finalreport

Project code: **BSC.027**

Prepared by: **Kevin F Lowe**

Department of Primary Industries and Fisheries

Date published: **9 December 2005**

ISBN: **1741910102**

PUBLISHED BY
Meat & Livestock Australia
Locked Bag 991
NORTH SYDNEY NSW 2059

Evaluation of the phenotypic variation in kikuyu populations

Abstract

A series of cutting experiments evaluated the phenotypic variation in populations of kikuyu in Australia to assess their capacity to provide sufficient variability to sustain a breeding and selection program. The aim of such a program would be to develop a high quality cultivar without compromising stand vigour. Single plants, generated either from seed of commercial cultivars or accessions, seed treated by mutagenic chemicals or from randomly-chosen runners from stands across Australia, were sown singularly in randomised block experiments under irrigation at two sites in subtropical eastern Australia.

There were significant differences between kikuyu cultivars in leaf, stem and runner dry matter yields, plant height and quality (crude protein, neutral detergent and acid detergent fibre content and *in vitro* digestibility). Variation between individual plants was also substantial, with 7, 3 and 3 % improvement over the population mean for crude protein content, *in vitro* digestibility, and metabolisable energy respectively, and a reduction of 5 and 8 % in neutral and acid detergent fibre content. It was also associated with a forage yield twice the population mean from these elite plants. Mutagenesis of seed from a commercial cultivar produced greater within-population variation for quality, but less for agronomic, traits compared with the natural population. Gene fingerprinting suggested that the material distributed across Australia came from two main sources, one related to cv. Whittet and the other to a 'common' lower yielding type. It was concluded that the currently-available material within Australia, although from limited genetic base, contained sufficient variation to achieve a significant improvement in quality without reducing plant vigour.

Executive Summary

The limitation to the breeding for improved quality in tropical species has always been the numbers of individuals needed to be screened, the need to grow plants to mature individuals before testing for quality and the cost of chemical analyses required for screening and the need to concurrently screen for both quality and agronomic characters. In general, this has proved a major hurdle which has not been successfully overcome. Molecular biology has provided a method of rapid screening of individuals for the presence of specific genes or gene sequences that control specific biological processes. In relation to quality, genes controlling lignin biosynthesis appear to be one of the most important in controlling digestibility. Molecular techniques are capable of rapidly screening large numbers of individuals in a very early stage of their development (eg. seedling stage), and, combined with traditional selection procedures, show promise in tropical pasture improvement.

Kikuyu (*Pennisetum clandestinum*) is an important summer-growing grass for the animal industries of Australia. It is high yielding and responsive to nitrogen and water. However, as with other tropical grasses, its quality is limited by the levels of lignin which reduce digestibility relative to temperate grasses. There have been attempts to improve the quality of tropical grasses but the difficulty of selecting for high yields, vigour and high quality concurrently has seen these attempts fail to achieve the goals either by improving quality at the expense of vigour or failing to deliver sufficient improvement in quality to substantially lift animal production. There have been concerns that any selection for improved quality in tropical species may be accompanied by a reduction in agronomic characters such as plant vigour or yield.

Kikuyu grows in many areas of Australia but little is known about the genetic variation existing in the natural populations. There are only a restricted number of cultivars and most were derived from an introduction which was used to develop the cultivar Whittet. Unlike most of the other tropical grass species, there is no collection of accessions in the Australian Tropical Genetic Resource Centre at Biloela. This series of experiments was designed to investigate Australian kikuyu resources to determine whether sufficient variation exists in it to sustain a breeding program for improved quality.

All experiments except one used single plants on a 1.5 m square grid as the experimental unit and a randomised block design with replicates varying from 3 to 10. The final experiment used a strip plot design, where single plants were sown on either a 0.5 or 1.5 m grid. All plants except the cultivar Crofts in Experiment 1 were genetically different individuals, either coming from seed or being selected at random from within swards of kikuyu. Experiment 1 investigated the variation in material generated from treating Whittet kikuyu seed with two chemicals known to cause mutagenesis in grasses and in a selection of commercial cultivars. Experiment 2 investigated variation in pre-release populations of four commercial cultivars and a breeding population from NSW. Experiments 4 and 6 investigated the variation in performance of ecotypes of kikuyu collected from regions within Australia. There were two sites, one at Wollongbar on a plateau area in northern NSW and a second at Mutdapilly in coastal south eastern Queensland. DNA was extracted from each plant and subjected to a modified DAF (DNA amplification fingerprinting) analysis to determine the genetic relatedness of the genotypes to each other. Experiments 5 and 7 investigated the variation within a separate population of mutagenised Whittet seed at the same two sites. The final experiment (Experiment 3) investigated the ability of kikuyu selections with varying ability to produce runners and with different growth habits, to colonise bare areas after establishment.

Selections, accessions and cultivars showed considerable variation in both physical and chemical attributes. In Experiment 1, Cultivars A and B, WK 9, WK 12, WK 39, WK 46 and WK 85 were higher yielding, Cultivar A and WK 42 produced more runners and Crofts, WK 9, Cultivars A and B and WK 42 were leafier than Whittet. In Experiment 2, Whittet was the highest yielding cultivar. The breeding line CND8 was consistently the lowest yielding, both in forage and runner yield and was a less erect type compared with Noonan, Breakwell, Whittet and a common, seeding selection. Noonan and Breakwell were only moderate yielding, a result consistent with their commercial performance. In experiments 4 and 6, significant differences in plant height and yield, runner development, and the crude protein, invitro digestibility and acid and neutral detergent fibre content of leaf was demonstrated between the 10 ecotypes and 6 cultivars evaluated. There was a 4-fold range in plant yield and a 10-fold range in runner production between the ecotypes. Crude protein ranged from 21 to 26%, *in vitro* digestibility from 67 to 80%, neutral detergent fibre from 53 to 45% and acid detergent fibre from 26 to 18%. Experiments 5 and 7 suggest that the two mutagenic chemicals (either used singly or in combination) randomly resulted in gene mutations but had no effect on specific plant parameters. There were no significant differences in any agronomic or quality parameter between the four mutagenic seed lots at Mutdapilly and only stem yield was significantly affected in one sampling at Wollongbar.

