

Phenotypic and genotypic variation within populations of kikuyu (*Pennisetum clandestinum*) in Australia

K.F. LOWE¹, T.M. BOWDLER¹, K. SINCLAIR², T.A. HOLTON³ AND S.J. SKABO³

¹Queensland Primary Industries and Fisheries, Mutdapilly Research Station, Peak Crossing, Queensland, Australia

²Industry & Investment, Wollongbar Primary Industries Institute, Bruxner Highway, Wollongbar, New South Wales, Australia

³Queensland Primary Industries and Fisheries, University of Queensland, Queensland, Australia

Abstract

Experiments at 2 sites in subtropical eastern Australia investigated the variation in agronomic attributes, quality and genetic structure existing within: naturally-occurring populations of kikuyu (*Pennisetum clandestinum*) from within Australia; selections produced from the treatment of Whittet seed with mutagenic chemicals; and available cultivars. Runners were collected from coastal areas extending from Western Australia to the Atherton Tableland in north Queensland. One experiment evaluated 10 mutagenic selections and 4 cultivars in a lattice design and the other evaluated 12 ecotypes and 3 cultivars in a randomised block design. The experimental unit was single plants, which were sown on a 1.5 m grid into a weed-free seed-bed (Mutdapilly) or a killed kikuyu stand (Wollongbar), both of which were kept clear of weeds and other kikuyu plants for the duration of the experiments. Foliage height, forage production and runner yield were assessed. Leaf material was analysed for concentrations of crude protein (CP), acid detergent fibre (ADF) and neutral detergent fibre (NDF) and for *in vitro* dry matter digestibility (IVDDM) in autumn, winter and spring. DNA was extracted from each plant in the ecotype comparison and

subjected to a modified DAF (DNA amplification fingerprinting) analysis to determine the level of genetic relatedness.

In the first experiment, none of the mutagenic lines derived from Whittet yielded significantly more or was more digestible than commercial Whittet material, although some selections were superior to the other commercial kikuyu cultivars, Noonan and Crofts, and 'common' kikuyu. However, there were significant differences in plant height and runner expansion. In the second experiment, significant differences in plant height, foliage yield, runner development, and leaf CP, ADF, NDF and IVDDM concentrations were demonstrated between the ecotypes, mutagenic selections and cultivars. There was a 4- to 6-fold difference in plant yield and a 6- to 10-fold difference in runner production between the ecotypes at the 2 sites. Quality of the leaf ranged from 200 to 270 g/kg (CP), from 700 to 770 g/kg (IVDDM), from 170 to 250 g/kg (ADF) and from 470 to 550 g/kg (NDF). Improvements in quality and agronomic attributes were not mutually exclusive.

Genetic fingerprint analysis of the kikuyu lines indicated that they formed 2 broad groupings. Most of the regional ecotypes were grouped with 'common' kikuyu as represented by the material collected from Wollongbar, and the Beechmont, Atherton Tableland and Gympie ecotypes were grouped with the registered cultivars Whittet, Noonan and Crofts. Two lines produced by mutagenesis from Whittet remained closely linked to Whittet. These results suggest that there was variation between populations of kikuyu in yield, quality and genetic diversity but that mutagenesis by treating seed with sodium azide and diethylene sulphide did not achieve a significant change in the digestibility of leaf over cv. Whittet.

Correspondence: Kevin F. Lowe, Queensland Primary Industries and Fisheries, Department of Employment, Economic Development and Innovation, Mutdapilly Research Station, MS 825, Peak Crossing, Qld 4306, Australia. E-mail: kevin.lowe@deedi.qld.gov.au

Introduction

The quality of tropical grasses is a major limitation to animal production in tropical and subtropical areas and this is primarily associated with the lower digestibility of these species relative to temperate species (Minson and McLeod 1970). The C₄ grasses have high fibre levels and a major improvement would require a reduction in lignin with a concomitant increase in the digestion of the neutral detergent fibre content of these plants (Clark and Wilson 1993). As the main advantage of tropical grasses lies in their ability to utilise the higher temperatures and light intensities of tropical environments to achieve high growth rates, any improvement in quality would need to be achieved without seriously affecting this ability (Clark and Wilson 1993). An earlier attempt to increase the quality of the tropical grass *Digitaria milanjiana* (Hacker 1986) resulted in a grass with more leaf, higher digestibility (at least in cutting studies) (Minson and Hacker 1986) and higher milk production. However, the grass did not persist under good grazing management in a subtropical environment (Lowe *et al.* 1991).

Kikuyu (*Pennisetum clandestinum*) is an important grass for the dairy and beef industries of the subtropics of Australia, South Africa and New Zealand (Mears 1970). Any improvement in quality of kikuyu in Australia will need to come from the material currently available on farms, as no accessions of kikuyu are held at the Australian Tropical Resource Centre (P. Lawrence, personal communication). Luckett *et al.* (1996) used mutagenesis to increase the variation within cv. Whittet, from which a potential new cultivar has been selected with tolerance to kikuyu yellows (*Verrucalvus flavofaciens*) (K. Sinclair, unpublished data).

The experiments discussed in this paper investigated the variation existing within natural populations selected from diverse regions within Australia, between cultivars and from within the mutagenic population produced in a previous project (Luckett *et al.* 1996). Foliage and runner yields, plus other physical and quality attributes of this material, were evaluated to establish if sufficient variation existed to sustain a breeding program for improved digestibility. Ecotypes were fingerprinted to establish the relatedness of kikuyu from different regions of Australia.

