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

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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.

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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 C$_4$ 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 (27º 34’ S, 152º 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 (27º 45’ S, 152º 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 (28º 50’ S, 153º 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 (Mutdapilly) and February 22 (Wollongbar), 2005. These plants were sown into a fully cultivated seed-bed at Mutdapilly 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 Mutdapilly 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 Mutdapilly. 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 Mutdapilly 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 matt’ of the main plant, approximately 30 cm in diameter, to avoid plants coalescing. At Mutdapilly only, plants were pruned back to the ‘core matt’ 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 Mutdapilly 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 (Mutdapilly 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′–CAGTGCCGGT–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 Mutdapilly, south-east Queensland (Experiment 1). Only significant quality responses presented.

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Harvest 1</th>
<th>Autumn 03</th>
<th>Summer 04</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Runners/plant (no.)</td>
<td>Plant height (cm)</td>
<td>Total foliage yield (g/plant)</td>
</tr>
<tr>
<td>Common</td>
<td>14.5</td>
<td>16</td>
<td>237</td>
</tr>
<tr>
<td>Crofts</td>
<td>14.0</td>
<td>26</td>
<td>343</td>
</tr>
<tr>
<td>Noonan</td>
<td>13.7</td>
<td>25</td>
<td>321</td>
</tr>
<tr>
<td>Whittet</td>
<td>13.0</td>
<td>23</td>
<td>362</td>
</tr>
<tr>
<td>Cultivar A</td>
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<td>13.3</td>
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<td>333</td>
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<td>WK 9</td>
<td>12.7</td>
<td>22</td>
<td>422</td>
</tr>
<tr>
<td>WK 12</td>
<td>16.2</td>
<td>34</td>
<td>407</td>
</tr>
<tr>
<td>WK 31</td>
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<td>333</td>
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<tr>
<td>WK 64</td>
<td>11.5</td>
<td>19</td>
<td>270</td>
</tr>
<tr>
<td>WK 85</td>
<td>14.7</td>
<td>24</td>
<td>372</td>
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<tr>
<td>LSD (P=0.05)</td>
<td>5.2</td>
<td>5</td>
<td>64.9</td>
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Phenotypic and genotypic variation in Australian kikuyu

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.

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Site</th>
<th>Cultivar</th>
<th>Plant height (cm)</th>
<th>Total foliage yield (g/plant)</th>
<th>Av. leaf content (%)</th>
<th>Total runner yield (g/plant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Australia</td>
<td>Vasse</td>
<td>common</td>
<td>18.8</td>
<td>141</td>
<td>71.1</td>
<td>1177</td>
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<tr>
<td>Victoria</td>
<td>Bairnsdale</td>
<td>common</td>
<td>14.9</td>
<td>111</td>
<td>74.0</td>
<td>881</td>
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<td>Victoria</td>
<td>Melbourne</td>
<td>common</td>
<td>17.7</td>
<td>111</td>
<td>72.4</td>
<td>1260</td>
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<tr>
<td>Southern NSW</td>
<td>Bega</td>
<td>common</td>
<td>16.3</td>
<td>109</td>
<td>72.5</td>
<td>1937</td>
</tr>
<tr>
<td>Central NSW</td>
<td>Bonalbo</td>
<td>Crofts</td>
<td>12.2</td>
<td>88</td>
<td>69.4</td>
<td>997</td>
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<tr>
<td>Central NSW</td>
<td>Bonalbo</td>
<td>Noonan</td>
<td>20.7</td>
<td>177</td>
<td>73.5</td>
<td>1173</td>
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<td>Northern NSW</td>
<td>Wollongbar</td>
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<td>17.9</td>
<td>159</td>
<td>69.3</td>
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<td>Wollongbar</td>
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<td>23.8</td>
<td>235</td>
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<td>Beechmont</td>
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<td>North Queensland</td>
<td>Atherton Tableland</td>
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<tr>
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<td>6.7</td>
<td>108.5</td>
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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.