Analysis of the genetic fingerprints of the ecotypes evaluated in Experiments 4 and 5 indicated that they formed two broad groupings. Most of the regional ecotypes grouped with “common” kikuyu as represented by the material collected from Wollongbar, whilst the Beechmont ecotype grouped with the cultivars Whittet, Noonan and Crofts. The only exception was the Atherton Tablelands ecotype that was not aligned with either group.

The variation between individual plants was also substantial. There was also greater variation in the populations generated by mutagenesis than from naturally-occurring populations or within regional ecotypes. In Experiment1, individual plants from Cultivar A, Cultivar B, WK 9, WK 12 and WK 42 demonstrated combinations of high yield and high quality during autumn, while WK 85 showed superior runner vigour but no superior quality, over Whittet. Cultivar A and B did not show the same superiority in the winter or late summer but the other entries continued to perform well. In Experiment 2, most of the ‘elite’ material (ie. high yielding combined with high quality attributes) came from Whittet, and to a lesser extent from Noonan and an accession of ‘common’ kikuyu. Generally the elite individuals from Experiments 4 and 6 came from the best performing cultivars (Whittet and Noonan) and ecotypes (Wollongbar, Numinbah Valley, Gympie and Victoria) but even within lower yielding ecotypes, outstanding plants emerged. The maximum improvement in quality attributes of individual plants was 14% in crude protein, 5% in *in vitro* digestibility and 6% in ME and a reduction in ADF and NDF of 20 and 16% respectively. However, the levels of improvement declined considerably from these values to around 3-5% when the added selection pressure for high forage yields and runner production was also taken into account. In Experiment 3, sward spread in the first two months reflected the potential runner production predicted from previously conducted experiments. Experiment 1 predicted that WK 42 and WK 64 would produce the most runner mass and this initially was the case. However in the longer term, runner production did not reflect a sward’s ability to produce foliage over the period from sowing to maximum sward coverage of 7 months. Selections performed similarly at the two densities; the lower density planting just took longer to cover the bare areas. Sward height was a better predictor but even it only accounted for 22.5% of the variation in forage yield. To select a successful kikuyu cultivar the following physical attributes would need to be assessed:- runner spread, runner yield and runner vigour, together with forage characteristics such as total and component yield and sward height.

It was concluded that, even from this limited evaluation of the material available, there was potential to produce elite lines of kikuyu which would show superior yield, runner production (measures of sward vigour) combined with higher quality parameters (lower fibre, higher digestibility and higher crude protein). The results also demonstrate that improvements in quality and agronomic attributes were not mutually exclusive and that a breeding program could be expected to achieve improved quality in kikuyu while retaining the vigour of current cultivars. Whilst no immediate use can be made of the results of these experiments, it does strengthen a case for a breeding program to improve the quality of kikuyu. Such improvements can be expected to have a substantial effect on improved returns from intensive animal industries which utilise kikuyu. In the long term, success in this area is likely to have far wider implications on the whole grazing industry because the principles derived here can be equally applied to other tropical grasses.

Contents

1 Background

1.1 General

The quality of tropical grasses is a major limitation to animal production in tropical and subtropical areas and this is mainly associated with the lower digestibility of these feeds relative to temperate grass species. This is mainly associated with higher fibre levels and a major improvement would be achieved by increasing the digestibility of the Neutral detergent fibre content of these plants. The main advantage of tropical grasses is in their more efficient photosynthetic ability and water use efficiency, particularly at higher light levels and higher temperatures. Any improvement in quality will need to be achieved without seriously affecting this ability as a previous attempt to increase the quality of the tropical grass *Digitaria milanjiana* (Hacker 1986; Minson and Hacker 1986; Masaoka *et al.* 1991; Lowe *et al.* 1991) resulted in a grass with more leaf, higher digestibility (at least in cutting studies) but which failed to persist under good management. One of the major limitations to achieving significant gains in quality is the cost of the chemical analyses required to determine differences in quality. If molecular markers for reduced lignin levels could be utilised to select elite lines, this would streamline the selection process, speed up cultivar development and reduce costs.

Kikuyu (*Pennisetum clandestinum*) is an important grass for the dairy and beef industries of the subtropics of Australia, South Africa and New Zealand. The commercial cultivars available in Australia appear to have come from a limited genetic base and there are no accessions in the Australian Tropical Resource Centre. Therefore, some form of genetic improvement appears to be the most appropriate way to progress quality improvement in this species.

Before commencing any research in this area, there is a need to establish that any improvement in quality will not necessarily result in a reduction in vigour or yield potential of the species.

1.2 Experiment 1

An attempt to improve the quality of kikuyu by trying to introduce the *brown midrib* trait into the cultivar Whittet was conducted in New South Wales (Luckett *et al.* 1996) and this project produced a selection of elite material. Experiment 1 utilised this elite material (Luckett *et al.* 1996) from experiments conducted at Mutdapilly (Lowe *et al.* 2002) to evaluate the variation in kikuyu cultivars and mutagenic selections.

1.3 Experiment 2

In Australia, kikuyu appears to have been spread vegetatively from material brought into the country in the early 20th Century. Mears (1970) provides a history of early kikuyu introductions in Australia. Any improvement in quality of kikuyu will need to come from the material currently available in Australia as there are no accessions of kikuyu held at the Australian Tropical Resource Centre (P. Lawrence, pers. comm.). A number of commercial cultivars have been released and these were developed by the New South Wales Department of Primary Industries (Oram 1990). They stemmed from the need to develop a seeding line to expedite pasture development and to increase tolerance to a fungal disease known as kikuyu yellows. Seed from these development programs was held at Grafton Research Station and is the only available source of experimental kikuyu in Australia. The only other source of genetic variation lies in local ecotypes which may or may not be prolific seeders.

Pre-release seed of these cultivars was sown in a spaced plant experiment at Mutdapilly Research Station to assess the potential variation in this material.

1.4 Experiment 3

The ability of a grass species to colonise or to maintain a dense stand is essential for achieving high pasture productivity. There is evidence that selection for higher quality reduces the ability of grasses to compete with other species and especially invaders. Kevin Lowe (unpublished data) has shown that a higher quality selection of tall fescue is less competitive than cultivars selected for agronomic traits and a previous attempt to increase the quality of the tropical grass *Digitaria milanjiana* (Hacker 1986; Minson and Hacker 1986; Masaoka *et al.* 1991; Lowe *et al.* 1991) resulted in a grass with more leaf, higher digestibility at least in cutting studies but which failed to persist under good management. Therefore it is important that, in any selection process, the ability of higher quality selections must also measure up in agronomic traits.