Materials and methods

Experiment 1

Single tillers of randomly selected plants from kikuyu stands within treatments in 2 previous experiments (Lowe *et al.* 2002) were established in 10 cm diameter plastic pots in November 2002. These plants were elite selections of kikuyu produced from seed treated with the mutagenic chemicals, sodium azide and diethylene sulphate (Luckett *et al.* 1996). Cultivar A and Cultivar B were selections from this material, which were earmarked for possible release. Common kikuyu was selected from material growing at Gatton Research Station (27° 34' S, 152° 20' E; elevation 95 masl) in subcoastal south-east Queensland. All plants were sown into a weed-free seed-bed on a Hypocalcic, Subnatric, Brown Sodosol (Isbell 1996) at Mutdapilly Research Station (27° 45' S, 152° 40' E; elevation 70 masl) in south-east Queensland on a 1.5 m grid in March 2003. The experiment was laid out as a lattice square with 6 replications. A mixed fertiliser (CK 88®; 150 g/kg N, 44 g/kg P, 115 g/kg K and 135 g/kg S) was applied at the base of each plant, at a rate equivalent to 500 kg/ha to adequately meet the high fertility requirement of kikuyu. Subsequently, urea was applied bi-monthly at 100 kg/ha N over the whole experimental area. The experimental plots were irrigated using Ezi-shift spray equipment to compensate for evapotranspiration losses.

Experiment 2

Runners from 14 kikuyu selections were collected between September and November 2004 by project staff or local agronomists from areas where kikuyu had been grown for over 40 years (Table 1). Areas known to have been sown to seeding cultivars were avoided, except where deliberately sampled at specific sites (*i.e.*, Noonan and Crofts). Runners selected from individual plants were cut into pieces (70 mm) with at least 2 nodes. These pieces were immediately planted into black polyvinyl bags (100 mm x 230 mm) containing a potting mixture composed of 50% hardwood sawdust, 30% composted pinebark fines and 20% coarse river sand, with micro and trace elements in a glasshouse at Wollongbar Primary Industries Institute (28° 50' S, 153° 25' E; elevation 140 masl). Only Whittet

and Noonan were established by seed. All were maintained in these polybags until required for sowing on January 19 (Muttapilly) and February 22 (Wollongbar), 2005. These plants were sown into a fully cultivated seed-bed at Muttapilly and into a sward of 'common' kikuyu pasture, which had been killed with an application of 2 L/ha of Roundup® (360 g/L active ingredient, glyphosate) at Wollongbar.

Entries were established as single-spaced plants on a 1.5 m grid as a randomised block with 3 replicates. The soil type at Muttapilly was a Hypocalcic, subnatric, brown sodosol, while that at Wollongbar was a Red Ferrosol (Isbell 1996). The experimental areas were irrigated every 3–5 days to ensure successful establishment. Irrigation to supplement rainfall continued throughout the measurement period (January–September 2005). Both mechanical and chemical weed control were required at Muttapilly. The residue from the previous kikuyu sward controlled weed invasion at Wollongbar and no regrowth of the existing kikuyu plants from this sward was recorded during the experimental period. A mixed fertiliser (CK 88®) was applied at a rate equivalent to 300 kg/ha to build up the fertility of the Muttapilly site. Urea was applied at 100 kg/ha N around the base of the plants initially and then as a 2-monthly application over the whole area. At Wollongbar, urea was applied at 100 kg/ha N after each harvest with superphosphate at 250 kg/ha and muriate of potash at 100 kg/ha applied as split dressings.

Measurements

In both experiments, all plants were defoliated to 5 cm using hand shears at monthly intervals, after an establishment period of 8 weeks. Dry matter (DM) yields were determined by drying harvested material at 60°C for 48 h. This contained both leaf and stem and is presented as total foliage yields. The ability of kikuyu to spread rapidly during the growing season made it necessary to prune runners back to a 'core mat' of the main plant, approximately 30 cm in diameter, to avoid plants coalescing. At Muttapilly only, plants were pruned back to the 'core mat' at the end of autumn and again at the end of winter to assess each plant's potential to colonise surrounding bare ground. This material had any leaves, roots and attached soil removed and was

recorded as 'Runner mass'. Other measurements at Muttapilly included leaf:stem ratio (by sorting the cut material into leaf lamina and stem), runner length (distance from plant centre to the tip of the longest runner) and foliage height.

Quality attributes of leaf were assessed in autumn, spring and late summer (on 2 replicates only in Experiment 1) and autumn, winter and spring (3 replicates in Experiment 2 at both sites). Leaf material was subjected to Near Infrared Reflectance Spectroscopy (NIR006) analyses for *in vitro* dry matter digestibility (IVDDM) and crude protein (CP), neutral detergent fibre (NDF) and acid detergent fibre (ADF) concentrations. Calibrations were previously derived according to procedures outlined by Smith and Flinn (1991). The reference methods used for NIR calibration were the Flinn and Saul (1983) method for CP, ADF and NDF, and a pepsin-cellulase technique (Clarke *et al.* 1982) for IVDDM. Analytical values were first adjusted using a linear regression based on similar samples of known *in vivo* DDM and checked by analysing a small number of samples using reference methods and comparing NIR and reference values (Callow *et al.* 2003).

