Case study 5: Research strategy for selecting 'best-bet' accessions of Napier grass for feeding to livestock

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Contents

Summary

The International Livestock Research Institute holds a collection of 53 accessions (genotypes) of Napier grass in its Forage Gene Bank in Addis Ababa. Napier grass has become a popular feed for livestock among smallholder farmers, because of its rapid growth, high nutritive value and yield, especially for dairy cattle. The collection shows considerable morphological variation, and so it was decided to evaluate a set of 'best-bet' accessions likely to be favoured by farmers. For example, strains with hairy leaves tend not to be favoured by farmers as they can be rough on their hands; also animals do not find hairy strains as palatable as less hairy strains.

A series of studies were therefore designed to gradually reduce the number of accessions to a manageable set that could receive final evaluation in a sheep-feeding study. This case study shows how a strategy for undertaking this research was developed, and then describes the different studies undertaken during the research process.

The first study took the form of a randomised block field experiment to compare a series of agronomic and morphological measurements in all 53 accessions. As this experiment was done in just one location it was then repeated in two locations to assess the extent of genotype x environment interactions. Nine of the better performing accessions were also evaluated in 10 trials across different countries in sub-Saharan Africa to compare their performance across different environments. The results of these three trials then finally led to a set of five 'best-bet' accessions to be evaluated when fed to sheep.

Results from the sheep study are included, as well as the protocol developed for the study to help students and young researchers to understand some of the important ingredients of a

study protocol.

Methods of principal component analysis and cluster analysis applied to the morphological data collected in the first study are also illustrated.

Glossary

Some terms with which the reader may not be familiar

Accession: A distinct, uniquely identifiable sample of germplasm representing a cultivar, breeding line or a population.

Dry matter: The total weight of plant material after removal of water by oven drying at 60 C, expressed as a percentage.

Clone/clonal: A genetically identical copy of living material.

Digestibility: A measure of the conversion of feed, in the rumen and intestines, into soluble and diffusible products, capable of being absorbed by the blood.

Degradability: The reduction of plant material to its component parts during digestion in the rumen.

Genotype: The genetic constitution of an individual plant or organism.

In vivo: Experimentation using the natural conditions in live animals.

In vitro: Experimentation in test tubes in artificial laboratory conditions outside the animal.

Organic matter: The carbon-based compounds that are non-mineral components of the plant.

Background

Napier or elephant grass *(Pennisetum purpureum)*, together with its hybrids with *Pennisetum glaucum*, is one of the major forages used in zero-grazing systems in many parts of sub-Saharan Africa. It is a tall, perennial grass that is indigenous to tropical Africa, and performs well from sea level up to an altitude of 2000m. It can withstand repeated cutting and regrows rapidly, producing a high biomass that is very palatable in the leafy stage. Since pests and disease problems are rare, it can be made into silage for feeding to livestock during the dry season. When grown in conjunction with leguminous trees, the plant's total yield and nutritive value are increased. Besides its use as fodder Napier grass can also be used for soil regeneration and mulch. In some countries it is used for the manufacture of paper pulp.

Napier grass has become a popular feed for livestock among smallholder farmers, because of its rapid growth, high nutritive value and yield, and ease of propagation, especially for dairy cattle, and especially in Kenya.

Source: ILRI

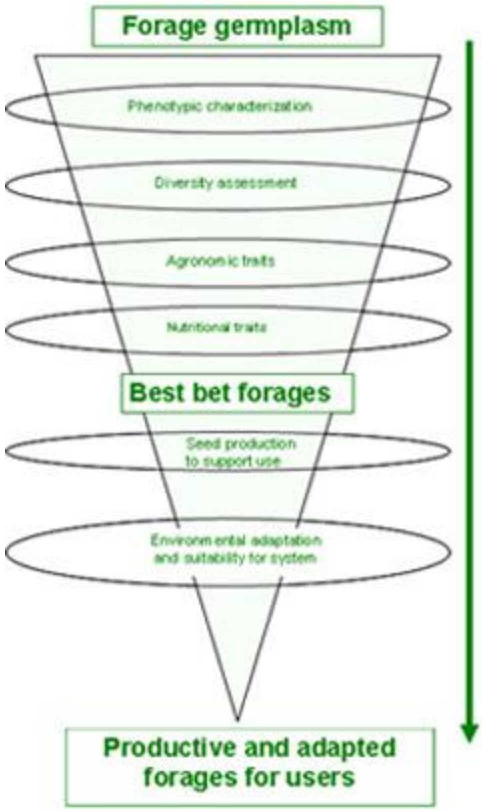
The International Livestock Research Institute (ILRI) holds 53 different accessions (genotypes) of *Pennisetum purpureum* in its Forage Gene Bank in Addis Ababa. This collection has been assembled from several African Countries and the USA and shows considerable morphological variation. Some of the accessions were donated by the International Centre for Research in the Semi-arid Tropics (ICRISAT) following evaluation of a number of them for dry matter yield in Zimbabwe. When the project came to an end the accessions were given to ILRI for inclusion in its Forage Gene Bank together with information on their yields.

The Napier grass accessions have different agronomic characters (such as dry matter yield, plant height), nutritional characters (such as protein concentration, digestibility) and morphological characters (such as leaf length, leaf hairiness, stem thickness). Different accessions tend to be suitable for different locations, different altitudes, soil types and climates.

Source: Jean Hanson

In the case of large germplasm collections it is impossible to fully evaluate each accession individually, for one would need to conduct many experiments to test how each accession grows in different locations, and how it performs when fed to livestock. One therefore needs to develop a research strategy that allows the number of accessions to be gradually reduced to a manageable sample for the final and ultimate evaluation.

This case study demonstrates how a research strategy was designed to conducting the necessary steps to be followed in the research process.



