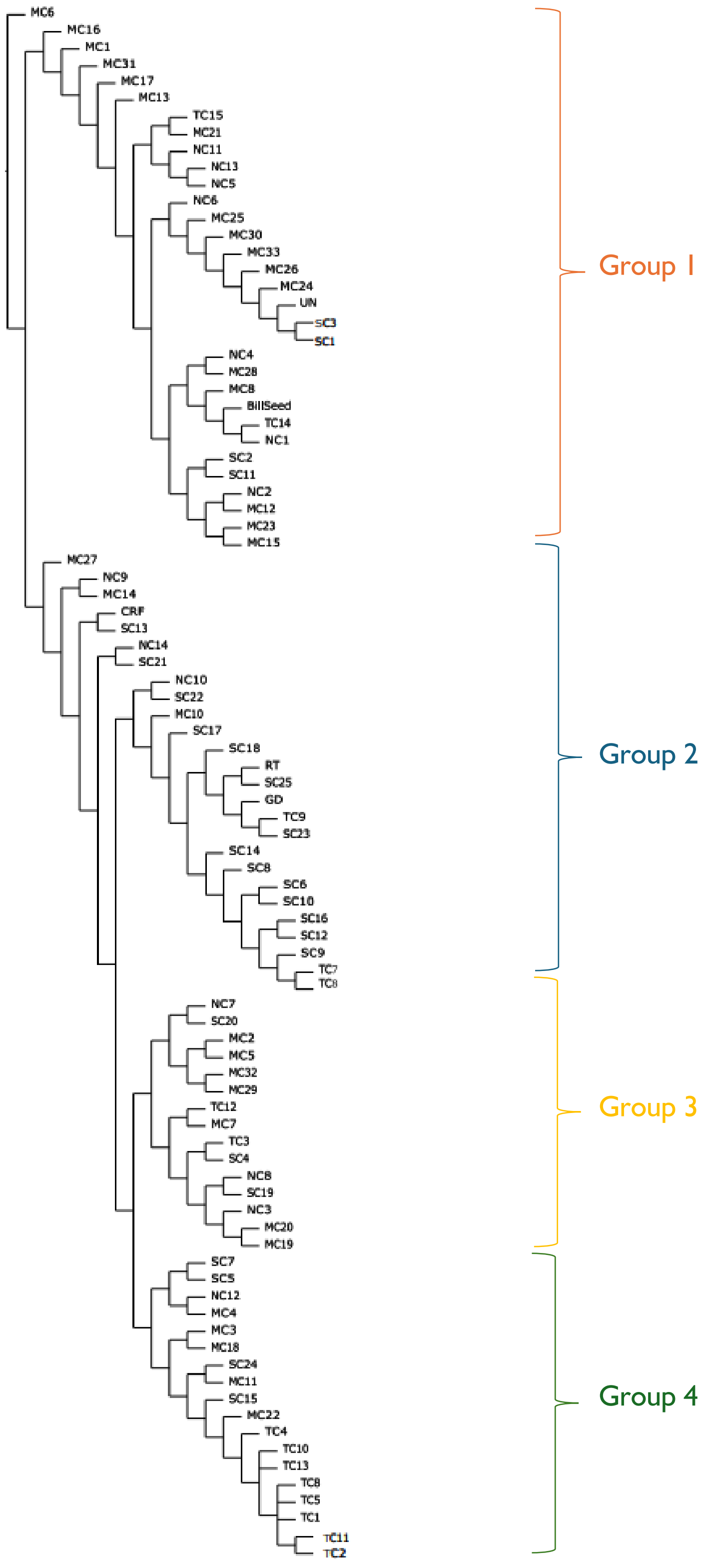

Figure 1. Dendrogram of kikuyu diversity at sampled sites in NSW. Clusters are indicated in four groups. A location/genotype key is given in Table 1.

7. Dry matter production of the new materials

Research team: Richard Trethowan, Laura Espinosa, Amit Singh, Vivien Tan

7.1 Background

The new kikuyu genotypes were identified from a previous study conducted by Duncan Fraser, a PhD student. He screened materials for drought and salinity tolerance (Fraser, 2020). During this process, 13 genotypes with pasture potential were identified based on biomass production and habit. These 13 genotypes were then compared to the cultivars Whittet and Fulkerson at one location and selected candidate lines subsequently evaluated across locations.

7.2 Objectives

- To evaluate the dry matter production of the new genetic materials throughout the growing season compared to Whittet and the Fulkerson varieties

7.3 Materials & Methods

(i) Initial evaluation of kikuyu genotypes

A small plot experiment was established at Landsdowne near Camden. Plots were generated from single runners of 15 genotypes (13 candidate lines plus the two checks) in 1 m^2 plots in a randomised complete block design with 4 replicates on the 17th of January 2021. The runners were grown in pots in the glasshouse and later transferred to the field and 1 m^2 plots established (Figure 2). Plots were harvested for biomass using a mower throughout the growing period and the fresh and dry weights determined. NDVI was assessed on the same date prior to mowing.