DNA was extracted from leaf samples of the different genotypes in Experiment 2 (Muttapilly site only). Genetic fingerprints of each kikuyu cultivar and ecotype were determined using a modified DAF analysis (Caetano-Anolles *et al.* 1991). Duplicate DNA samples were amplified using 4 different oligonucleotide primers and fragment sizes determined by denaturing polyacrylamide gel electrophoresis. The oligonucleotide sequences of the DAF primers used in this study were:

I-08:	5'-TTTGCCCGGT-3'
V-15:	5'-CAGTGCCCGGT-3'
AE-11:	5'-AAGACCGGGA-3'
BB-18:	5'-CAACCGGTCT-3'

Statistical analyses

Simple ANOVA analyses were used to determine significant differences between treatments (Payne *et al.* 2007) for both experiments. There were no improvements in analyses by data transformation. For DNA, DAF profiles were analysed by PHYLIP (Felsenstein 2005) to determine the genetic relatedness of each of the individuals.

Results

Agronomic traits

Experiment 1. Over the study period, total foliage yield ranged from 422 g/plant (WK 9) to 237 g/plant (common) ($P < 0.05$) (Table 1). WK 12, Cultivar A, WK 85 and Whittet produced similar ($P > 0.05$) yields to WK 9, while the cultivars, Crofts and Noonan, and the other mutagenic lines, Cultivar B, WK 31, WK 39, WK 42, WK 46 and WK 64, produced significantly lower ($P < 0.05$) yields than WK 9. There appeared to be seasonal growth differences between the entries, with Crofts giving lower yields ($P < 0.05$) in autumn but higher yields in winter than the rest of the entries (data not presented).

There was a range of growth habits in the high-yielding selections, with both prostrate and erect types evident in the mutagenic lines, as indicated by height at the initial defoliation (Table 1). Cultivar A and WK 42 appeared to develop the greatest number of runners extending from the central biomass of the plant early in plant development (data not presented) and this is reflected in the total yield of runners recorded. WK 42, WK 12 and Cultivar A were significantly ($P < 0.05$) different from common kikuyu, which produced the lowest yield of runners (Table 1).

The harvested foliage was separated into leaf and stem on 3 occasions, April 29 and July 1, 2003 and March 16, 2004. Significant differences in leaf:stem ratios (data not presented) existed between lines and ratios varied with season, with site means of 1.8 in autumn and winter and 1.0 in summer. Average leaf content varied from 51% to 61% ($P > 0.05$) (Table 1).

Experiment 2 (Mutdapilly site). Plant height varied from 12.2 cm (Crofts) to 24.6 cm (Whittet) ($P < 0.05$) (Table 2). The most erect plants were from the Numinbah Valley ecotype and Whittet, while the most prostrate ones came from Crofts, and from the Bairnsdale, Bega, Mt Mee and Atherton Tableland ecotypes. The difference in height between these two groups was significant ($P < 0.05$).

No differences in leafiness ($P > 0.05$) could be detected between phenotypes. The leaf:stem ratio was extremely variable and no significant differences were detected at any of the samplings (data not presented).

Total foliage yield varied from 252 g/plant (Whittet) to 60 g/plant (common from Mt Mee) ($P < 0.05$). The ecotypes from Numinbah Valley, Gympie and Wollongbar, Cultivar A and both Noonan sources were not different from Whittet (Table 2). Lowest yielding, apart from the South Australian ecotype, which survived only one harvest, were ecotypes collected from Bairnsdale, Bega, Crofts, Mt Mee and the Atherton Tableland.

Total runner production did not differ significantly ($P > 0.05$) between ecotypes, despite mean yields ranging from 600 to 2000 g/plant (Table 2). At the first cut in February, the Numinbah Valley ecotype produced the longest ($P < 0.05$) runners and the highest runner mass (data not presented). While this trend continued in later samplings, differences were not significant. Cultivar A and the ecotypes from Bairnsdale, the Atherton Tableland and Beechmont displayed poor runner production.

Experiment 2 (Wollongbar site). Overall, growth at Wollongbar was much lower than at Mutdapilly. Whittet produced the highest yields (51.7 g/plant; $P < 0.05$) (Table 3), while ecotypes from South Australia, Western Australia, Mt Mee and Numinbah Valley and cv. Noonan established from seed produced the lowest yields (3.2–13.2 g/plant). There were no significant ($P > 0.05$) differences in leafiness between entries (59–70%; Table 3) or in leaf:stem ratios (data not presented), although Cultivar B had the highest ratio of 2.7, averaged over the 3 harvests. While there were few significant differences in runner production between most of the common ecotypes, Whittet produced the greatest weight ($P < 0.05$) of runners, almost 5 times that of the Mt Mee ecotype (Table 3).

Forage quality

Experiment 1. Leaf crude protein concentration in autumn varied from 324 to 279 g/kg, with few significant differences between ecotypes or cultivars (Table 1). With the differences in DM yields between ecotypes, yield of crude protein per plant varied significantly (9.3–1.1 g/plant; $P < 0.05$). ADF concentration in autumn varied from 285 to 257 g/kg with no significant differences, and ADF yields tended to parallel DM

Table 1. Plant height and runner number at the first harvest, total foliage and runner yields over a 12-month period and concentrations and yields of crude protein (CP), acid detergent fibre (ADF) and *in vitro* digestible dry matter (IVDDM) of the leaf blade of 4 cultivars and 13 mutagenically treated selections of kikuyu over the period March 2003 - March 2004 at Muidapilly, south-east Queensland (Experiment 1). Only significant quality responses presented.