Research strategy

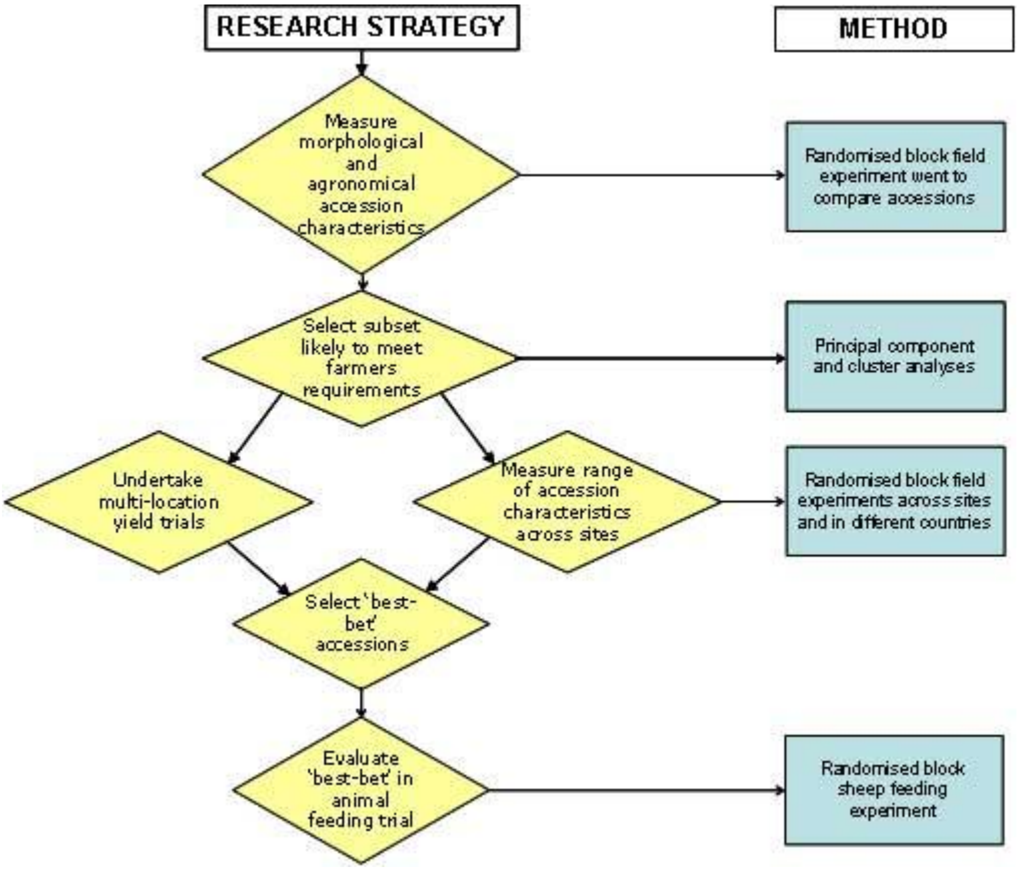
Before developing a research strategy one needs first to consider the agronomic, nutritional and morphological characters that smallholder farmers look for.

- Obviously they need strains of Napier grass that produce high yields and are used efficiently by cattle, e.g. strains with high digestibility.
- But taller strains may be particularly advantageous in certain environments than others.
- Smallholder farmers tend to prefer strains without hairy leaves since they are rough on their hands.
- Also animals do not find hairy strains as palatable as less hairy strains.

It is probable that there are different groups of accessions that share similar trait characteristics and some may even be virtual duplicates of each other. By applying multivariate methods to the range of morphological and agronomic characteristics of the available accessions it should be possible to group the accessions into different 'types' and select individual accessions within these different types for further evaluation.

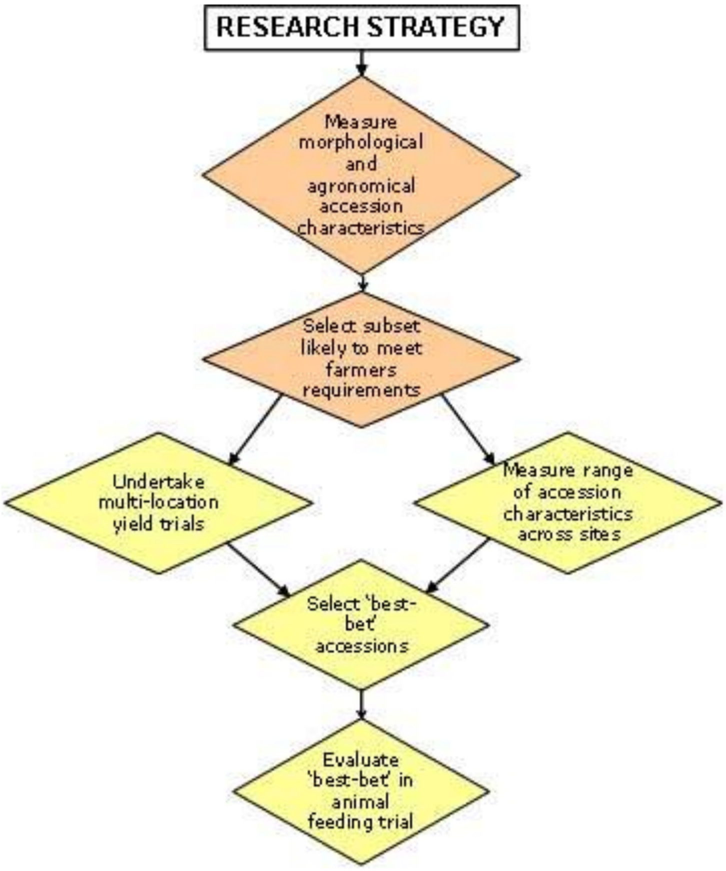
Source: Jean Hanson

A series of experiments were thus planned to select the accessions that best met the above characteristics. As we shall see, we shall essentially divide the research process into four studies. These will be described as Studies A, B1, B2 and C.