This experiment assessed how useful the measurements of runner development, runner extension and runner yield were in assessing a plant's ability to colonise bare areas.

1.5 Experiments 4 and 5

Mears (1970) reviewed the history of kikuyu and 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 late 1930s. A further 60 or so years after Parker's 1941 research in South Australia, intermixing of natural and pastures sown to kikuyu cultivars has created even further variation. There is little information on where the introductions of kikuyu went in Australia although it is assumed that most 'naturalised' kikuyu is fairly similar.

This experiment assessed kikuyu taken from a wide range of climatic environments within Australia to see if the regional ecotypes are phenotypically and genetically different.

1.6 Experiments 6 and 7

The project of Luckett *et al.* (1996) to improve the quality of kikuyu by attempting to create the brown midrib trait in the cultivar Whittet produced seed which had been treated by the two mutagenic chemicals, sodium azide and diethylene sulphate. This seed had been cryogenically stored in a cold room and was still viable. Seed from the four different seed treatments was used in these experiments to assess the variation in the populations achieved by mutagenesis. The variation within the plants established from this seed was expected to be different to that assessed in Experiment 1 because that material had been selected as elite lines from a population of around 40 000 plants screened in the project. On the other hand, Experiments 6 and 7 examined a random selection from the base population of treated seed.

2 Project Objectives

2.1 Comparison of mutagenised kikuyu selections and commercial cultivars (Experiment 1)

2.1.1 Complete the chemical analysis of cloned material being evaluated in Experiment 1

Experiment 1 commenced prior to the MLA funding but only limited analysis of the quality attributes of the kikuyu accessions had been made. Objective 1 provided an avenue to increase the data available on the quality differences in this material and to further assess the relationships between yield and quality within the mutagenic populations produced from the previous project DAN 063.

2.2 Comparison of pre-commercial kikuyu selections and accessions (Experiment 2)

2.2.1 Cultivate plants from seed collection

Experiment 2 also commenced prior to the MLA funding but no data had been collected. Objective 2 evaluated the performance of spaced plants derived from seedlings established from pre-release seed of five breeding lines from NSW Department of Primary Industries, the precursors of the Australian cultivars Whittet, Breakwell and Noonan and two seeding accessions (common and CND8).

2.2.2 Complete the chemical analysis of material as in Experiment 1

Experiment 2 assessed the between 'cultivar' and between individual plant variation in the physical and chemical characteristics of these populations of kikuyu. The relationships between yield and quality were evaluated within these populations.

2.3 Comparison of ability of diverse agronomic kikuyu selections to colonize bare ground (Experiment 3)

2.3.1 Cultivate plants from plant/soil cores taken from Experiment 1

The objective in Experiment 3 was to determine the ability of kikuyu plants of varying growth habits to colonise an area. To achieve this, kikuyu plants with different habits and abilities to produce runners, as assessed in Experiment 1, were planted at two density spacings.

2.3.2 Assess how rapidly each colonized when planted at two plant spacings

The development of swards, both in yield and basal cover, was assessed over time.

2.4 Comparison of regional kikuyu ecotypes and commercial cultivars (Experiments 4 and 5)

2.4.1 Collect genetically diverse cultivars from around Australia

The objective was to determine whether there are any phenotypic and genetic differences in kikuyu plants selected from regions within Australia. To achieve this, kikuyu runners were collected from the Atherton Tableland in north Queensland to the south-western corner of Western Australia.

Sixteen kikuyu selections were collected by project staff or local agronomists from areas considered to have grown kikuyu for over 30 years. Areas, known to have been sown to seeding cultivars, were avoided except where deliberately sampled at specific sites (ie Noonan, Crofts, Whittet).

2.4.2 Establish experiments at Wollongbar and Mutdapilly sites

Experiments were established on a red ferosol soil at Wollongbar on a plateau area in northern NSW and on a yellow podzolic soil on an upper terrace of an alluvial plain in southeast Queensland. Both areas are centres for dairying and beef enterprises in the subtropics.

2.4.3 Complete the agronomic and chemical analysis of material as in Experiment 1

Experiments assessed the 'between cultivar' and 'between individual plant' variation in physical and chemical characteristics of these populations of kikuyu. The relationships between yield and quality parameters were also evaluated.

2.4.4 Complete the genetic fingerprinting analysis of material

DNA was extracted from each plant and subjected to a modified DAF (DNA amplification fingerprinting) analysis to determine the genetic relatedness of the genotypes to each other.

2.5 Comparison of plants from mutagenised Whittet kikuyu seed (Experiments 6 and 7)

2.5.1 Establish plants from mutagenised seed produced from a previous kikuyu breeding project (DAN063)

The objective was to determine the phenotypic and genetic differences within kikuyu plants grown from the remaining seed produced by the mutagenesis treatment of commercial Whittet kikuyu seed in Project DAN063. There were two chemicals, sodium azide and diethylene sulphate, used to treat the seed and these were used alone or in combination.

2.5.2 Establish experiments at Wollongbar and Mutdapilly sites

Experiments were established on a red ferosol soil at Wollongbar on a plateau area in northern NSW and on a yellow podzolic soil on an upper terrace of an alluvial plain in southeast Queensland. Both areas are centres for dairying and beef enterprises in the subtropics

2.5.3 Complete the agronomic and chemical analysis of material as in Experiment 1

Experiments assessed the 'between cultivar' and 'between individual plant' variation in physical and chemical characteristics of these populations of kikuyu. The relationships between yield and quality parameters were evaluated within these populations.

2.5.4 Complete the genetic fingerprinting analysis of material

Gene fingerprinting of the plants from the four seed lots were conducted in order to assess whether the mutations achieved by the different mutagenic chemical treatments resulted in differences.