Ecotype	Harvest 1			Total runner yield (g/plant)	Autumn 03		Summer 04			
	Runners/plant (no.)	Plant height (cm)	Total foliage yield (g/plant)		Av. leaf content (%)	CP		IVDDM		
						Conc. (g/kg)	Yield (g/plant)	Conc. (g/kg)	Yield (g/plant)	
Common	14.5	16	237	51.2	264	324	1.1	257	727	37.1
Crofts	14.0	26	343	60.0	360	304	2.7	276	725	26.6
Noonan	13.7	25	321	60.9	353	305	5.6	264	724	40.5
Whittet	13.0	23	362	57.9	342	298	4.4	269	728	45.9
Cultivar A	19.5	28	387	58.6	458	311	7.4	268	728	46.8
Cultivar B	13.3	33	333	58.1	309	279	8.1	267	727	32.1
WK 9	12.7	22	422	59.5	392	288	8.2	265	716	43.2
WK 12	16.2	34	407	56.1	504	305	8.4	285	718	52.9
WK 31	11.2	24	342	60.1	337	317	2.5	270	708	43.8
WK 39	15.8	27	339	53.2	429	301	4.3	260	720	30.0
WK 42	18.3	27	331	57.1	596	305	7.3	276	730	35.2
WK 46	16.3	26	333	57.1	393	312	5.1	261	727	27.8
WK 64	11.5	19	270	55.1	371	319	2.7	283	729	35.2
WK 85	14.7	24	372	55.1	314	291	9.3	274	724	41.5
LSD (P=0.05)	5.2	5	64.9	NS	153	21	3.3	24.3	14	15.4

Table 2. Plant height, total foliage and runner DM yields and average leaf content of 16 regional kikuyu ecotypes or cultivars grown as spaced plants at Mutdapilly in south-east Queensland over a 9-month period (January – September 2005) (Experiment 2). Flaxley ecotype from South Australia did not establish at this site.

Ecotype	Site	Cultivar	Plant height (cm)	Total foliage yield (g/plant)	Av. leaf content (%)	Total runner yield (g/plant)
Western Australia	Vasse	common	18.8	141	71.1	1177
Victoria	Bairnsdale	common	14.9	111	74.0	881
Victoria	Melbourne	common	17.7	111	72.4	1260
Southern NSW	Bega	common	16.3	109	72.5	1937
Central NSW	Bonalbo	Crofts	12.2	88	69.4	997
Central NSW	Bonalbo	Noonan	20.7	177	73.5	1173
Northern NSW	Wollongbar	common	21.8	173	69.7	1340
Northern NSW	Wollongbar	Cultivar A	21.3	171	73.0	831
Northern NSW	Wollongbar	Cultivar B	20.3	131	75.8	1338
Northern NSW	Wollongbar	Noonan	17.9	159	69.3	1401
Wollongbar	Wollongbar	Whittet	24.6	252	72.7	1795
SE Queensland	Numinbah Valley	common	23.8	235	61.6	1998
SE Queensland	Beechmont	common	18.3	111	77.2	952
SE Queensland	Mt Mee	common	15.3	60	76.4	1316
SE Queensland	Gympie	common	19.2	156	70.8	1195
North Queensland	Atherton Tableland	common	14.8	87	79.0	578
LSD (P=0.05)			6.7	108.5	NS	NS

Table 3. Total foliage and runner DM yields and average leaf content of 15 regional kikuyu ecotypes and cultivars grown as spaced plants at Wollongbar in northern NSW over an 8-month period (February – September 2005) (Experiment 2). There was insufficient material to establish the Bega and Melbourne ecotypes at this site.

Ecotype	Site	Cultivar	Total foliage yield (g/plant)	Av. leaf content	Total runner yield
Western Australia	Vasse	common	12.9	70.4	231
South Australia	Flaxley	common	3.2	-	-
Victoria	Bairnsdale	common	25.4	66.0	425
Central NSW	Bonalbo	Crofts	24.2	66.8	346
Central NSW	Bonalbo	Noonan	19.5	62.1	428
Northern NSW	Wollongbar	common	24.9	66.9	192
Northern NSW	Wollongbar	Cultivar A	19.3	63.2	161
Northern NSW	Wollongbar	Cultivar B	30.8	70.3	219
Northern NSW	Wollongbar	Noonan	14.1	59.2	375
Wollongbar	Wollongbar	Whittet	51.7	69.3	518
SE Queensland	Numinbah Valley	common	13.2	60.6	181
SE Queensland	Beechmont	common	23.5	67.5	262
SE Queensland	Mt Mee	common	8.8	62.3	112
SE Queensland	Gympie	common	22.1	62.2	231
North Queensland	Atherton Tableland	common	20.7	70.2	342
LSD (P=0.05)			13.6	NS	211

yields. All ecotypes were highly digestible in summer (708–730 g/kg).

Experiment 2 (Mutdapilly site). Crude protein concentration was high in all ecotypes and cultivars in all seasons (255–211 g/kg in autumn; 292–255 g/kg in winter; 273–231 g/kg in spring) (Table 4). Some significant differences ($P < 0.05$) between ecotypes occurred in each season.