Study A

The first step is to carry out a comparative study of the agronomic and morphological characteristics of the 53 different accessions.



A randomised blank experiment was undertaken at ILRI's Debre Zeit field station in Ethiopia. The study was carried out by Van de Wouw et al. (1999).

Conducting an experiment only at one location, however, has certain limitations - strictly the results cannot be extrapolated beyond that location. Thus the patterns determined among the accessions in Study A can only be assumed to apply to the environment where the study was carried out.

Replication across sites is therefore strictly necessary to determine those accessions that perform well in different environments and also to test what is known as genotype x environment interactions.

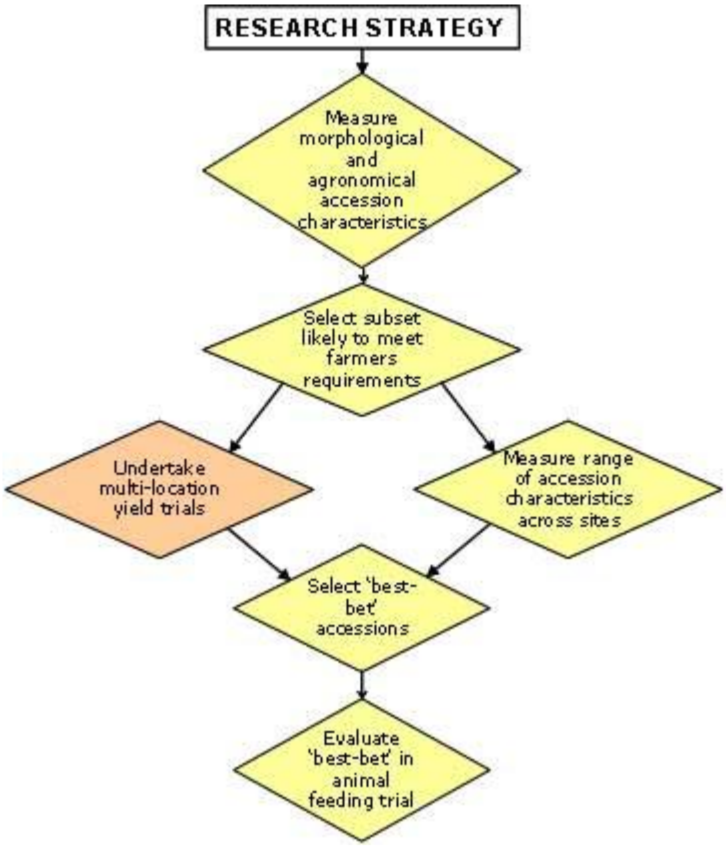
Napier grass has the advantage that it propagates clonally. In other words one can divide a plant and know that the two halves are genetically identical in every respect. Thus any statistically significant differences observed when the two halves are evaluated at different sites should theoretically be due entirely to the influence of environment.

Two separate studies were undertaken: Study B1 and Study B2.

Source: Solomon

Study B1

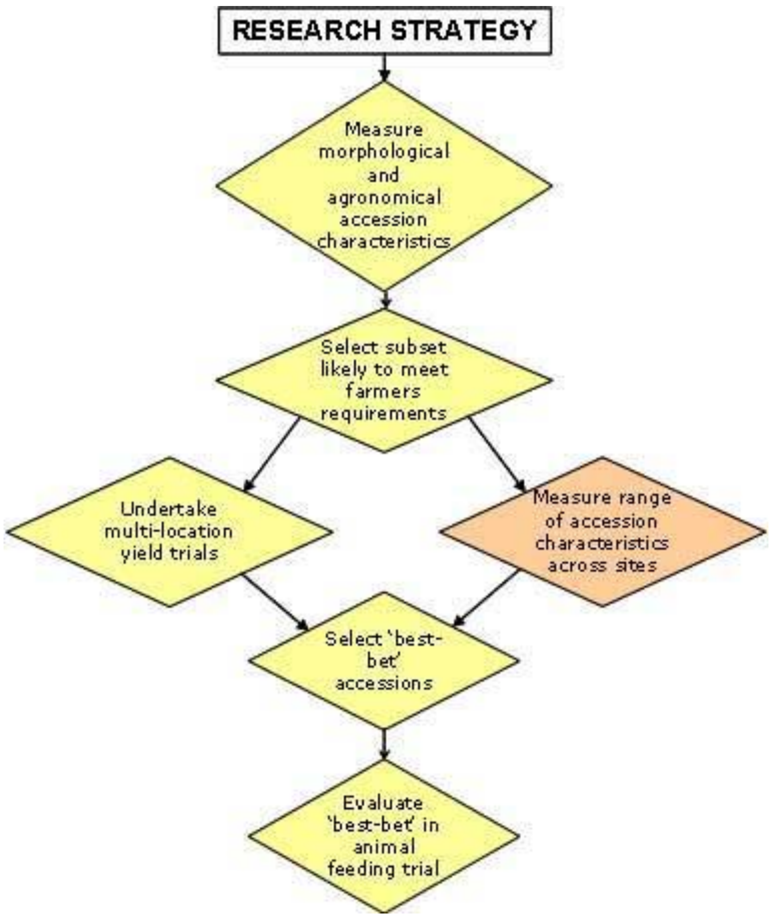
Ten trials were organised by AFRNET in different countries, the former ILRI/NARS African Feed Resources Network (Ndikumana and Kamidi), to evaluate nine accessions recommended by ICRISAT. The purpose was to compare the performance of the different genotypes over a range of climates and environments.