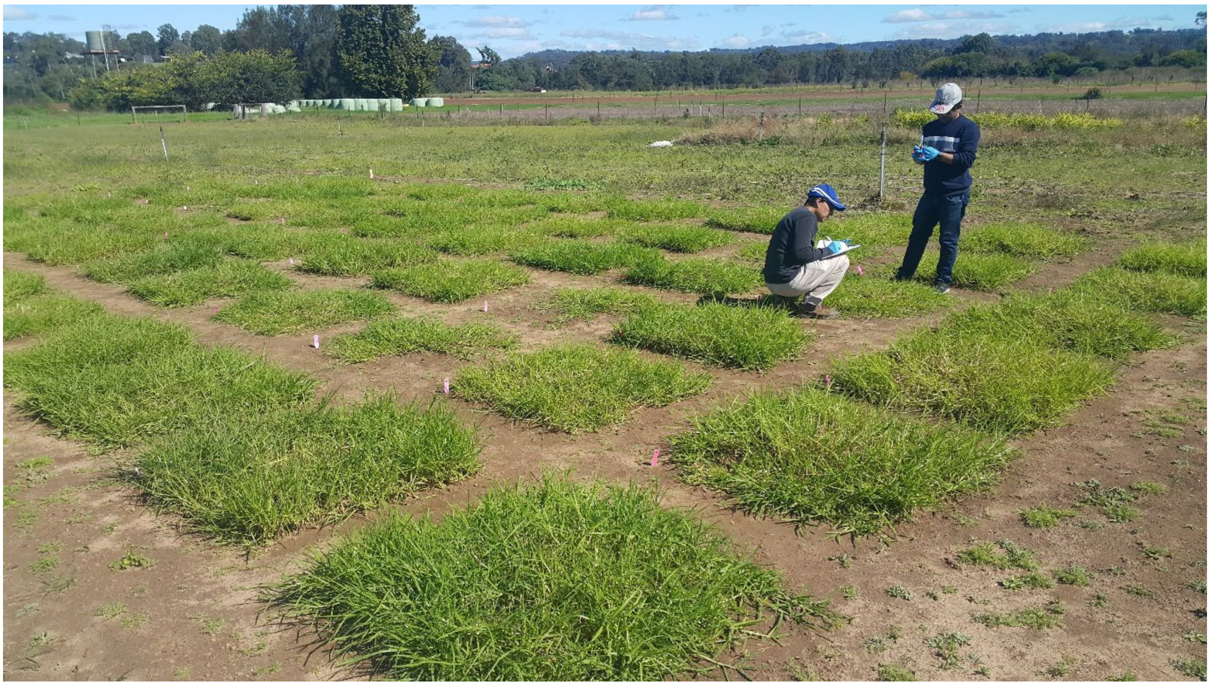

Figure 2. Small plot trial at Lansdowne in April 2022

(ii) Multi-environment strip trials of selected kikuyu genotypes

Based on small plot testing at Lansdowne in 2022-2023, three genotypes with potential were identified (L2, L8 and L10). On-farm strip trials were established of these three lines plus Whittet (L15) at three locations (Berry, Bega and Corstorphine near Camden) in the Eastern of NSW.

The strip trials were replicated and divided laterally using electric fencing to provide grazed and mowed treatments. Trials were established successfully (see Figure 3). Kikuyu biomass was harvested from the mowed treatment in each strip on a 1.5 m^2 area using a hand mower and dry matter estimated (see Figure 5). The sub-samples were subsequently analysed for nutritional traits using NIR and wet chemistry. The mowing and grazing treatments were conducted as close as possible to the same date. The trials were sampled at up to 30 different dates between November 2024 and May 2026. NDVI and a Plate Meter were used to capture estimates of greenness (chlorophyl) and biomass prior to cutting.

Figure 3. Cows grazing at Berry on the strip plot trial (photo: Laura Espinosa)

7.4 Key Findings

(i) Small plot experiment at Lansdowne

NDVI and dry matter was assessed across a 12-month period to identify lines with consistent dry matter production (Figure 4). The cumulative dry matter across the same period was also calculated (Figure 5). Three candidate lines, L2, L8 and L10 were selected for further testing in different environments with and without grazing pressure based on dry matter production at different dates of sampling and cumulative biomass production in a 12-month period.

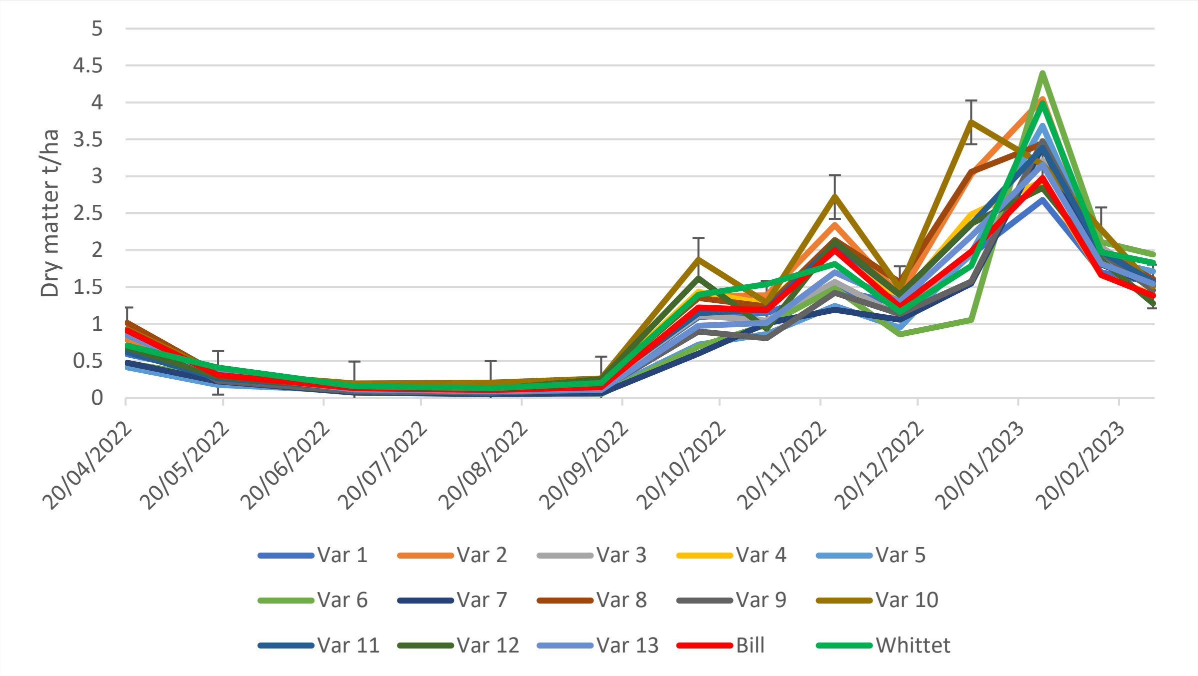

Figure 4. Dry matter production over time for 15 genotypes including Whittet and the Bill Fulkerson varieties