IVDDM was high in both autumn (768–696 g/kg) and winter (769–729 g/kg) with some significant differences between ecotypes in each season. In autumn, ADF concentration varied from 246 to 181 g/kg and from 264 to 210 g/kg in winter, with some differences between ecotypes. Similarly, NDF concentration varied from 500 to 454 g/kg in winter and from 507 to 445 g/kg in

Table 4. Seasonal crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and *in vitro* digestible dry matter (IVDDM) concentrations and yields of ecotypes and cultivars of kikuyu grown as spaced plants at Mutdapilly between January and September 2005 (Experiment 2). Only significant quality responses presented.

Ecotype	Autumn				Winter				Spring			
	CP		IVDDM		ADF		NDF		CP		NDF	
	Conc. (g/kg)	Yield (g/plant)	Conc. (g/kg)	Yield (g/plant)	Conc. (g/kg)	Yield (g/plant)	Conc. (g/kg)	Yield (g/plant)	Conc. (g/kg)	Yield (g/plant)	Conc. (g/kg)	Yield (g/plant)
Vasse	226	2.5	760	8.4	201	8.4	245	483	255	744	239	8.4
Bairnsdale	224	2.0	741	6.1	214	6.1	253	493	263	729	231	8.6
Melbourne	211	0.5	696	1.5	246	1.5	247	454	264	756	273	4.0
Bega	251	2.5	768	7.0	208	7.0	237	470	278	748	251	11.4
Croftis	255	2.1	751	6.2	183	6.2	215	457	292	762	269	5.6
Noonan (Bernalbo)	246	4.3	731	13.1	233	13.1	220	472	285	766	255	11.3
Wollongbar common	230	4.4	760	14.2	197	14.2	232	462	267	753	244	10.6
Cultivar A	229	9.8	763	32.2	196	32.2	210	455	281	767	245	8.9
Cultivar B	246	2.7	765	8.3	181	8.3	240	460	276	749	260	10.2
Noonan (seed)	238	3.4	750	10.9	193	10.9	227	456	276	758	250	10.4
Whittet	241	6.9	768	21.6	200	21.6	228	464	275	769	249	14.7
Numinbah Valley	232	7.1	756	23.2	201	23.2	245	500	265	747	243	10.5
Beechmont	216	2.6	742	8.9	214	8.9	250	485	257	738	238	5.8
Mt Mee	231	2.2	748	7.2	210	7.2	264	479	257	738	239	6.3
Gympie	241	3.7	734	11.3	224	11.3	219	486	280	761	258	9.6
Atherton Tableland	243	1.7	760	5.6	182	5.6	215	480	273	735	262	6.7
LSD (P=0.05)	22	3.7	26	11.7	32	11.7	17	26	21	25	22	5.6

Table 5. Seasonal crude protein (CP), acid detergent fibre (ADF), neutral detergent fibre (NDF) and *in vitro* digestible dry matter (IVDDM) concentrations and yields of ecotypes and cultivars of kikuyu grown as spaced plants at Wollongbar between February and September 2005 (Experiment 2). Only significant quality responses presented.

Ecotype	Autumn			Winter				
	CP	NDF	ADF	CP		IVDDM		ADF
	Conc.	Conc.	Conc.	Conc.	Yield	Conc.	Yield	Conc.
	(g/kg)	(g/kg)	(g/kg)	(g/kg)	(g/plant)	(g/kg)	(g/plant)	(g/kg)
Vasse	243	483	211	230	0.64	737	2.06	225
Flaxley	233	477	209	258	0.25	754	0.76	181
Bairnsdale	211	489	213	223	1.04	737	3.42	223
Crofts	241	466	212	234	0.99	748	3.17	213
Noonan (Benalbo)	250	454	193	249	0.73	751	2.20	200
Wollongbar common	226	472	200	229	0.93	741	3.03	226
Cultivar A	234	472	199	247	0.74	751	2.17	197
Cultivar B	247	446	203	246	1.03	749	3.16	206
Noonan (seed)	240	466	212	253	0.68	757	2.04	194
Whittet (seed)	230	496	194	251	1.22	767	3.71	195
Numinbah Valley	226	486	224	233	0.58	742	1.86	220
Beechmont	241	471	179	245	1.20	755	3.13	206
Mt Mee	205	513	254	210	0.68	729	2.89	230
Gympie	238	461	216	234	1.12	746	3.60	203
Atherton Tableland	209	505	219	249	0.95	756	2.89	201
LSD (P=0.05)	24	39	33	25	0.52	18	1.61	20

spring, with some significant differences between ecotypes.

Experiment 2 (Wollongbar site). As at Mutdapilly, crude protein concentrations were high in all ecotypes in both seasons (250–205 g/kg in autumn; 258–210 g/kg in winter) (Table 5) with some significant ecotype differences. NDF concentration varied between ecotypes in autumn (513–446 g/kg), while ADF concentration also varied (254–179 g/kg in autumn; 230–181 g/kg in winter). IVDDM concentration in winter was high in all ecotypes (767–729 g/kg) with some significant differences.