3 Methodology

3.1 Experiments 1, 2, 4, 5, 6 and 7

3.1.1 Establishment

Single plants were established in 10 cm diameter plastic pots from either runners or seed and then planted out into the field on a 1.5 m grid. Experiments were sown into a sandy loam duplex soil at Mutdapilly Research Station (27º 45' S, 152º 40' E, 70 m) in south east Queensland or a red clay loam (red ferrosol) at Wollongbar (28º48 S, 1543º20 E) in northern New South Wales. All experiments, except Experiment 3, were randomised block designs; Experiment 3 was a strip plot, randomised block. Plants were sown into a fully cultivated seedbed at Mutdapilly and into a sward of degraded kikuyu pasture which had been killed with an application of 2 L/ha of Roundup® at Wollongbar. A mixed fertiliser (CK 88) was applied at the base of each plant, with a rate equivalent to 500 kg/ha to satisfy the high fertility requirement of kikuyu. Subsequently an application of urea at 50 kg N/ha was applied after each defoliation. The experiments were fully irrigated using lawn size, spray equipment until mid 2004 and then with Ezi-shift equipment which had a substantially higher application rate.

3.1.2 Assessment of agronomic characteristics

Dry matter (DM) yields were assessed by defoliating each plant to 5 cm using hand shears and drying at 80ºC. Other measurements included leaf to stem ratio by sorting the cut material into leaf laminae and stem, runner length and yield by destructively sampling the runners extending from the 'core matt' of the main plant, approx 30 cm in diameter (see Plate 1), and foliage height. Plants were pruned back to this 'core mat' at the end of autumn and again at the end of winter and in mid-spring to prevent plants coalescing into neighbouring swards and to assess 'spreadability', i.e. a plant's ability to produce stolons to colonise surrounding bare ground.

3.1.3 Assessment of chemical characteristics

Quality attributes of leaf were assessed in autumn 2003, spring 2003 and late summer 2004 by submitting material to NIR analyses for crude protein (CP), *in vitro* digestibility (IVDMD), neutral detergent fibre (NDF) and acid detergent fibre (ADF) content and the derived estimate of metabolisable energy (ME). Only replicates 1 and 4 were analysed in autumn 2003 but in spring 2003 and late summer 2004, four replicates (Reps 1, 2, 4, 5) were used.

3.1.4 Gene fingerprinting

DNA was extracted from leaf samples of the different genotypes. Genetic fingerprints of each of the kikuyu cultivars and ecotypes were determined using a modified DAF analysis (Caetano-Anolles *et al*., 1991). Duplicate DNA samples were amplified using four different oligonucleotide primers and fragment sizes determined by denaturing polyacrylamide gel electrophoresis. DAF profiles were analysed by PHYLIP (Felsenstein, 2005) to determine the genetic relatedness of each of the individuals.

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'

Table 1. Details of kikuyu experiments.

MRS – Mutdapilly Research Station, WAI – Wollongbar Agricultural Institute

Experiment No.	No.of treatments	Treatments	Reps	Site	Estab. date
1	16	Mutagenic selections from DAN063, Cultivars Whittet, Noonan and Crofts and common (from Gatton)	8	MRS	Mar. 2003
2	5	Pre-breeders' seed of Whittet, Breakwell and Noonan and 2 accessions	10	MRS	Dec. 2003
3	10	Selections from Experiment 1, showing a range of abilities to produce runners	3	MRS	Dec. 2004
4	15	Ecotypes from West Aust., South Aust., Vic., central and northern NSW, southern and northern Qld and cultivars Whittet, Noonan and Crofts	3	WAI	Feb 2005
5	17	Ecotypes from West Aust., South Aust., Vic., southern, central and northern NSW, southern and northern Qld and cultivars Whittet, Noonan and Crofts	3	MRS	Jan 2005
6	4	Seed from 4 mutagenic seed treatments	6	WAI	Feb 2005
7	4	Seed from 4 mutagenic seed treatments	8	MRS	Jan 2005

3.1.5 Statistical assessment

Yield and quality attributes were analysed by ANOVA (GenStat *et al.* 1993). Interplant variation was presented in XY graphs, plotting individual plant yield at each harvest against individual agronomic and quality attributes (CoPlot 2002). Population means for each attribute were plotted on the respective axes; elite material fell in the top right hand quadrant of the graph (i.e. higher than average quality and higher than average yield, except for acid and neutral detergent fibre measurements where they would fall in the lower right hand quadrant.

3.2 Experiment 3

3.2.1 Establishment

Five kikuyu selections, ranging from the most prostrate to the most erect as assessed in Experiment 1, were chosen. The five selections were also selected on their ability to produce runners. They were (ordered in increasing runner production): Cultivar B, common, WK85, WK64 and WK 42. There were two planting densities: plants sown on a 0.5 m or a 1.5 m grid. There were 9 plants per treatment on a 3 by 3 square layout. The experiment was laid out as a strip plot, randomised block design with 3 replicates. The strip plot design was necessary as plot size of the grids differed and it was impossible to randomly allocate treatments within replicates (see Appendix 3). Kikuyu selections were randomly allocated within strips.

The experiment was sown on 13 December 2004. Cores, 10 cm diameter by 10 cm depth, were dug from the existing swards (originating from a single plant) in Experiment 1 and planted out into a fully cultivated seedbed at Mutdapilly Research Station. The soil type was similar to that used in Experiment 1. The experimental area was irrigated every 3 – 5 days to ensure successful establishment. Irrigation to supplement rainfall has continued throughout the measurement period (January to June 2005).

3.2.2 Assessment of agronomic characteristics

Measurements taken included basal area of kikuyu within the grid and yield from the 9 plants (monthly after an initial 2-month establishment period). Basal area was assessed by taking a transact across both diagonals of the square using a 0.25 m by 1 m quadrant divided into one hundred, 5 cm by 5 cm squares. Basal area was assessed on the percentage of these squares containing a kikuyu runner.

3.2.3 Statistical assessment

Yield and density attributes were analysed by ANOVA (GenStat *et al.* 1993). Transformation of the density data did not improve analysis so all data was analysed without transformation.

4 Results and Discussion

4.1 Experiment 1

Individual plants of kikuyu (*Pennisetum clandestinum*), selected from two old experiments, which had evaluated selections from mutagenised cv. Whittet, were sown as spaced plants to assess the variation in physical and chemical characteristics within kikuyu. Measurements taken on individual plants included total foliage yield, leaf and stem yield, leaf to stem ratio, runner length and yield and foliage height. Leaf samples from two replicates at the first and third harvests in autumn and winter 2003, respectively and from four replicates at the sixth harvest in late summer 2004, were submitted for Near Infra Red (NIR) analysis for NDF, ADF and crude protein concentrations and *in vitro* digestibility.