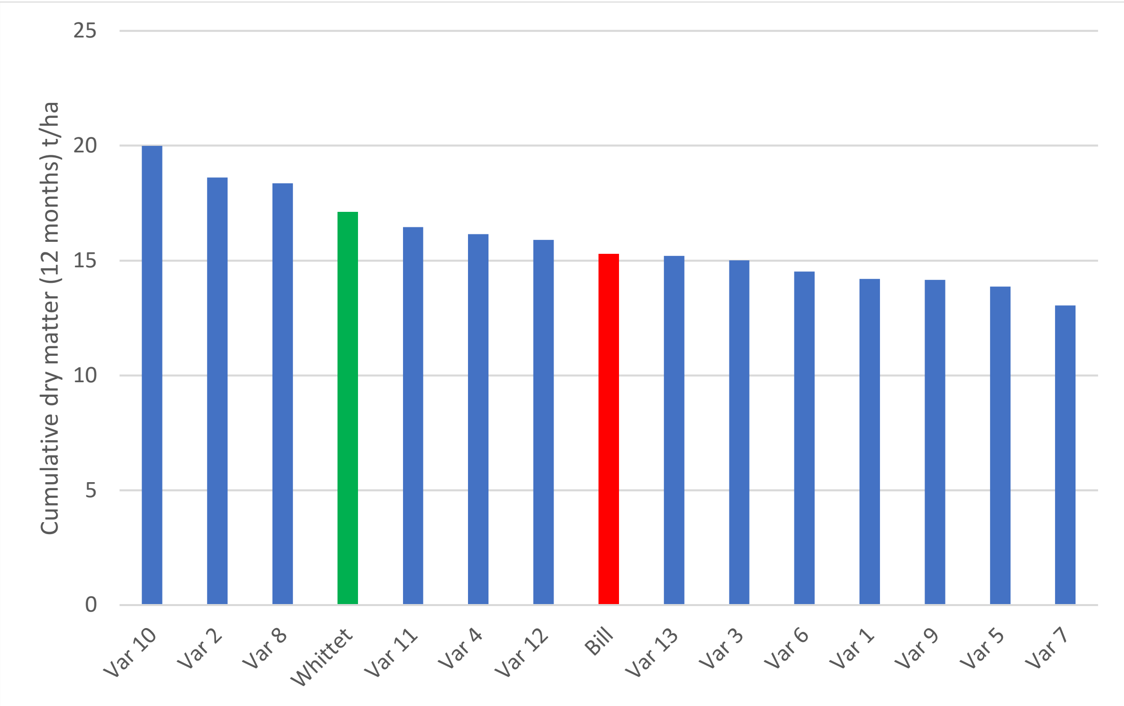

Figure 5. Cumulative dry matter production over time for 15 genotypes

(ii) Multi-environment strip trials of selected kikuyu genotypes

Based on small plot testing at Lansdowne in 2022-2023, three genotypes with potential were identified (L2, L8 and L10). These candidate lines and Whittet also referred as L15 were evaluated at three sites in strip trials across two years. The dry matter production over time at Corstorphine, Berry, and Bega are shown on Figure 6. (Kikuyu grew under irrigated condition in Corstorphine while conditions were rainfed in Berry and Bega). As expected, the dry matter production peaked in summer and declined into winter. The 4 genotypes followed a similar pattern at all three sites.

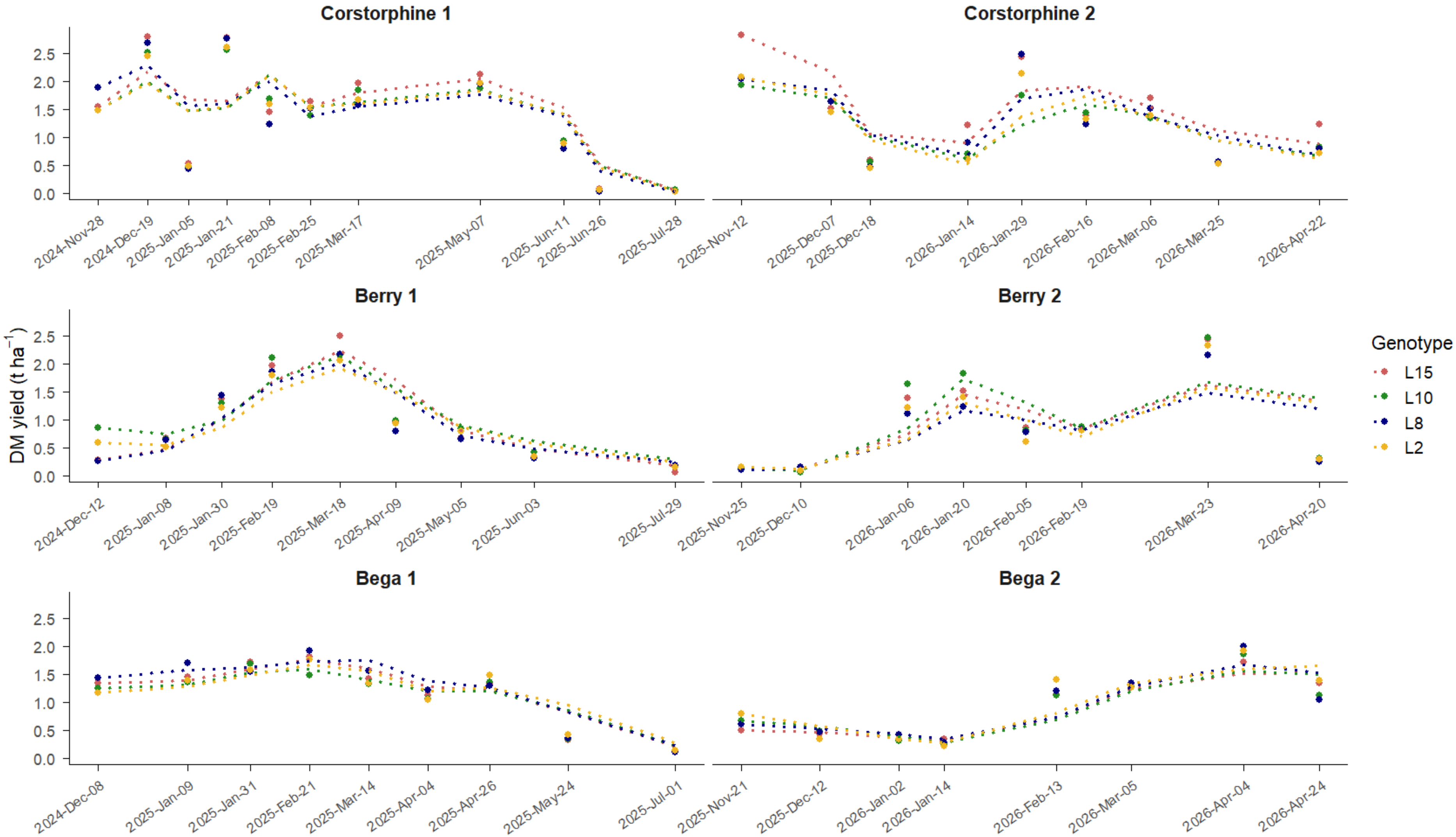

Figure 6. Dry matter production over two years (summer) at Corstorphine, Berry, and Bega. Points represent observed mean dry matter (DM) yields. Dotted lines represent the moving-average trend for each genotype within each summer season.

NDVI and the plate meter were assessed on each strip throughout the growing period in each year. No difference between treatments (moved and grazed) were observed. The plate meter was much more discriminating than NDVI for most effects (Table 2). For the plate meter, year, location and genotype main effects were highly significant, as were all two-way interactions with genotype (Table 2), indicating differences in genotype performance among environments. Whittet and L8 had the highest plate meter values across sampling dates, sites and years (Figure 7). Figure 8 shows the genotype responses at each site in each year with changes in ranking at each site over time.