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Site</th>
<th>Cultivar</th>
<th>Total foliage yield (g/plant)</th>
<th>Av. leaf content (%)</th>
<th>Total runner yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>Western Australia</td>
<td>Vasse</td>
<td>common</td>
<td>12.9</td>
<td>70.4</td>
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<td>South Australia</td>
<td>Flaxley</td>
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<td>Victoria</td>
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<td>Bonalbo</td>
<td>Crofts</td>
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<td>Gympie</td>
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<td>Atherton Tableland</td>
<td>common</td>
<td>20.7</td>
<td>70.2</td>
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</tr>
<tr>
<td>LSD (P=0.05)</td>
<td></td>
<td></td>
<td>13.6</td>
<td>NS</td>
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</tr>
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</table>

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>
<thead>
<tr>
<th>Ecotype</th>
<th>Autumn</th>
<th>Winter</th>
<th>Spring</th>
</tr>
</thead>
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<tr>
<td></td>
<td>CP</td>
<td>IVDDM</td>
<td>ADF</td>
</tr>
<tr>
<td>Vasse</td>
<td>226</td>
<td>2.5</td>
<td>760</td>
</tr>
<tr>
<td>Bairnsdale</td>
<td>224</td>
<td>2.0</td>
<td>741</td>
</tr>
<tr>
<td>Melbourne</td>
<td>211</td>
<td>0.5</td>
<td>696</td>
</tr>
<tr>
<td>Bega</td>
<td>251</td>
<td>2.5</td>
<td>768</td>
</tr>
<tr>
<td>Crofts</td>
<td>255</td>
<td>2.1</td>
<td>751</td>
</tr>
<tr>
<td>Noonan (Benalbo)</td>
<td>246</td>
<td>4.3</td>
<td>731</td>
</tr>
<tr>
<td>Wollongbar common</td>
<td>230</td>
<td>4.4</td>
<td>760</td>
</tr>
<tr>
<td>Cultivar A</td>
<td>229</td>
<td>9.8</td>
<td>763</td>
</tr>
<tr>
<td>Cultivar B</td>
<td>246</td>
<td>2.7</td>
<td>765</td>
</tr>
<tr>
<td>Noonan (seed)</td>
<td>238</td>
<td>3.4</td>
<td>750</td>
</tr>
<tr>
<td>Whittet</td>
<td>241</td>
<td>6.9</td>
<td>768</td>
</tr>
<tr>
<td>Numinbah Valley</td>
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<td>7.1</td>
<td>756</td>
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<tr>
<td>Beechmont</td>
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<td>Atherton Tableland</td>
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<td>760</td>
</tr>
<tr>
<td>LSD (P=0.05)</td>
<td>22</td>
<td>3.7</td>
<td>26</td>
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</tbody>
</table>

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.
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.

### 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.

<table>
<thead>
<tr>
<th>Ecotype</th>
<th>Autumn</th>
<th>Winter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP Conc. (g/kg)</td>
<td>NDF Conc. (g/kg)</td>
</tr>
<tr>
<td>Vasse</td>
<td>243</td>
<td>483</td>
</tr>
<tr>
<td>Flaxley</td>
<td>233</td>
<td>477</td>
</tr>
<tr>
<td>Bairnsdale</td>
<td>211</td>
<td>489</td>
</tr>
<tr>
<td>Crofts</td>
<td>241</td>
<td>466</td>
</tr>
<tr>
<td>Noonan (Benalbo)</td>
<td>250</td>
<td>454</td>
</tr>
<tr>
<td>Wollongbar common</td>
<td>226</td>
<td>472</td>
</tr>
<tr>
<td>Cultivar A</td>
<td>234</td>
<td>472</td>
</tr>
<tr>
<td>Cultivar B</td>
<td>247</td>
<td>446</td>
</tr>
<tr>
<td>Noonan (seed)</td>
<td>240</td>
<td>466</td>
</tr>
<tr>
<td>Whittet (seed)</td>
<td>230</td>
<td>496</td>
</tr>
<tr>
<td>Numinbah Valley</td>
<td>226</td>
<td>486</td>
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<tr>
<td>Beechmont</td>
<td>241</td>
<td>471</td>
</tr>
<tr>
<td>Mt Mee</td>
<td>205</td>
<td>513</td>
</tr>
<tr>
<td>Gympie</td>
<td>238</td>
<td>461</td>
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<tr>
<td>Atherton Tableland</td>
<td>209</td>
<td>505</td>
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<tr>
<td>LSD (P=0.05)</td>
<td>24</td>
<td>39</td>
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</tbody>
</table>
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 (Luckett 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 (Luckett 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


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