Genetic fingerprinting

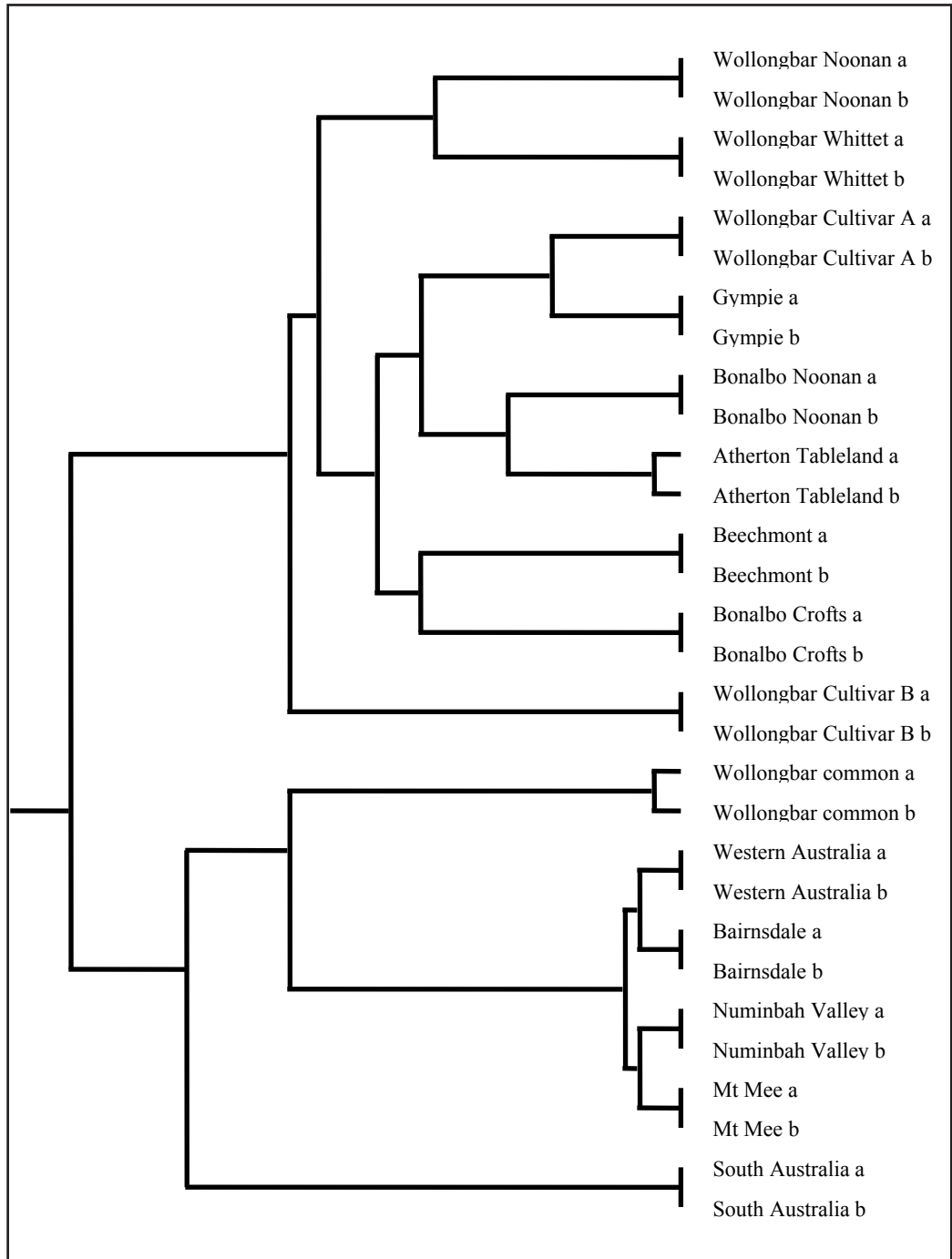
DAF profiling using 4 different oligonucleotide decamer primers identified 40 polymorphic loci across the kikuyu cultivars and ecotypes tested. Polymorphisms were scored as either present or absent. Analysis of the polymorphisms using PHYLIP (Felsenstein 2005) enabled the construction of a dendrogram showing the genetic relatedness of the individual genotypes, which is presented in Figure 1. Analysis of the genetic fingerprints of the ecotypes indicated that they formed 2 broad groupings. Most of the regional

ecotypes were grouped with 'common' kikuyu as represented by the material collected from Wollongbar, while the Beechmont, Gympie and Atherton Tableland ecotypes were grouped with the registered cultivars Whittet, Noonan and Crofts.

Discussion

After reviewing the history of kikuyu, Mears (1970) concluded that it would be difficult to recognise the original ecotypes introduced into Australia from Africa, even though Parker (1941) recognised clonal variation in Australian material in the 1930s. Sixty years have elapsed since Parker's (1941) research in South Australia, and intermixing of local populations and pastures sown to registered cultivars (such as cv. Whittet) has created what was believed to be a homogenous population known as 'common' kikuyu, albeit one which still exhibited natural variation. However, the results of these experiments demonstrate that there is significant variation in the agronomic and quality attributes of kikuyu in Australia. This variation extends down to individual plants. For example, mean total foliage yield of kikuyu lines in Experiment 1 varied from 237 to 422 g/plant

Figure 1. Genetic relationships between ecotypes and cultivars, determined by DAF profiling using four different oligonucleotide decamer primers. 'a' and 'b' indicate the results of duplicate samples.



but individual plants ranged from 163 to 532 g/plant, with runner yields of individual plants varying from 55 to 955 g/plant compared with mean values of 255–596 g/plant. This suggests that it would be possible to select kikuyu cultivars with improved agronomic characteristics from within the naturally-occurring populations and available cultivars.