Table 2. Probabilities from tests of fixed effects for NDVI and Plate Meter over years using date as a random term

	NDVI	**Plate Meter**
Fixed term	F pr	F pr
Year	<0.001	<0.001
Site	0.044	<0.001
Treatment	0.199	0.866
Genotype	0.296	<0.001
Year.FarmName	0.927	<0.001
Year.Treatment	0.305	0.235
FarmName.Treatment	0.01	<0.001
Year.Genotype	0.345	<0.001
FarmName.Genotype	0.193	<0.001
Treatment.Genotype	0.414	<0.001
Year.FarmName.Treatment	0.076	<0.001
Year.FarmName.Genotype	0.38	<0.001
Year.Treatment.Genotype	0.404	0.277
FarmName.Treatment.Genotype	0.345	0.015
Year.FarmName.Treatment.Genotype	0.393	<0.001

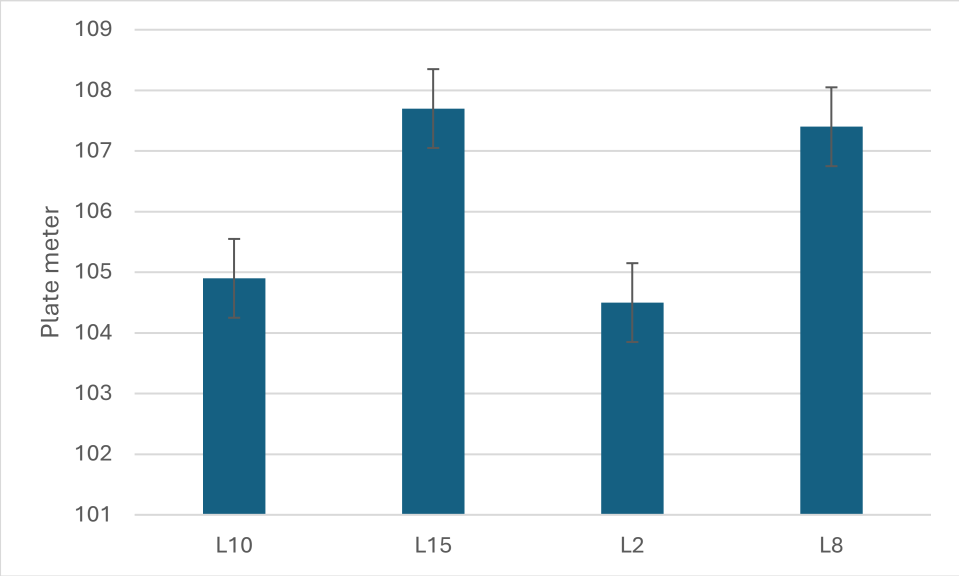

Figure 7. Plate meter results for each genotype across years, dates and locations

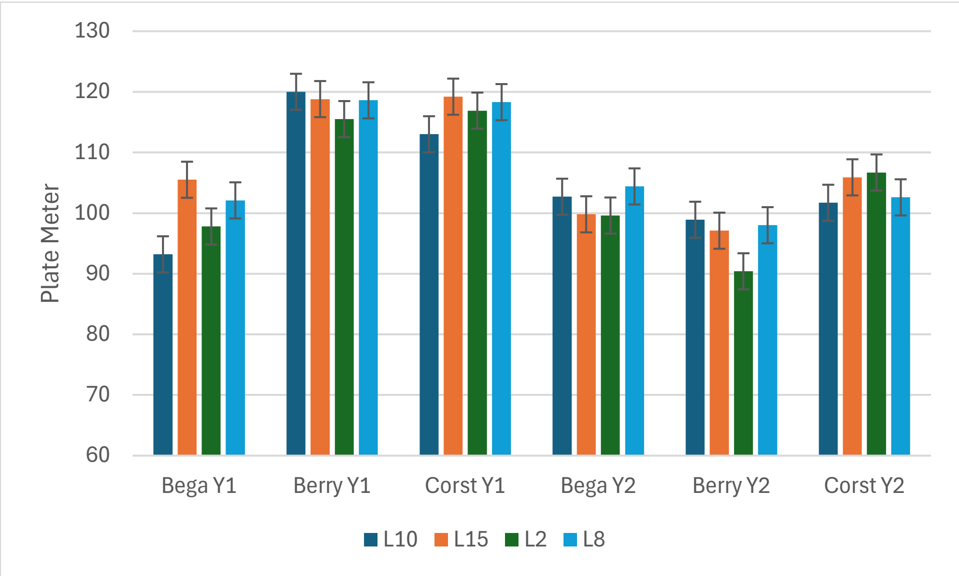

Figure 8. Plate meter results for each genotype in each year (Y1 and Y2) and location

Dry matter was assessed at regular intervals across both seasons using a lawn mower to collect fresh biomass that was later dried and weighed. No differences between seasons were observed, however, location and genotype main effects were significant as was the two-way interaction location x genotype (Table 3).

Table 3. Test of fixed effects for dry matter across years and locations using date as a random term

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Year	0.61	1	0.61	159	0.213
Location	14.58	2	7.29	159	0.013
Genotype	9.63	3	3.21	477	0.006
Year.Location	2.1	2	1.05	159	0.746
Year.Genotype	6.39	3	2.13	477	0.499
Location.Genotype	19.08	6	3.18	477	<0.001
Year.Location.Genotype	14.78	6	2.46	477	0.086

Mean dry matter across all sites, years and sampling dates showed that Whittet was in general was slightly higher than other genotypes (Figure 9).

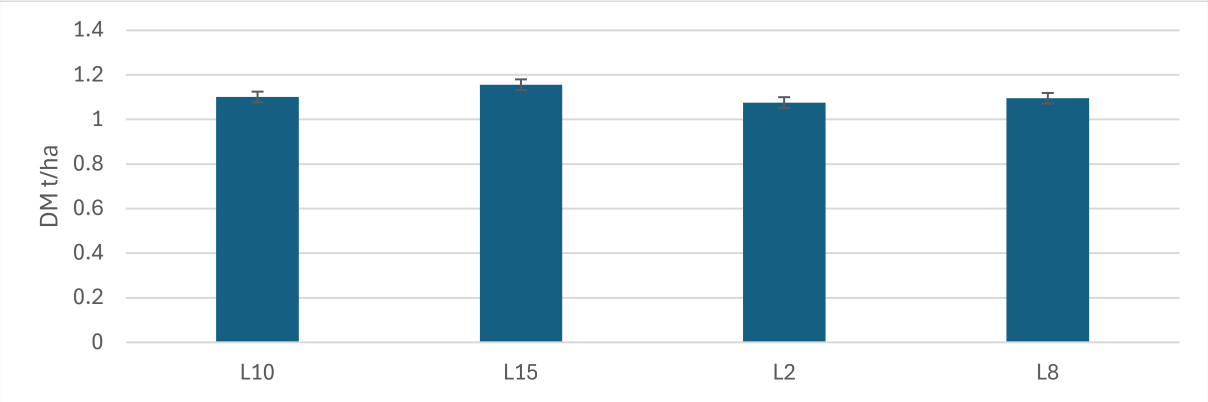

Figure 9. Mean dry matter for each genotype across years, site and dates

However, the slightly higher mean dry matter of Whittet was driven by exceptional biomass production and Corstophine in the second season (Figure 10).