Selections showed considerable variation in both physical and chemical attributes, relative to the original cultivar from which they had been derived and from other commercial cultivars of kikuyu. Cultivars A and B, WK 9, WK 12, WK 39, WK 46 and WK 85 were higher yielding than Whittet.

Cultivar A and WK 42 produced more runners than Whittet and therefore should be more aggressive in colonising areas. Crofts, WK 9, Cultivar A, Cultivar B and WK 42 produced greater leaf yields than Whittet, while Cultivar B, WK 9 and Noonan had the highest leaf to stem ratio.

The variation between individual plants was even greater than that between selections. Plants from Cultivar A, Cultivar B, WK 9, WK 12 and WK 42 demonstrated high yields and high quality characteristics during autumn, while WK 85 showed superior runner vigour but no superior quality, over Whittet. Cultivar A and B did not show superiority in the winter or late summer but the other entries continued to perform well. It was concluded that, even from this limited evaluation of the mutagenic population produced from Whittet, there was very good chance of producing elite lines of kikuyu which would show superior yield, runner production (measures of sward vigour) and higher quality (lower fibre, higher digestibility and higher crude protein).

4.2 Experiment 2

Individual plants of kikuyu (*Pennisetum clandestinum*), established from pre-release seed of three Australian cultivars and two accessions, were sown as spaced plants to further assess the variation in physical and chemical characteristics within kikuyu. Measurements taken on individual plants included total foliage yield, leaf and stem yield, leaf to stem ratio, runner length and yield and foliage height. Leaf samples from the first and second harvests in late summer and autumn 2004 were submitted for Near Infra Red (NIR) analysis for NDF, ADF and crude protein concentrations and *in vitro* digestibility.

Cultivars showed considerable variation in both physical and chemical attributes. Whittet was the highest yielding cultivar in the five harvests taken in 2004/05. Winter temperatures were substantially lower in 2004 and no growth was recorded over winter, which contrasted to the conditions experienced in 2003. CND8 was consistently the lowest yielding, both in forage and runner yield and was a less erect type compared with the other four. Noonan and Breakwell were only moderate yielding, a result consistent with their commercial reputation.

The variation between individual plants was greater than that between cultivars. While plants from all cultivars demonstrated high yields and high quality characteristics during late summer, autumn and spring, most of the ‘elite’ material (i.e. high yielding combined with high quality attributes) came from Whittet, (and to a lesser extent from Noonan and Common). While the variation recorded in quality attributes was lower than that recorded in the mutagenically-produced lines in Experiment 1, there was still a good chance of producing elite lines of kikuyu which would show superior yield, runner production (measures of sward vigour) and higher quality (lower fibre, higher digestibility and higher crude protein) from these cultivars.

4.3 Experiment 3

Cores, taken from swards of five selections of kikuyu (*Pennisetum clandestinum*) with differing growth habits and runner-producing capabilities, were planted on a square grid at two densities (0.5 and 1.5 m) to assess which characteristics controlled the ability of kikuyu to spread into bare areas. The experiment was sown under irrigation at Mutdapilly on a sandy duplex soil.

Sward spread in the first two months reflected the potential runner production predicted from previously conducted spaced-plant experiments. Experiment 1 predicted that WK 42 and WK 64 would produce the most runner mass and these initially achieved the best spread. As the

experiment progressed, common kikuyu exceeded expectations and covered the bare areas more rapidly. However, despite ending the 7 months with a sward with 90% coverage at the higher density, common kikuyu was the lowest yielding line. Selections performed similarly at the two densities; the lower density planting just took longer to cover the bare areas. Runner production did not reflect a sward's ability to produce foliage over the time from sowing to maximum sward coverage. Sward height was a better predictor of forage yield but this parameter only accounted for 22.5% of the yield variation. It was concluded that to select an agronomically-successful kikuyu cultivar the following physical attributes would need to be assessed:- runner spread, runner yield and runner vigour, together with forage characteristics such as total and component yield and sward height.

4.4 Experiment 4 and 6

Experiments at two sites in subtropical eastern Australia investigated the variation in agronomic attributes, quality and genetic structure existing within naturally-occurring Australian populations of kikuyu and from within available cultivars. Runners were collected from coastal areas extending from Western Australia through to the Atherton Tablelands in northern Queensland. These were established in replicated experiments and sampled approximately monthly from January to September 2005. Foliage height, forage production and runner yield was studied on these ecotypes which were sown as single plants on a 1.5 m grid. The leaf material was analysed for crude protein, *in vitro* digestibility and acid and neutral detergent fibre content in autumn, winter and spring. Leaf material was also gene fingerprinted to determine the closeness of the relationship between the ecotypes and cultivars.

There were significant differences in plant height and yield, runner development, and the crude protein, *in vitro* digestibility and acid and neutral detergent fibre content of leaf between the 10 ecotypes and 6 cultivars. There was a 4-fold range in plant yield and a 10-fold range in runner production between the ecotypes. Crude protein ranged from 21 to 26%, *in vitro* digestibility from 67 to 80%, neutral detergent fibre from 53 to 45% and acid detergent fibre from 26 to 18%. These results suggest that considerable variation existed within populations of kikuyu in quality and yield.

Analysis of the genetic fingerprints of the ecotypes evaluated in Experiments 4 and 5 indicated that they formed two broad groupings. Most of the regional ecotypes grouped with "common" kikuyu as represented by the material collected from Wollongbar, whilst the Beechmont ecotype grouped with the cultivars Whittet, Noonan and Crofts. The only exception was the Atherton Tablelands ecotype that was not aligned with either group.

There was an even greater variation in the agronomic and quality attributes of individual plants within these cultivars and ecotypes. Generally, the elite material came from the best performing cultivars and ecotypes but even within lower yielding ecotypes, outstanding plants emerged. The maximum improvement in quality attributes of individual plants was 14% in crude protein, 5% in *in vitro* digestibility and 6% in ME and a reduction in ADF and NDF of 20% and 16% respectively. However, the levels of improvement declined considerably from these values when the added selection pressure for high forage yields and runner production also had to be taken into account. Our results show that improvements in quality and agronomic attributes were not mutually exclusive and that a breeding program could be expected to achieve improved quality in kikuyu while retaining the vigour of current cultivars.