Study B2

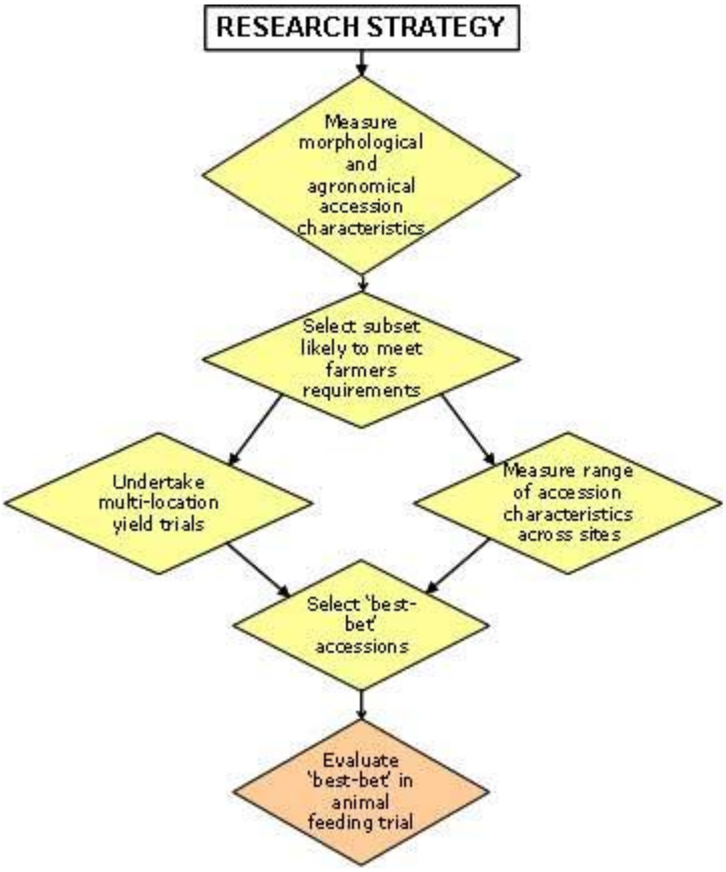
This experiment was undertaken following a similar design to Study A to compare 57 genotypes but replicated at two sites in Ethiopia: Debre Zeit, where the previous experiment was undertaken, and at Zwai.

Morphological and agronomic characteristics were measured as in Study A.



Study C

The ranking of accessions based on agronomic measurements of dry matter yield, protein concentration and in vitro estimates of digestibility do not necessarily correlate with growth rate achieved by animals when fed these accessions. Thus, a final selection of accessions was included in a sheep feeding experiment to compare their effects on animal performance.



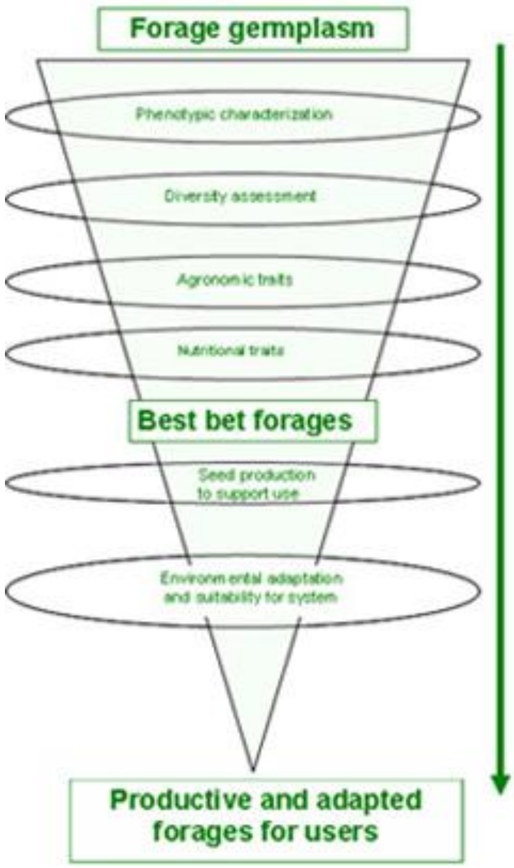
Animal experiments are expensive and so they can only be included at the end of the research process when a short list of 'best bets' likely to be favoured by farmers has been obtained from the earlier experiments. The short list was selected for a number of traits including yield, digestibility, plant height and level of leaf hairiness.

Source: Richard Fulss

In summary, we have described here the research strategy involved in assessing the suitability of different Napier grass accessions as a feed for animals in different environments. The overall process is one which

- begins with the available population of accessions,
- gradually acquires information about their characteristics
- ends with a smaller sample suitable for evaluation in animal feeding experiments.

If a biometrician becomes involved in a project such as this then he/she needs to understand the whole process and how each study helps to complete a part of the jigsaw.



Objectives

Having considered our research strategy we can now formulate an objective for the whole study, namely:

- To identify Napier grass accessions from the ILRI Forage Gene Bank suitable for growth under different environmental and climatic conditions.

Each study will, of course, have its own separate objective, namely:

- Study A Compare the agronomic and morphological characteristics of 53 accessions of Napier grass from the ILRI Forage Gene Bank. Use multivariate methods to describe groups of accessions and select a subset of genotypes for further evaluation.
- Study B1 Compare a selection of Napier grass accessions for yield and acceptability in 10 different locations covering a range of environmental and climatic conditions in East and southern Africa.
- Study B2 Study variation in morphological and agronomic traits of 56 accessions grown at two locations and determine evidence for genotype x environment interactions.
- Study C Having determined a shortlist of 'best bet' Napier grass accessions from the previous three studies that are likely to be found favour with farmers, compare growth rates of sheep fed the different accessions

Study design

Contents

Study A
A randomised block field experiment to evaluate a range of accessions with the aim of selecting a set of 'superior' or 'first best bet' accessions suitable for further evaluation.

Study B1
A cross-country evaluation of some of these 'first best bet' accessions, together with additional ones previously evaluated and recommended by ICRISAT.

Study B2
Two randomised block experiments at two sites in Ethiopia to examine genotype x environment interactions between the range of accessions.