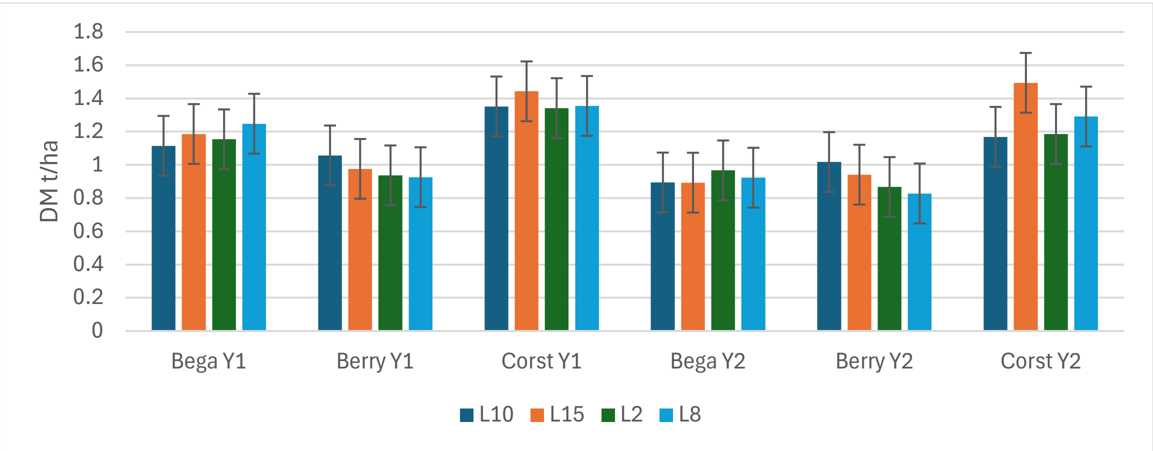

Figure 10. Mean dry matter for each genotype in each location and year

Cumulative dry matter across both seasons at each location was also calculated. There was a significant year and location main effect and genotype x location interaction (Table 4). However, the genotype main effect was not significant.

Table 4. Test of fixed effects for cumulative dry matter

Fixed term	Wald statistic	n.d.f.	F statistic	d.d.f.	F pr
Year	53.35	1	53.35	12	<0.001
Location	151.56	2	75.78	12	<0.001
Genotype	7.1	3	2.37	36	0.087
Year Location	5.94	2	2.97	12	0.089
Year.Genotype	0.98	3	0.33	36	0.806
Location.Genotype	22.26	6	3.71	36	0.006
Year.Location.Genotype	5.27	6	0.88	36	0.52

As genotype main effects were not significant, the cumulative dry matter production of each genotype in year and location was assessed (Figure 11). Once again, there was little difference among the genotypes at all sites in all years with the exception of Corstorphine in year 2, where Whittet produced significantly more dry matter across the season.

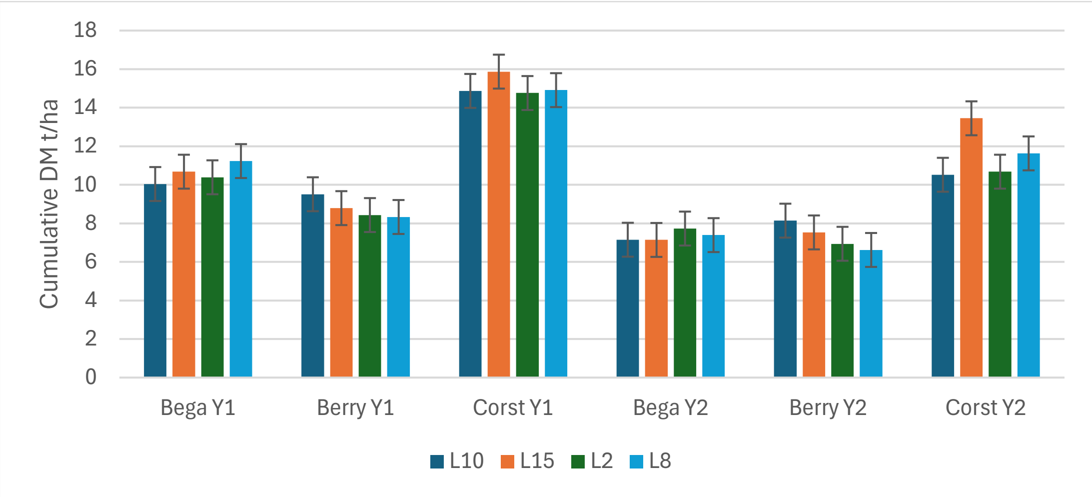

Figure 11. Cumulative dry matter for each genotype in each year and location

To examine the usefulness of NDVI and the plate meter as indirect assessments of dry matter, they were correlated across sampling dates for each genotype at each site (Table 13). The plate meter was best correlated with dry matter at Corstophine and Berry. However, the NDVI was also correlated at Corstophine. No significant associations were found in Bega, although the plate meter correlations were positive. These results suggest that the plate meter can be used to assess dry matter with a reasonable degree of accuracy.

Table 13. Correlation between dry matter and NDVI and the Plate meter at each location for each genotype across dates of sampling.

Site	**Genotype**		**NDVI**	**Plate Meter**
Corstophine	L10	Dry Matter	0.690**	0.676**
	Whittet	Dry Matter	0.467*	0.577**
	L2	Dry Matter	0.607**	0.643**
	L8	Dry Matter	0.585**	0.542*
	Mean	Dry Matter	0.65**	0.550*
Berry	L10	Dry Matter	-0.294 ns	0.804***
	Whittet	Dry Matter	0.427ns	0.777**
	L2	Dry Matter	0.451ns	0.781***
	L8	Dry Matter	0.435ns	0.819***
	Mean	Dry Matter	-0.84ns	0.801***
Bega	L10	Dry Matter	-0.004ns	0.25ns
	Whittet	Dry Matter	0.069ns	0.477ns
	L2	Dry Matter	0.056ns	0.397ns
	L8	Dry Matter	-0.028ns	0.376ns
	Mean	Dry Matter	0.0222ns	0.376ns

8. Nutritional value

Research team: R Trethowan, Laura Espinosa, Sergio Garcia, Rafiq Islam

8.1 Background

The new candidate materials have potentially competitive biomass and better disease resistance profiles compared to Whittet and the Fulkerson varieties. However, while they were screened earlier for drought and salinity tolerance by a PhD student (Duncan Fraser), nothing is known of their nutrition profile and suitability for feeding to dairy cattle.

8.2 Objectives

To evaluate the selected candidate lines for nutritional quality using biomass sampled at multiple sites

8.3 Materials & Methods

Dry matter sampled at each site over time was retained and ground for chemical analysis. The chemical analysis including NIR and physical testing using NDFD 24h was conducted by the Department of Primary Industries and Regional Development Feed Quality Service.