4.5 Experiment 5 and 7

Experiments at two sites in subtropical eastern Australia investigated the variation in agronomic attributes and quality existing within plants derived from seed of *Pennisetum clandestinum* cv. Whittet which had been treated with sodium azide or diethylene sulphate, chemicals known to cause mutagenesis in monocotyledonous species. There were four treatments; seed was treated with either of the two chemicals applied alone or both chemicals applied in either order. These were established in replicated experiments and sampled approximately monthly from January to September 2005. Foliage height, forage production and runner yield was studied on these ecotypes which were sown as single plants on a 1.5 m grid. The leaf material was analysed for crude protein, *in vitro* digestibility and neutral and acid detergent fibre content in autumn, winter and spring.

There were no significant differences in any agronomic or quality character between the four seed lots at Mutdapilly and only stem yield was significantly affected in one sampling at Wollongbar. This suggests that the chemicals causing mutagenesis randomly resulted in gene mutations and had no effect on specific plant attributes. Variation within the individual plants was substantial and showed an improvement in crude protein, *in vitro* digestibility and metabolisable energy of 11%, 3% and 4% respectively and a reduction in ADF and NDF of 14% and 8% respectively over the site mean values at Mutdapilly. A similar level of improvement was demonstrated at Wollongbar. However in any selection process to improve quality, compromises need to be made when selecting for both high quality and high yields: selecting for multiple characters reduced the improvement in quality below these values in some parameters.

5 Success in Achieving Objectives

5.1 Comparison of mutagenically-produced kikuyu selections and commercial cultivars (Experiment 1)

Experiment 1 commenced prior to the MLA funding but only limited analysis of the quality attributes of the kikuyu accessions had been made. Milestone 1 provided an avenue to increase the data available on the quality differences of this material and to further assess the relationships between yield and quality within the mutagenic populations produced from a previous project DAN 063.

Leaf samples of kikuyu, taken from the spaced plants in spring 2003 and autumn 2004, were submitted to the Department of Primary Industries' (Victoria) Feedtest Laboratory for NIR analysis. The following quality parameters were assessed: *in vitro* digestibility, acid detergent fibre, neutral detergent fibre and Metabolisable Energy (derived).

Selections showed considerable variation in both physical and chemical attributes, relative to the original cultivar from which they had been derived and from other commercial cultivars of kikuyu. Cultivars A and B, WK 9, WK 12, WK 39, WK 46 and WK 85 were higher yielding than Whittet. Cultivar A and WK 42 produced more runners than Whittet and, therefore, should be more aggressive in colonising areas. Crofts, WK 9, Cultivar A, Cultivar B and WK 42 produced higher leaf yields than Whittet, while Cultivar B, WK 9 and Noonan had the highest leaf to stem ratio.

The variation between individual plants was even greater than that between selections. Plants from Cultivar A, Cultivar B, WK 9, WK 12 and WK 42 demonstrated high yields and high quality characteristics during autumn; while WK 85 showed superior runner vigour but no superior quality

over Whittet. Cultivar A and B did not show the same superiority in winter or late summer but the other entries continued to perform well. It was concluded that, even from this evaluation of a limited range of the mutagenic population produced from Whittet, there was very good chance of producing elite lines of kikuyu which would show superior yield, runner production (measures of sward vigour) and higher quality (lower fibre, higher digestibility and higher crude protein).

5.2 Comparison of pre-commercial kikuyu selections and accessions (Experiment 2)

Experiment 2, which evaluated plants grown from pre-release seed of five NSW Agriculture breeding lines, the precursors of the Australian cultivars Whittet, Breakwell and Noonan and two seeding lines (Common and CND8) commenced prior to the MLA funding but no data had been collected.

Leaf samples of kikuyu, sampled from the spaced plants in late summer, autumn and spring 2004, were submitted for NIR analysis. The following quality analyses were performed: crude protein (CP), *in vitro* digestibility (IVDMD), acid detergent fibre (ADF), neutral detergent fibre (NDF) and Metabolisable Energy (ME - calculated).

Cultivars showed considerable variation in both physical and chemical attributes. Whittet was the highest yielding cultivar in the five harvests taken in 2004/05. CND8 was consistently the lowest yielding, both in forage and runner yield and was a less erect type compared with the other four. Noonan and Breakwell were only moderate yielding, a result consistent with their commercial reputation.

The variation between individual plants was greater than that between cultivars, suggesting that genetic variation between cultivars was not large. The major variation between individual plants was most likely due to environmental and sampling variation. While plants from all cultivars demonstrated high yields and high quality characteristics during late summer, autumn and spring, most of the ‘elite’ material (i.e. high yielding combined with high quality attributes) came from Whittet, (and to a lesser extent from Noonan and Common). While the variation recorded in quality attributes was lower than that recorded in the mutagenically-produced lines in Experiment 1, there was a good chance of producing elite lines of kikuyu which would show superior yield, runner production (measures of sward vigour) and higher quality (lower fibre, higher digestibility and higher crude protein) from this source.

5.3 Comparison of ability of diverse agronomic kikuyu selections to colonize bare ground (Experiment 3)

Experiment 3 studied the ability of selections of kikuyu to colonise an area by runners. It suffered a setback when overvigorous use of Roundup® damaged plants and suppressed growth, especially in 1 replicate. However it recovered well and has demonstrated cultivar differences in the ability of kikuyu selections to colonise an area, especially related to their ability to produce runner mass. The experiment has indicated that no single agronomic attribute can give a good assessment of the ability of a selection to invade an area or maintain ground cover and to produce utilisable forage. Forage yield, plant height and potential runner production, as well as quality attributes, are all necessary to assess the performance of a kikuyu selection.

5.4 Comparison of regional kikuyu ecotypes and commercial cultivars (Experiments 4 and 5)

Experiments 4 and 5 studied the phenotypic and genetic variation in kikuyu ecotypes collected from within Australia. Phenotypic characters of these ecotypes were recorded in autumn, winter and spring 2005. Genotypic variation was assessed on material collected at Wollongbar after some initial pilot studies to determine which primers provided best discrimination within kikuyu.

The experiment has demonstrated that there is considerable phenotypic variation in ecotypes but cv. Whittet was higher yielding than any local ecotype. Most of the elite plants from these populations came from Whittet, which suggests that it will form the basis for any selection or breeding program.