There were substantial differences in the performance of kikuyu between Experiments 1 and 2. Experiment 1 was conducted over a full 12-month period, including a very mild winter, when kikuyu grew vigorously. In comparison, the second experiment measured growth during the January–September period over a cold winter with a very much reduced leaf production. The agronomic performance of the ecotypes and cultivars in Experiment 2 was similar at both sites in Experiment 2, with similar ranges in quality parameters. While Whittet performed well at both sites, ecotypes from Gympie, Mt Mee, Beechmont and Atherton Tableland performed poorly. On the other hand, some ecotypes such as Bairnsdale performed better at Wollongbar than at Mutdapilly, and Cultivar A and the Numinbah Valley ecotype performed poorly at Wollongbar but not at Mutdapilly. The South Australian ecotype failed to survive at either site. The variable performance of ecotypes at the two sites may be related to environmental differences at the two sites, relative to those at the collection site. Equally, it could be a reflection of differences in vigour between plants generated from the supplied runners.

Selections from the mutagenesis program at Wagga Wagga (Lockett *et al.* 1996) showed variable performance relative to Whittet, from which they were produced. None was significantly higher-yielding nor more digestible than Whittet in Experiment 1, although some had higher crude protein concentrations. At both sites in Experiment 2, Whittet outyielded Cultivar A and Cultivar B selected out of this program and displayed better quality (more digestible and with lower ADF and NDF concentrations). This result suggests that the mutagenic chemicals used in the Wagga Wagga project did not achieve the desired genetic mutations to produce the *bmr* gene, as most studies indicate that intake of genotypes of other species containing the *bmr* gene is higher than that of 'normal' genotypes and that they have greater *in vivo* digestibility (Cherney *et al.* 1991). The use of different chemicals in the

development of a new mutagenic population in the current program proposed by Bernard *et al.* (2004) seems warranted.

While mean quality data for the ecotypes and cultivars demonstrated limited variation, data obtained from single plants within this study (K.F. Lowe, unpublished data) suggested much greater variation. For example, in Experiment 1, the IVDDM in late summer of the best individual was 738 g/kg, compared with the overall mean of 722 g/kg. In Experiment 2 at Mutdapilly, the highest CP value of an individual plant in autumn was 267 g/kg, compared with the ecotype average of 250 g/kg; individual plant values for a single harvest in autumn were even greater (287 g/kg). Our data showed considerably higher levels of CP and IVDDM than those in grazed swards (Mears 1970; Reeves *et al.* 1996a) but were similar to those for intensively managed kikuyu in plot experiments (Reeves *et al.* 1996b). Since our material was managed on an individual plant basis, plants had not built up an underlying runner mass present in a full kikuyu sward. There seemed to be an inverse relationship between quality and vigour. Cultivar B, selected out of the kikuyu improvement program in NSW (Lockett *et al.* 1996), generally was higher in CP and IVDDM and lower in ADF and NDF concentrations than the other entries at both sites, but was not the most vigorous entry. Crofts and Cultivar A, on the other hand, were lower in most quality attributes but more vigorous, at least at the Mutdapilly site.

Edwards (1937) recognised 3 ecotypes of kikuyu in Kenya, but Mears (1970) concluded that there was little chance of distinguishing these in current Australian material. Our genetic fingerprinting shows that there are at least 2 distinct ecotypes. The classification of Whittet and Noonan, derived from commercial seed, in the same group was not unexpected as Noonan was selected from Whittet (Oram 1990). Similarly, the mutagenically-produced lines (Cultivars A and B) were in the same group as Whittet, suggesting that genetic mutations did not drastically change the genetic makeup of the selections. The other group appeared to have a different derivation, and ecotypes in this group were generally sourced from commercial farms which had grown kikuyu for generations. It is of interest that the ecotypes from Beechmont, Gympie and the Atherton Tableland did not fit in this group. These ecotypes might be different from the mate-

rial in other regional areas, but the more likely possibility is that these areas were contaminated by oversowing with Whittet at some time in their history.

To make progress through a selection program, there must be sufficient variation in the original material within which selection will occur. Our results suggest there is significant variation in yield, quality and genetic diversity (especially on an individual plant basis) within the natural populations within Australia and the commercial cultivars, which could sustain a breeding program to improve kikuyu quality.

Acknowledgements

Ecotypes from Vasse, Flaxley, Bairnsdale, Bega, Bonalbo, Mt Mee, Gympie and the Atherton Tableland were collected by Richard Morris, Greg Sweeney, Peter Stapleton, Dick Beusnel, Bede Clarke, Kerry Moore, Tom Bowdler, Ross Warren and John Lindsay, respectively. We thank them for their contribution to the project. The technical assistance of Tom Bowdler, Nikki Casey and Sandra Nolan (Mutdapilly) and Dick Bryant (Wollongbar) is acknowledged. The project was partially funded by Meat and Livestock Australia and Dairy Australia.