Study C
A randomised block sheep feeding experiment to evaluate final 'best bet' accessions.

Study design/Study A

The experiment was carried out in the Ethiopian highlands at the ILRI Debre Zeit Research Station south of Addis Ababa at an altitude of 1850m above sea level (Van de Wouw et al., 1999). The area has an average annual rainfall of around 850 mm, of which 80% falls between June and September.

The 53 accessions were planted from stem cuttings in three replicates in 1.5 x 2.7 m plots, each replicate subdivided for ease of management into three blocks (see below), using 22 plants per plot with a spacing of 50 cm within rows and 60 cm between rows. The accessions were randomised to plots within each replicate (see next page).

The plants were irrigated and allowed to grow undisturbed for one year. One hundred kg/ha N (as urea) was applied in a single application during the establishment period. Plant height of each accession was recorded and all plots were cut back to 20 cm at the start of the rains.

Replicate 1	Block 1	Block 2	Block 3
Replicate 2	Block 1	Block 2	Block 3
Replicate 3	Block 1	Block 2	Block 3

Different agronomic and morphological measurements were observed in each plot 10-15 weeks after cutting. Whenever possible, measurements were made on 10 plants per plot. Leaf measurements were taken on the third leaf below the first completely unrolled leaf at the top of the plants.

Randomisation of accession numbers for Replicate 1

Block 1	Block 2	Block 3
15743	16799	16793
14982	16840	16821
16836	16808	14355
16838	16817	16800
16816	16782	16809
16621	14983	16789
16805	16785	16837
16790	16801	16788
16810	16794	16804
16786	16797	16803
16839	16813	14984
15357	16815	14389
16811	16783	16792
16819	16793	16806
16791	16822	16795
16787	16835	16798
16814	16812	16818
16784	16802	16807
16834	16902	

The data were analysed as a randomised block to determine mean accession values.

Source of variation	d.f.
Replicate	2
Accession	52
Residual	101
Total	155

Multivariate methods were applied to the 53 accession means using cluster and principal component analyses (Van de Wouw et al.,1999).

Both the randomised block analysis and the multivariate methods of analysis are shown under Statistical modelling.

Study design/Study B1

The multi-location evaluation of a selection of Napier grass accessions was undertaken by the African Feed Resources Network across a number of locations as shown in the table below (Ndikumana and Kamidi). The nine accessions had been recommended by ICRISAT. Except for one location promising 'local' varieties commonly grown in the particular region were also included in the experiments as shown in the table. Experiments were laid down as randomised blocks but details of the numbers of replications have not been given in the publications.

Accession	Location									
	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10
16834	x	x	x	x	x	x		x		x
16835	x	x	x	x	x	x		x	x	x
16837	x	x	x	x	x	x	x	x	x	x
16838		x	x	x	x	x		x	x	x

16840		x		x	x				x	x
16786	x	x	x	x	x	x	x	x		x
16791	x	x	x	x	x	x	x	x		x
16798	x	x	x	x	x	x	x	x	x	x
15743		x		x	x	x		x	x	x
Local varieties	4	1	1	3	6	0	1	2	1	1

L1: Cote d'Ivoire; L2: Ghana, L3: Tanzania; L4: Tanzania; L5: Kenya; L6: Ethiopia; L7: Uganda; L8 Ethiopia; L9: Zimbabwe; L10 Malawi

The sites covered a wide range of agro-climatic conditions in the sub-humid (L3, L4, L7, L9 and L10), humid (L1 and L2) and highland (L5, L6 and L8) ecological zones.

The altitudes covered by the sites range from 66m above sea level at Tang (L5) to 2400 m above sea level at Holetta (L8). The annual rainfall at the sites ranges from 900 mm at Morogoro (L2) to 1900 mm at Kakamega (L5) all in a bimodal pattern except at Bunda which has a unimodal pattern. Frosts occur at Bunda (L6) and Holetta at some nights during the dry season.

The methodology for the agronomic evaluation was standardised across sites. The accessions/hybrids were planted in randomised complete block designs at an inter-row spacing of 0.75m and within row spacing of 0.40m. Each accession was planted on lines 6m long and the harvesting was to be done on the middle 2m length when the plants had reached 1.5m height or at the end of the first growing season.

The data that were collected included rainfall, date of planting, days to flowering, plant height at intervals of 20 days, dry matter yield, crude protein, NDF content, leaf:stem ratio and number of tillers per plant at harvesting.

Fifty six Napier grass accessions were grown at two sites in Ethiopia: Debre Zeit (as in Study A) and Zwai. Both sites are ILRI research stations (several kilometres apart), with Zwai at an altitude of 1650 mm, 200 mm lower than Debre Zeit. The Zwai Research Station also experiences slightly lower rainfall and slightly higher temperatures than Debre Zeit.

The accessions were planted in randomised blocks in similar arrangements to Study A with accessions randomised to plots. Plot shape, however, was slightly different. Here, each plot was 5 metres in length and contained a single row of 10 plants, each spaced 0.5 metres apart. Rows were planted one metre apart. Similar agronomic and morphological traits were measured as in Study A.


Source: Solomon Teka

Study design/Study C

The results for the previous three trials were then considered together and five 'best-bet' accessions chosen based on yield and adaptation. These were:

14984 Best accession in Study B2
16803 Best accession in Study A
16786 Best accession in Study B1
16835 Second best accession in Study B1
16837 Second best accession in Study A

Forty eight young male lambs, weighing up to 20 kg were purchased in local markets. They were quarantined for six weeks, treated for internal parasites, vaccinated against common diseases and adapted to consuming green Napier grass before starting the experimental phase.

The experiment is described by Hanson and Fernandez-Rivera (2005) but one or two aspects of the design of the experiment are unclear. These are investigated further in some of the Study questions. We think the following closely represents the steps taken and describes more explicitly the way such animal experiments should be designed.