Gene fingerprinting was carried out on this material and it indicates that kikuyu in Australia is from two distinct genetic groups, one related to Whittet and the other related to a 'common' type. There was generally good agreement between the members of these genetic groups in terms of phenotypic characters.

5.5 Comparison of plants from mutagenised Whittet kikuyu seed (Experiments 6 and 7)

Experiments 6 and 7 studied a new range of mutagenic kikuyu plants from the four mutagenic treatments imposed on Whittet kikuyu seed by the earlier NSW Department of Primary Industries project (Dairy Australia, DAN063). An assessment of this material in terms of both agronomic and quality attributes suggests that there is considerably more inter-plant variation in this material than in the ecotype selections. There were, however, no significant differences between plants produced by the four seed treatments. This suggests that the gene mutations were randomly produced within the original population and that no one chemical, or combination of chemicals, was more efficacious in creating mutations.

6 Impact on Meat and Livestock Industry – now & in five years time

6.1 Impact on Meat and Livestock Industry – now

This pilot project sought to investigate the variation within the populations of kikuyu and to determine where the most likely elite material could be found for a future breeding program. As such, it will have no immediate effect on the Meat and Livestock or Dairy Industries.

The finding that Whittet is the most productive and useful cultivar is not new. However, it does confirm current knowledge, albeit that we used single plants which do not closely relate to kikuyu's performance in a sward situation. The leaf quality measured in this series of experiments was generally higher than those recorded in literature and confirms the recommendations made from previous research conducted at Wollongbar that more intense management to maintain kikuyu continuously in a leafy state will optimise animal production.

6.2 Impact on Meat and Livestock Industry – in five years time

What impact these results will have in the future depends on whether a breeding program to improve the digestibility of C_4 grasses commences in the next few years. The current experiments have demonstrated that there is considerable phenotypic variation between individuals of existing populations in terms of yield and the most useful quality indicators (crude protein, NDF, ADF, *in vitro* digestibility and ME). While the 3 - 5% improvement over the population mean in these attributes is not perhaps the 'quantum leap' that the industry might like, it has to be taken into context. We have been dealing with very small numbers of plants in this experiment, probably in the order of 400. In the previous project (DAN063) numbers in the order of 40,000 were screened and even this is small in comparison to breeding population in other crops.

A breeding program which combines gene marker selection to rapidly screen large numbers of seedlings for lignin levels (targeting genes controlling the lignin biosynthetic pathway) with traditional selection for agronomic traits will allow a more rapid and reliable development of elite material than traditional selection processes of growing plants to maturity before screening for quality. We have also demonstrated that gene technology for lignin developed for sorghum and maize can successfully adapted to other C4 species. Kikuyu then will be a test species and there is no apparent reason why the technology developed for it can not be used for other grasses.

We have already demonstrated that a 1 digestibility unit increase in the digestibility of *Digitaria milanjiana* and underlying changes to morphological characters (such as leaf content) translated into a 1 L/cow/day (5%) increase in short term milk production (Lowe *et al.* 1991). This suggests that the level of improvement in digestibility of kikuyu we recorded here could result in considerably greater increases in milk or meat production. The concern with the *Digitaria* breeding program was that its agronomy was not maintained at the same time as quality was improved. However we have demonstrated that at least with kikuyu, we have been able to record improvements in both quality and production. Gene marker technology should ensure that we are able to concurrently select for quality and yield.

7 Conclusions and Recommendations

7.1 Conclusions

The documented variation within kikuyu in Australia is sufficient to justify the commencement of a breeding program designed to develop a cultivar with improved quality. Whittet seems to be the most promising source of variation of the commercial cultivars. There is some scope for further, and wider, collection of material from natural populations but this was generally more likely to yield material with a lower growing habit and more aggressive runner production, rather than higher foliage production.

Mutagenesis of seed lots appears to provide greater variability within a population than occurs naturally and this is the most likely source of high quality material. The *brown mid rib* trait was not produced in the mutagenesis populations generated by the NSW Department of Primary Industries project. However, this trait is the most promising route to providing a large increase in digestibility of kikuyu.

There appears to be two different sources of genetic material present in Australia today. One is related to cv. Whittet which tends to be higher yielding, more up right and quite variable in runner development. The second is what has been known the 'common' type, with a relatively prostrate growth habit, more vigorous runner production and moderate forage production. All the material collected from southern areas of Australia fall into the latter category. The 'Whittet' type contains the other commercial cultivars, Noonan and Crofts (Breakwell was not tested). There were three exceptions but whether these had been contaminated by Whittet seed or were different sources is unclear.

7.2 Recommendations

It is recommended that a genetic improvement program for kikuyu be commenced for the following reasons:-

- Gene mutation works effectively in kikuyu and we have demonstrated that it can lead to increases in yield and quality. Further increases in quality may be made by screening larger numbers of mutant kikuyu plants.
- *Brown mid-rib* mutants have been identified in maize, sorghum and pearl millet (a close relative of kikuyu). These mutations result in large increases in digestibility (10-33%) and are due to mutations within lignin biosynthesis genes. Recent analyses have shown that large digestibility increases can be obtained through the brown mid-rib trait without compromising agricultural productivity.
- *DPI&F have undertaken an EST sequencing programme in kikuyu and have identified the majority of genes involved in lignin biosynthesis*
- The technology and expertise is available within DPI&F to screen large numbers of plants for lignin biosynthesis markers. Screening for lignin gene mutants at the genotypic level should be much more efficient than phenotypic screening and involve the need for fewer plants.
- Mutations in a couple of key lignin genes have been demonstrated to create the brown-mid-rib trait in other C4 grasses. We would like to create and select mutations in lignin genes in kikuyu to identify new sources of genetic variation. In parallel we would also like to use sorghum as a model system to identify other genes which may be useful or better targets for mutagenesis. A mutant sorghum population has already been created and is ready for "mining" for new mutations.
- If Australia does not invest in this technology it will be left behind the rest of the World as the South American countries have already invested significantly in C4 quality improvement technology.
- Adoption will not be restricted by public resistance to GMO technology or IP issues because new genes are not being introduced into the plant. Novel genetic variation will be created by chemical mutatgenesis and selected via molecular marker technology (TILLING).
- A spin-off of the Luckett *et al.* (1996) program was the identification of an accession which was more tolerant of kikuyu yellows than cv. Whittet. Further work in the area of disease tolerance should be incorporated into a future breeding program.
- Climate change suggests that spring conditions in subtropical eastern Australia are likely to be hotter in the future. This is likely to further restrict the use of ryegrass in this region and increase the likelihood of using a 'high quality' kikuyu under irrigation for spring forage. Furthermore, kikuyu demonstrates more efficient water use efficiency than C3 grasses such as ryegrass.