8.4 Key Findings

A preliminary chemical analysis of the original 15 lines was conducted using dry matter harvested per plot in 2022. The ranks of these lines for a series of nutritional traits are given in Table 5. Three candidate lines, L2, L8 and L10 were selected for further testing in different environments with and without grazing pressure using biomass, disease information and the preliminary chemical analysis. These three candidate lines and Whittet and Fulkerson were tested again in 2023 for nutritional traits (Table 6).

Table 5. Ranks of each genotype from the chemical analysis of the Lansdowne trial in 2022

RANK	DM	CDM	NDVI	ADF	CP	DMD	DOMD	ME	NDF	WSC
1	L 10	L 10	L 10	L12	L 10	L 12	L 12	L 12	L12	L 13
2	L 2	L 2	L 2	L2	L 12	Whittet	Whittet	Whittet	L11	L 12
3	L 8	L 8	L 8	L8	L 8	L 11	L 10	L 10	L8	L 9
4	Whittet	Whittet	L 11	L11	Whittet	L 8	L 11	L 11	L10	L 5
5	L 11	L 11	Whittet	L4	L 2	L 10	L 8	L 8	L13	L 1
6	L 4	L 4	L 4	Whittet	L 11	L 2	L 2	L 2	Whittet	L 2
7	L 12	L 12	L 12	L10	L 5	L 5	L 5	L 1	L5	L 3
8	Bill	Bill	L 3	L1	Bill	L 9	L 1	L 5	L4	L 11
9	L 13	L 13	L 13	L13	L 4	L 1	L 9	L 9	L2	L 10
10	L 3	L 3	L 1	L3	L 3	L 13	L 13	L 13	L1	L 7
11	L 6	L 6	Bill	L5	L 7	L 3	L 3	L 3	L3	Whittet
12	L 1	L 1	L 6	L7	L 6	Bill	Bill	Bill	L9	L 4
13	L 9	L 9	L 7	L9	L 13	L 4	L 4	L 4	L7	L 6
14	L 5	L 5	L 5	Bill	L 9	L 7	L 7	L 7	Bill	L 8
15	L 7	L 7	L 9	L6	L 1	L 6	L 6	L 6	L6	Bill

Note: DM, CDM, NDVI, adf, ash, cp, me, dmd, domd, me, ndf, wsc are dry matter, cumulative dry matter, NDVI, acid detergent fibre, ash content, crude protein, metabolizable energy, dry matter digestibility, dry organic matter digestibility, neutral detergent fibre, water soluble carbohydrates, respectively.

Of the three candidate lines, L8 and L10 both showed equivalent or slightly better chemical profiles than Whittet based on the NIR analysis (Table 6). All lines were superior to the Fulkerson variety with the exception of ash content.

Table 6. Significant traits from chemical analysis of candidate kikuyu lines and check cultivars from Lansdowne 2022 and 2023.

Variety	**adf**	**ash**	**cp**	**me**	**ndf**	**wsc**	**om**	**Av Rank**
L8	31.78 (2)	19.98 (5)	17.13 (2)	8.138 (2)	61.91 (1)	2.700 (4)	80.02 (1)	2.43
L10	31.99 (4)	21.03 (2)	17.30 (1)	8.096 (3)	62.02 (2)	3.338 (2)	78.97 (4)	2.57
Whittet	31.87 (3)	20.39 (4)	17.12 (3)	8.188 (1)	62.52 (3)	3.189 (3)	79.61 (2)	2.71
L2	31.68 (1)	20.54 (3)	16.72 (4)	7.981 (4)	62.92 (4)	3.419 (1)	79.46 (3)	2.86
Fulkerson	34.49 (5)	23.26 (1)	16.30 (5)	7.827 (5)	64.77 (5)	2.544 (5)	76.74 (5)	4.43
Std error	0.8544	1.242	0.5156	0.1297	0.9043	0.2416	1.072	

Note: the rank is given in brackets. adf, ash, cp, me, ndf, wsc, and om are acid detergent fibre, ash content, crude protein, metabolizable energy, neutral detergent fibre, water soluble carbohydrates and organic matter, respectively.

The three candidate lines plus Whittet were sown in multi-environment strip trials in 2024 and 2025 and dry matter retained from each plot and different sampling times. These materials were analysed for nutritional traits using NIR and the probabilities from the test of fixed effects are given in Table 7. Genotype main effects were all significant as were location effects with the exception of NDF. All two-way interactions were significant except for inorganic ash and water-soluble carbohydrates.

Chemical analysis of multi-environment trial samples

Table 7. Probabilities from tests of fixed effects for chemical traits assessed on the multi-environment trial in one year using NIR

	Trait (probability)								
Fixed term	**ADF**	**CP**	**DMD**	**DOMD**	**Inorganic ash**	**ME**	**NDF**	**OM**	**WSC**
Location	0.052	<0.001	<0.001	<0.001	<0.001	<0.001	0.071	<0.001	<0.001
Genotype	0.031	<0.001	0.006	0.006	0.016	0.006	<0.001	<0.001	0.002
Location.Genotype	0.032	0.004	0.017	0.012	0.382	0.012	0.008	0.004	0.24

Note: adf, ash, cp, me, ndf, wsc, and om are acid detergent fibre, ash content, crude protein, metabolizable energy, neutral detergent fibre, water soluble carbohydrates and organic matter, respectively

The means of each nutritional trait for each genotype are given on Table 8. Little difference was observed among genotypes with L8 showing highest WSC and Whittet lower NDF.

Table 8. Means of each genotype from the chemical analysis of multi-environment trial samples assessed using NIR

	Genotype				
Trait	L10	Whittet	L2	L8	Std error
ADF	23.85	23.40	23.95	23.54	0.1985
CP	20.29	21.26	20.09	20.47	0.2171
DMD	64.71	65.44	64.68	65.20	0.2482
DOMD	61.99	62.61	61.93	62.42	0.2190
Inorganic ash	10.39	10.60	10.36	10.42	0.0844
ME	9.58	9.71	9.57	9.67	0.0444
NDF	56.07	54.85	56.21	55.28	0.2944
OM	20.29	21.26	20.09	20.47	0.2170
WSC	4.64	4.54	4.67	4.92	0.1016

Note: adf, cp, dmd, domd, ash, me, ndf, wsc are acid detergent fibre, crude protein, dry matter digestibility, dry organic matter digestibility, ash content, metabolizable energy, neutral detergent fibre, organic matter, water soluble carbohydrates, respectively.

However, a subset of materials was select for wet chemistry analysis to confirm NIR observations. All genotype and location main effects were significant (Table 9). However, the two-way interactions were generally not significant indicating similar ranking at each environment.