Lambs were weighed, sorted according to their body weight and put into eight groups of six so that the six heaviest lambs were in group 1, the next six in group 2 and so on. Five lambs within each group were then randomly allocated to each of the five accessions and a sixth to be slaughtered at the beginning of the experiment to determine initial carcass characteristics. The lambs on experiment were then assigned in group order to pens (thus group 1 to pens 1-5, group 2 to pens 6-10 etc,) so that any variations due to the position of a pen within the building can be confounded with the lamb's initial weight grouping. The lambs were fed for 12 weeks and weighed at the beginning and at two-week intervals during the experiment.

Lambs were individually fed with wilted green Napier grass at libitum adjusting the level of offer weekly to allow for a refusal of approximately 15% of fodder offered. Refusals were collected and weighed daily.

Hanson and Fernandez-Rivera (2005) suggested that the data should be analysed by analysis of covariance with initial weight as a covariate. Having designed the experiment as a randomised block (groups of lambs based on initial weight) the analysis should strictly be that for a randomised block with a term for replicate. A covariate for initial weight (see below) can also be included if this accounts for additional residual variation.

Response variables included voluntary intake of dry matter, in vivo digestibility, digestible organic matter, average daily gain and carcass weight. The sheep adapted poorly to the diet during the early stages of the experiment and overall growth rate was poor. This may have influenced the conclusions that can be drawn from the experiment. It would have been preferable to have used cattle for this experiment, being the livestock to which Napier grass is generally fed, but this would have been more expensive.

Source	d.f.
Accessions	4
Replicates	7
Initial weight	1
Residual	27
Total	39

Source: Salvador Fernandez-Rivera

Study protocol

More details for the design of Study C are given in the accompanying study protocol (Hanson and Fernandez-Rivera, 2005). Students should be encouraged to read this protocol, and tackle some of the study questions that refer to it. It shows the student or young researcher how to go about developing a study protocol - the headings are given below. Case Study 13 also deals with study protocol writing.

Main heading	Sub-heading
Justification	
Objective	
Null hypothesis	
Expected outputs	
Materials and Methods	Accessions
	Animals
	Feeding of animals
	Monitoring of experiment
	Response variables
	Experimental design and analysis
Calendar	
Budget	
References	

Source material

Dry matter yields for the 53 accessions compared in Study A are contained in CS5Data1 and documented in CS5Doc1. These are used to describe the variation in the data as determined by analysis of variance. Corresponding morphological data are contained in CS5Data2. These are mean values for the three replications which are used for principal component and cluster analyses. The morphological measurements are described in CS5Doc2.

Individual lamb body weights and average daily weight gains for Study C are shown in CS5Data3 as described in CS5Doc3.

Statistical modeling

Study A

Here we look at some analysis of data from Study A. Individual yields of Napier grass recorded in the randomised block experiment are contained in CS5Data1. Using Stats → Analysis of Variance… and choosing 'One-way ANOVA (in Randomized Blocks)' for the **Design**, and ticking %cv in the dialog box after clicking the **Options…** button, the analysis of variance below can be obtained for total dry matter yield.

We note first that that there is no variation across replicates (variance ratio = 0.85) and if we look at the bottom of the table we see that the coefficient of variation is 41.4%, implying a large variation across plots and indicating the difficulties in controlling the variation in a randomised block experiment that has to accommodate so many accessions.

Despite this there are significant differences in dry matter yields among the 53 accessions (P<0.001).

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***** Analysis of variance *****

Variate: Totalyield

Source of variation        d.f.      s.s.       m.s.      v.r.  F pr.

Replicate stratum             2     1484.8      742.4     0.85

Replicate.*Units* stratum
Accno                        52    94160.2     1810.8     2.07  <.001
Residual                    104    90825.1      873.3