7.3 Future Research

We propose that the development of an elite, high quality kikuyu cultivar be conducted in two stages. The first stage, over 3 years, would encompass research on both kikuyu and sorghum. Sorghum would be used as a model system: highly digestible brown mid-rib mutants already exist in sorghum and have been shown to result from specific "knock-out" mutations in lignin genes, although they were initially identified visually by "brown mid-rib" phenotype. A large mutant sorghum population suitable for TILLING of new alleles has already been created and very large numbers of expressed gene sequences have already been analysed to identify sorghum lignin genes. This resource will be used to identify other genes which may be useful or better targets for mutagenesis. Genomic DNA extracted from the sorghum mutant population will already have been isolated at the start of this project. Concurrently, available kikuyu accessions will be screened for allelic variation in lignin genes and a mutagenised kikuyu population suitable for TILLING will be created, full-length lignin gene sequences will be developed and gene expression patterns identified. Stage 2 will start about 2 years into Stage 1 and will involve screening of the mutagenised kikuyu population for new alleles in selected lignin biosynthesis genes, followed by analysis of lignin composition, digestibility and agronomic traits of homozygous mutant individuals. Elite quality plants would be selected and incorporated into a traditional breeding programme. Using such a schedule, it would be expected that an elite kikuyu cultivar could be ready for commercial development in 7-8 years. Negotiations to bring a commercial partner into the project would be done very early in the process.

Stage 1 project (3 years) will incorporate the following steps:

• Examine gene expression profiles for kikuyu and sorghum via oligonucleotide microarray analysis using array elements developed from in-house kikuyu ESTs and published sorghum ESTs. These experiments will be used to identify developmental gene expression profiles, tissue-specificity and effects of environmental stresses on gene expression, particularly for the lignin pathway genes.

• Isolate full-length gene sequences from kikuyu lignin genes. These sequences will be used to develop PCR-based markers for direct screening of allelic variation in available kikuyu germplasm collection (EcoTILLING).

• Further examine and quantify mutation level in Luckett *et al.* (1996) mutagenised seed collection. This analysis will be used to determine whether a new mutant population will have to be created.

• Generate new mutant kikuyu population suitable for isolating large numbers of mutant alleles of target lignin genes via TILLING approach.

• Develop PCR-based markers from full-length gene sequences and use these to screen mutant sorghum population for induced allelic variation for target lignin biosynthesis genes.

• Germinate seeds from plants identified as containing mutations in target lignin genes and assay homozygous mutants for changes in lignin composition, digestibility and phenotype.

• Examine the effects of mutations in multiple lignin genes within individual plants.

• Determine which lignin genes are the most suitable targets for mutagenesis to create improved digestibility.

Stage 2 Kikuyu (3 years: could start 2 years into Stage 1 project)

- Extract DNA from mutant kikuyu population
- Use information from stage 1 project to screen kikuyu mutant population (2000-3000 plants) for novel alleles in target lignin genes using TILLING approach
- Identify plants containing novel mutations in target lignin genes

- Create homozygous loci for individual mutant lignin genes
- Assay homozygous mutant plants for changes in lignin composition, *in vitro* digestibility and agronomic traits such as yield and runner development
- Set up crosses from suitable individuals to create high quality cultivar.

.

8 Bibliography

Anon. (2002) The CoPlot manual, Version 6.101. (Cohort Software: Monterey California)

GenStat, 5, Committee (1993) 'GenStat 5 Release 3 Reference Manual. Software version 5 release 3.2.' (Oxford University Press: Oxford)

Hacker JB (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.

Lowe KF, Moss RJ, Cowan RT, Minson DJ, HackeR JB (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 [ed full sibs.* **31**, 603-608.

Lowe SA, White JA, Lowe KF, Bowdler TM (2002) 'Evaluation of kikuyu cultivars. Final Report. Project Report Series QO 02013.' Department of Primary Industries, QO 02013, Brisbane.

Luckett D, Kaiser A, 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, DAN 063, Orange, NSW.

Masaoka Y, Wilson JR, Hacker JB (1991) Selecting for nutritive value in *Digitaria milanjiana* 3. Relation of chemical composition and morphological and anatomical charateristics to the difference in digestibility of divergently selec. *Australian Journal of Experimental Agriculture [ed full sibs..* **31**, 631-638.

Minson DJ, Hacker JB (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 RN (1990) *Pennisetum clandestinum* Hochst. ex Chiov. (kikuyu grass) cv. Whittet. In 'Register of Australian Herbage Plant Cultivars'. p. 73. (CSIRO: Melbourne)

9 Appendices

9.1 Appendix 1: Evaluation of the mutagenic populations generated by Project DAN063 (Experiment 1)

(See File *Experiment 1 Final Appendix.doc* or *Experiment 1 Final Appendix.pdf*)

9.2 Appendix 2: Evaluation of pre-release populations of commercial kikuyu cultivars (Experiment 2)

(See File *Experiment 2 Final Appendix.doc* or *Experiment 2 Final Appendix.pdf*)

9.3 Appendix 3: The effect of plant habit and runner production on the ability of kikuyu to colonise bare ground (Experiment 3)

(See File *Experiment 3 Final Appendix.doc* or *Experiment 3 Final Appendix.pdf*)

9.4 Appendix 4: Performance of, and variation within, ecotypes of *Pennisetum clandestinum* selected from regions throughout Australia (Experiments 4 and 5)

(See File *Experiment 4 & 5 Final Appendix.doc* or *Experiment 4 & 5 Final Appendix.pdf*)

9.5 Appendix 5: Performance of, and variation within, populations of *Pennisetum clandestinum* cv. Whittet treated with two chemicals known to cause mutagenesis (Experiments 6 and 7)

(See File *Experiment 6 & 7 Final Appendix.doc* or *Experiment 5 & 6 Final Appendix.pdf*)

9.6 Appendix 6: Publication of results from project

(See File *Publications Final Appendix.doc* or *Publications Final Appendix.pdf*)