Table 9. Probabilities from tests of fixed effects for chemical traits assessed on the multi-environment trial in one year using wet chemistry

	DMD	**dNDF_DM_24**	**dNDF_OM_24**	**DOMD_24**	**NDF_DM**	**NDF_OM**	**NDFD_24_DM**	**NDFD_24_OM**	**OMD_24**	**uNDF_DM_24**	**uNDF_OM_24**
Fixed term											
Location	0.001	0.019	0.016	0.009	0.003	0.002	0.007	0.006	0.003	0.001	<0.001
Genotype	0.02	0.02	0.023	0.008	0.059	0.08	0.025	0.022	0.022	0.018	0.023
Location xGenotype	0.052	0.077	0.096	0.011	0.104	0.135	0.103	0.1	0.03	0.111	0.09

The means from the physical analysis of samples is given in Table 10. This analysis showed that the candidate lines were superior to Whittet for most traits including DMD, DOMD, NDFD, OMD and NDF. Lines 2 and 8 in particular have a better nutritional profile than Whittet.

Table 10. Means of each genotype from the chemical analysis of multi-environment trial samples assessed using wet chemistry

	Genotype				
Trait	L10	L15	L2	L8	Std error
DMD	0.612	0.588	0.627	0.613	0.0117
dNDF_DM_24	30.03	27.66	30.08	29.19	0.8119
dNDF_OM_24	29.50	27.17	29.48	28.63	0.7996
DOMD_24	0.514	0.493	0.530	0.523	0.0101
NDF_DM	57.13	56.12	55.53	56.05	0.5642
NDF_OM	55.82	54.85	54.13	54.97	0.6197
NDFD_24_DM	0.526	0.494	0.542	0.523	0.0146
NDFD_24_OM	0.529	0.497	0.546	0.521	0.0149
OMD_24	0.577	0.554	0.596	0.581	0.0125
uNDF_DM_24	27.10	28.47	25.46	26.85	0.8748
uNDF_OM_24	26.32	27.68	24.65	26.34	0.9080

Note:

9. Disease resistance

Research team: Richard Trethowan, Percy Wong, Vivien Tan, Laura Espinosa

9.1 Background

The disease resistance of the new candidate materials was unknown beyond field observations during the PhD study of Duncan Fraser. The value of the new materials will only be understood if their disease reactions is known.

9.2 Objectives

To confirm the disease resistance of the new kikuyu materials under kikuyu yellows and black spot inoculations.

9.3 Materials & Methods

(i) Controlled environment testing

The three candidate lines plus Whittet and the Fulkerson variety were grown in pots under disease inoculation in controlled conditions to induce an epidemic. Black spot and Kikuyu Yellows diseases were assessed using standard inoculation procedures and the incidence and severity were scored.

(ii) Field evaluation

The candidate materials and the Whittet and the Fulkerson varieties were inoculated in the field on the small plot trial described in section 7. Incidence and severity were assessed as possible.

9.4 Key Findings

(i) Controlled environment testing

A. Black spot (under inoculation)

Scoring of each plot was done by manually counting the number of pustules on 10 randomly selected leaves in each pot. Lines 2, 8 and 10 showed significantly fewer symptoms compared to Whittet and Fulkerson (Figure 14).

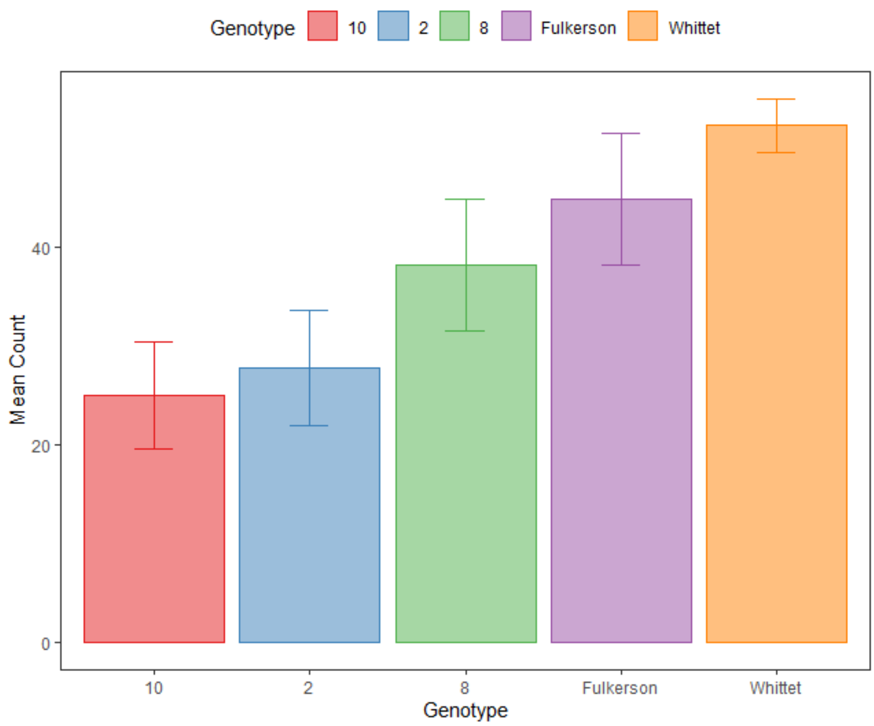

Figure 14. Blackspot disease expression on 5 genotypes

B. Kikuyu Yellows (under inoculation)

Although no significance difference between the 5 genotypes was noted for disease severity, there was a significant difference in disease progression. Line 2 had significantly slower disease progression compared to Whittet (Figure 9). The lack of differences in disease severity reflected the excessive level of infection achieved under controlled conditions. For this reason a field assessment was conducted in the 2025/26 summer.