Total                       158   186470.1


*** Stratum standard errors and coefficients of variation ***

Variate: Totalyield

Stratum                    d.f.        s.e.       cv%

Replicate                     2       3.743       5.2
Replicate.*Units*           104      29.552      41.4
```

There are now alternative designs for dealing with experiments in which large numbers of plant varieties need to be compared. These designs are known as row-column designs which can efficiently handle experimental spatial variation in both directions. The late Mr Harvey Dicks provided the following solution for 54 (53+1) accessions in a 3-replicated 18x3 row-column design which he presented during a training course in Gaborone in June 2009 in which this case study was discussed.

Column		1	2	3	1	2	3	1	2	3
Rep	Row									
1	1	27	35	7	6	42	38	29	37	2
1	2	20	4	23	25	31	11	41	48	45
1	3	44	32	13	3	46	21	53	30	16
1	4	51	14	1	22	5	40	26	50	52
1	5	28	36	54	33	39	24	43	18	12
1	6	15	8	47	34	9	10	17	19	49
2	1	7	41	22	15	2	9	18	32	40
2	2	48	5	36	53	3	54	49	38	20
2	3	26	24	19	35	51	6	46	45	47
2	4	42	34	52	10	43	4	27	1	30
2	5	12	13	21	31	8	50	25	28	29
2	6	33	37	44	23	11	16	14	17	39
3	1	46	18	8	7	28	53	52	6	11
3	2	43	21	33	49	35	1	37	20	9
3	3	22	51	45	29	48	39	32	10	13
3	4	50	42	15	24	16	25	47	41	36
3	5	23	17	5	4	40	19	3	12	27
3	6	38	54	30	44	34	2	31	14	26

The numbers from 1-54 correspond to 54 accessions, one more than the 53 tested in the original experiment. Should an additional accession not be available then one of the 53 could have been duplicated. (Naturally a design with the prime number 53 is not possible.)

Note that each accession occurs once in each replicate and the columns are 'latinised' in the sense that no one accession occurs twice in any one column. This is clearly a more efficient design than the conventional randomised block since variation can be accounted for in two directions.

We can also take the opportunity to demonstrate methods of principal component and cluster analysis to attempt to characterise the different accessions (see Van de Wouw et al. (1999)). We shall use the morphological spreadsheet contained in CS5Data2.

Principal components

Using **Stats → Multivariate Analysis → Principal Components…** and clicking the **Options…** button in the dialog box to ensure that **Number of Dimensions** is 2 (to indicate we are only interested in the first two components), to tick **Scatter Plot Matrix of Principal Component Scores** and to enter 'Code' into the **Display Labels** window so that each point is identified by its accession code (note that Code must be declared as text), we obtain the scatter plot shown on the next page.

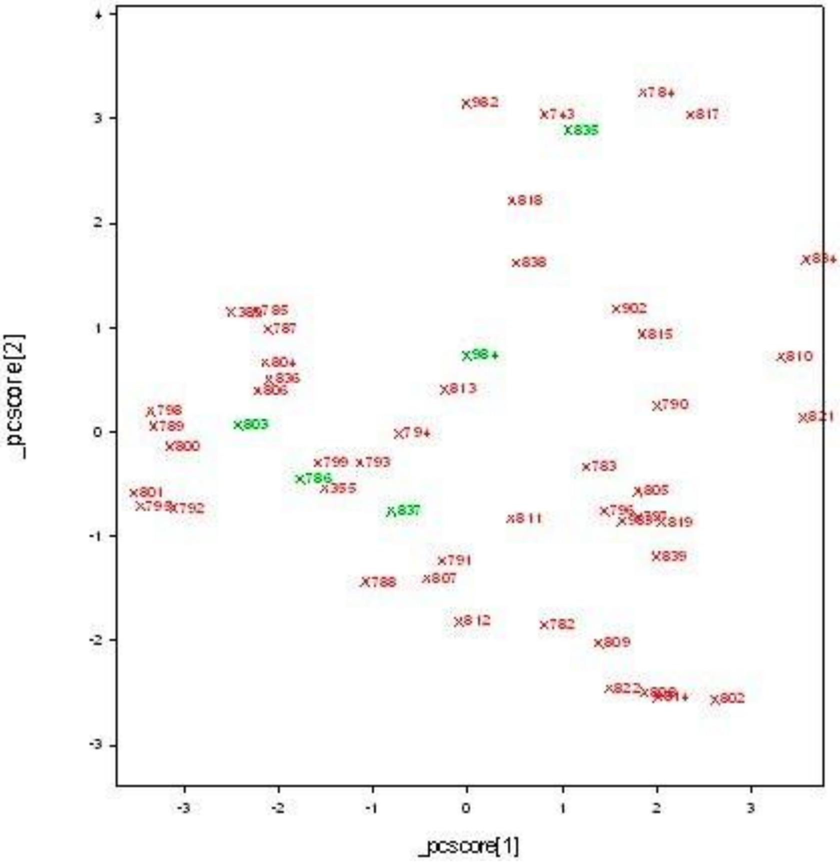
The output alongside shows the two principal components and their vector loadings (or coefficients). We can see that Growth, Length and Stem contribute high negative weights to the first principal component together with variables describing the extent of hairiness. In contrast, Ligule and Rhizome have slightly lower weightings but in the opposite direction. Highest weightings for the second component are provided by Roughness, Rhizomes, Stem and Internode. It is often possible to interpret a principal component biologically. Thus, the first component tends to separate accessions on the basis of size and degree of hairiness.

*** Latent Vectors (Loadings) ***

	1	2
Growth	-0.27774	0.07547
Internode	-0.00917	-0.33470
Leafhair	-0.39914	0.19338
Length	-0.37341	-0.29174
Ligule	0.23012	-0.23549
Nodehair	-0.34845	0.10751
Rhizome	0.24335	-0.41061
Roughness	0.06816	-0.50754
Sheathedge	-0.29179	-0.23581
Sheathhair	-0.34903	-0.03453
Sheathlen	-0.24451	-0.15888
Stem	-0.29922	-0.39974
Teeth	-0.17179	0.15722

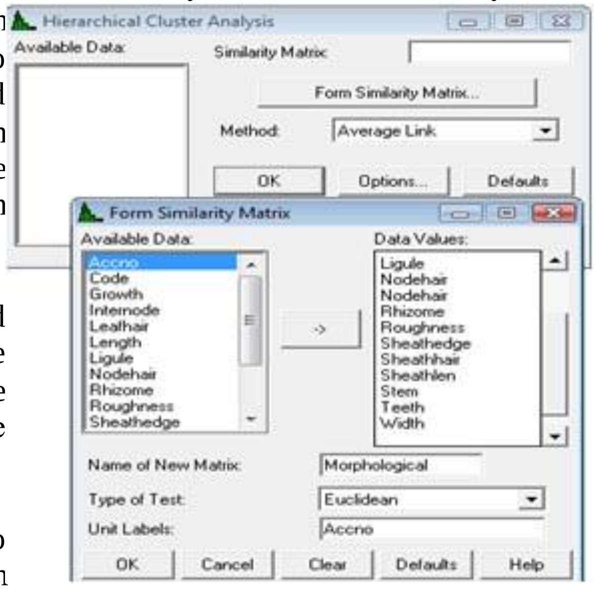
The GenStat output (not shown here) also reports the first component to have accounted for 32% and the second component 17% of the total variation expressed amongst the individual variables.

The five eventually selected 'best-bet' accessions are shaded in green in the scatter plot of the two principal components. Four (coded 786, 803, 837 and 984) are in close proximity in the central left of the diagram suggesting that they had similar morphological characteristics. As shown by from their principal component coefficients these strains tend to have less hairy leaves. These tend to be favoured by farmers as they are not so rough on their hands; also animals find less hairy strains more palatable. The fifth, accession no. 16835 (code 835) is in the top right of the diagram, away from the other four, tending to have smooth leaves and small rhizomes.