References

- BERNARD, M., LOWE, K.F. and HOLTON, T.A. (2004) Isolation and analysis of expressed gene sequences from kikuyu (*Pennisetum clandestinum*). In: *Dairy Science 2004. Proceedings of the Australian Dairy Science Symposium, Shepparton, Victoria, 26–27 February, 2004*, 1, 24.
- CAETANO-ANOLLES, G., BASSAM, B.J. and GRESSHOFF, P.M. (1991) DNA amplification fingerprinting using very short arbitrary oligonucleotide primers. *Biotechnology*, 9, 553–557.
- CALLOW, M.N., LOWE, K.F., BOWDLER, T.M., LOWE, S.A. and GOBIUS, N.R. (2003) Dry matter yield, forage quality and persistence of tall fescue (*Festuca arundinacea*) cultivars compared with perennial ryegrass (*Lolium perenne*) in a subtropical environment. *Australian Journal of Experimental Agriculture*, 43, 1093–1099.
- CHERNEY, J.H., CHERNEY, D.J.R., AKIN, D.E. and AXTELL, J.D. (1991) Potential of brown-midrib, low lignin mutants for improving forage quality. *Advances in Agronomy*, 46, 157–198.
- CLARK, D.A. and WILSON, J.R. (1993) Implications of improvements in nutritional value on plant performance and grassland management. *Proceedings of the XVII International Grassland Congress, Palmerston North, New Zealand, 1993*, 1, 543–550.
- CLARKE, K.F., FLINN, P.C. and MCGOWAN, A.A. (1982) Low-cost pepsin-cellulase assays for prediction of digestibility of herbage. *Grass and Forage Science*, 37, 147–150.
- EDWARDS, D.C. (1937) Three ecotypes of *Pennisetum clandestinum* (Hochst) kikuyu grass. *Empire Journal of Experimental Agriculture*, 5, 371–374.
- FELSENSTEIN, J. (2005) *PHYLLIP (Phylogeny Inference Package) Version 3.6*. Distributed by the author. (Department of Genome Sciences, University of Washington: Seattle).
- FLINN, P. and SAUL, G.R. (1983) Measurement of hay quality and its effect on intake and liveweight gain of weaner sheep. In: Robards, G.E. and Packham, R.G. (eds) *Feed Information and Animal Production*, pp. 259–263. (Commonwealth Agricultural Bureaux: Farnham Royal, UK).
- HACKER, J.B. (1986) Selecting for nutritive value in *Digitaria milanjiana*. 1. Breeding of contrasting full-sib clones differing in leaf digestibility. *Australian Journal of Experimental Agriculture*, 26, 543–549.
- ISBELL, R.F. (1996) *The Australian Soil Classification*. (CSIRO Publishing: Melbourne).
- LOWE, K.F., MOSS, R.J., COWAN, R.T., MINSON, D.J. and HACKER, J.B. (1991) Selecting for nutritive value in *Digitaria milanjiana*. 4. Milk production from an elite genotype compared with *Digitaria eriantha* ssp. *pentzii* (pangola grass). *Australian Journal of Experimental Agriculture*, 31, 603–608.
- LOWE, S.A., WHITE, J.A., LOWE, K.F. and BOWDLER, T.M. (2002) Evaluation of kikuyu cultivars. *Final Report. Project Report Series QO 02013. Department of Primary Industries, Brisbane*.
- LUCKETT, D., KAISER, A. and VIRGONA, J. (1996) Innovative breeding of high-digestibility kikuyu cultivars to increase milk production. *Final Report on Project DAN 063 to the Dairy Research and Development Corporation. NSW Agriculture, Orange, NSW*.
- MEARS, P. (1970) Kikuyu (*Pennisetum clandestinum*) as a pasture grass - a Review. *Tropical Grasslands*, 4, 139–152.
- MINSON, D.J. and MCLEOD, M.N. (1970) The digestibility of temperate and tropical grasses. *Proceedings of the XI International Grassland Congress, Surfers Paradise, 1970*, pp. 719–722.
- MINSON, D.J. and HACKER, J.B. (1986) Selecting for nutritive value in *Digitaria milanjiana*. 2. Intake and digestibility of divergently selected full-sibs compared with *Digitaria decumbens*. *Australian Journal of Experimental Agriculture*, 26, 551–556.
- ORAM, R.N. (1990) *Pennisetum clandestinum* Hochst. ex Chiov. (Kikuyu grass) cv. Noonan. In: *Register of Australian Herbage Plant Cultivars*. (CSIRO: Melbourne).
- PARKER, D.L. (1941) Strain variation and seed production in kikuyu grass (*Pennisetum clandestinum*) Hochst. *Journal of Agriculture of South Australia*, 45, 55.
- PAYNE, R.W., HARDING, S.A., MURRAY, D.A., SOUTAR, D.M., BAIRD, D.B., WELHAM, S.J., KANE, A.F., GILMOUR, A.R., THOMPSON, R., WEBSTER, R. and WILSON, G.T. (2007) *The Guide to GenStat Release 10, Part 2: Statistics*. (VSN International: Hemel Hempstead).
- REEVES, M., FULKERSON, W.J. and KELLAWAY, R.C. (1996a) Production responses of dairy cows grazing well-managed kikuyu (*Pennisetum clandestinum*) pastures to energy and protein supplementation. *Australian Journal of Experimental Agriculture*, 36, 763–770.
- REEVES, M., FULKERSON, W.J. and KELLAWAY, R.C. (1996b) Forage quality of kikuyu (*Pennisetum clandestinum*): the effect of time of defoliation and nitrogen fertiliser application and in comparison with perennial ryegrass (*Lolium perenne*). *Australian Journal of Agricultural Research*, 47, 1349–1359.
- SMITH, K. and FLINN, P. (1991) Monitoring the performance of a broad-based calibration for measuring the nutritive value of two independent populations of pasture using near infrared reflectance (NIR) spectroscopy. *Australian Journal of Experimental Agriculture*, 31, 205–210.