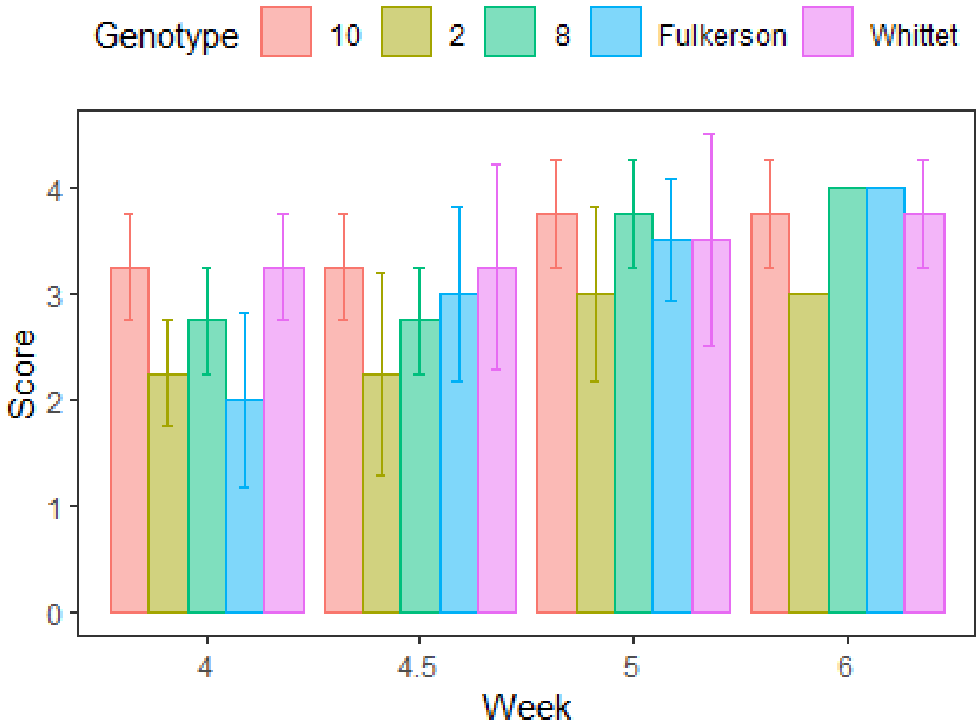

Figure 15. Kikuyu yellows expression on 5 genotypes

(ii) Field evaluation of kikuyu yellows disease at Lansdowne

Unfortunately, disease severity could not be assessed as the season cooled too quickly post the first incidence of infection. However, observations on first incidence in each replicate of the field experiment was possible. Whittet and L10 showed the first incidence of yellows disease around week 9, this was followed by L8 and L2 at week 11. The Fulkerson variety did not show incidence until week 14 when all four replicates recorded disease incidence. Based on these field results, it appears that Whittet and L10 are more susceptible than L2 and L8, which are less resistant than the Fulkerson variety. However, these are incidence only and severity still needs to be assessed.

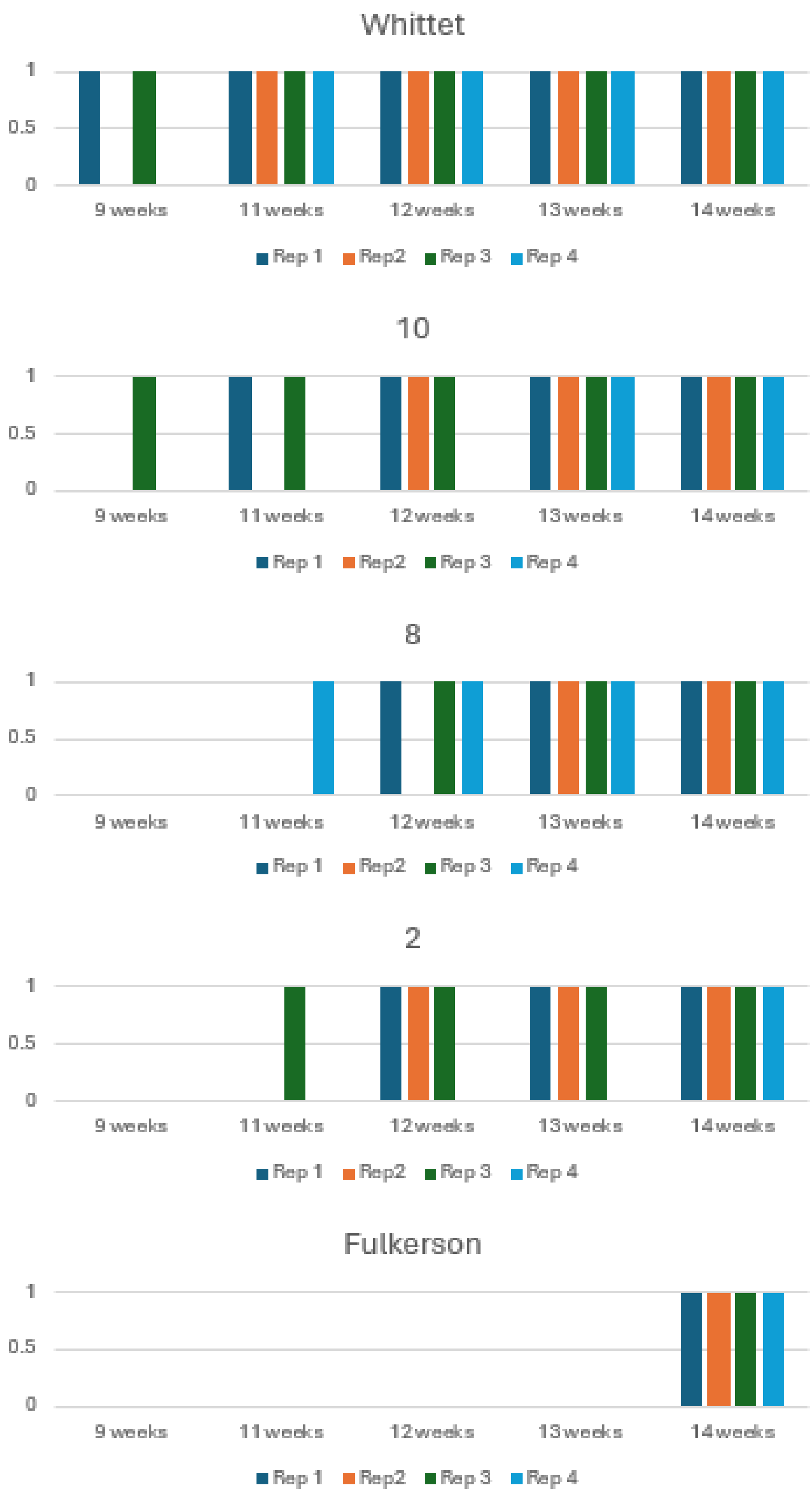

Figure 16. Boxplots of kikuyu yellows disease incidence in the field at Camden. 1 = positive and 0 = negative.

10. Key Outputs

- Results confirm the uniqueness of the new genetic materials compared to kikuyu pastures in NSW
- The diversity of kikuyu pastures across NSW was confirmed
- Two candidate lines with potential for release to dairy farmers based on dry matter production, disease resistance and nutritional profile identified

11. Conclusions and Recommendations

- Significant genetic diversity in kikuyu pastures was observed across the sampled sites in NSW with just 35% of sites clustering with Whittet and the Fulkerson line
- The new candidate kikuyu lines are genetically different and distinct from Whittet and the Fulkerson variety and generally different to most other pastures
- A relationship between kikuyu toxicity and kikuyu genotype was suggested with most instances occurring in Group 1.
- The genotypes did not differ significantly for dry matter at each date of sampling nor cumulative dry matter across two summer seasons.
- L2 and L8 have potential for release as new varieties based on genetic diversity, dry matter production (equivalent to Whittet), better black spot resistance (better than Whittet), resistance to yellows disease (better then Whittet and equivalent to Fulkerson) and superior chemical profile (better than both Whittet and Fulkerson).

Recommendations for future investment should prioritise:

- L2 and L8 should be evaluated further in the Hunter region and the NSW north coast
- The kikuyu yellow severity and incidence of the new lines need to be retested and confirmed
- The suggested link between kikuyu genotype and toxicity incidence should be investigated