There are options in the dialog box to base the principal components analysis on either a matrix of sums of squares and products or the variance covariance matrix or a correlation matrix. Because the variables vary in magnitude we have used the correlation matrix, which has the advantage that the derived principal component is not unduly weighted to those variables with the largest values and correspondingly large sums of squares.

To undertake a cluster analysis we use **Stats → Multivariate Analysis → Cluster Analysis → Hierarchical…** A hierarchical cluster is developed by each accession first being defined as its own cluster. The two closest clusters are then merged into one larger cluster, and this continues until finally all the accessions have been formed into a single cluster. This process, as shown on the next page, is represented by a hierarchical tree with each node indicating the merges that occur at each step.



There are different clustering methods that can be applied that can result in slight differences in the final result, but we have chosen the 'Average link' method. Clicking the Options… button allows us to enter Accno to label the dendogram shown on the next page.

We need to first create a similarity matrix which we can do by clicking the **Form Similarity Matrix…** button and then putting the fourteen variables into the **Data Values:** window and the accession code into the **Unit Labels** box. We need to give a name to the matrix and we choose the name 'Morphological'.

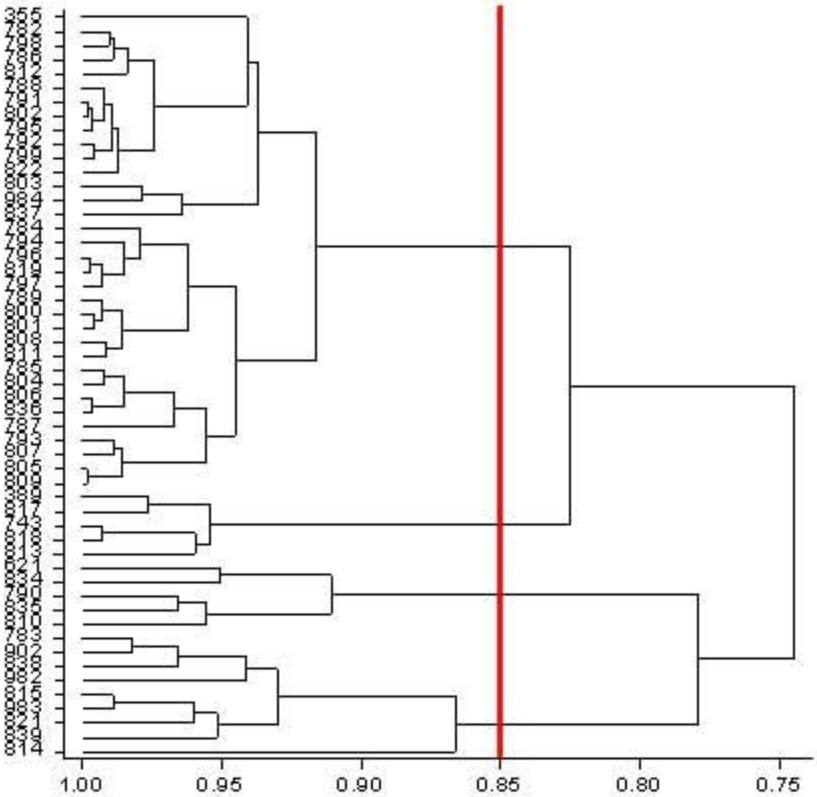
There are several methods for defining similarity coefficients. We shall keep the default Euclidean Method which is generally suitable for quantitative variables.

The dendogram illustrates the hierarchical cluster structure. As we move across the diagram from right to left we can see how the number of clusters increases. We can decide arbitrarily how many clusters we wish to define.

We can draw by hand a vertical line (shown here in red) and position it so that an appropriate number of clusters are defined. We have chosen to separate the data into five clusters.

We can see that the four 'best-bet' accessions 16803, 16786, 14984 and 16837 that fall close together in the principal component scatter diagram all appear in the first cluster. Accession number 16835 appears in the third cluster.

Thus, the two analyses support each other and draw similar conclusions.



Findings, implications and lessons learned

- This case study demonstrates the importance of developing a research strategy before embarking on a research project.
- A flow chart has been shown to illustrate the different stages in the research process. Ideally, a time frame also needs to be planned so that the project can be completed within the time allotted for the research.

- The details required in the preparation of a study protocol are illustrated. Researchers are not always good at writing protocols; a place for this in biometrics and research method courses will be beneficial.
- One step in the research strategy is perhaps missing - the process of how results should be disseminated to smallholders.
- Methods of analysis and interpretation of principal component and cluster analyses have been demonstrated.

Source: Jean Hanson

From a biological point of view the case study demonstrates how important it is not only to establish how well a Napier grass accession grows but also to determine how well animals perform when fed the accession. The following table shows the results for Study C. The reader can verify the results for average daily gain by analyzing the values stored in CS5Data3.

Accession	Dry matter yield (tonnes/ha)	Average animal daily gain (g/d)
14984	15.68	32.3
16803	10.84	35.2
16786	11.35	7.0
16835	14.42	22.4
16837	17.62	11.9

Considerable variation occurred both in dry matter yield and nutritive value of accessions. The table shows how it is necessary to consider both variables when selecting accessions for promotion with farmers. Accession 14984 had both a high dry matter yield and a good nutritive quality that resulted in good growth rates when fed to sheep. However, as mentioned earlier, the lambs were slow to adjust to the Napier grass feed and this may have affected the overall conclusions that can be drawn.

Study questions

1. Carry out an analysis of variance of the two individual columns of dry matter yields contained in CS5Data1. Do your results confirm that accessions 16803 and 16837 performed the best?
2. Discuss when it is appropriate to use a multiple range test. Apply Duncan's multiple range test to the mean values for dry matter yield in Study A and write a brief report. Which accessions would you recommend for a preliminary shortlist for further investigation based on their dry matter yields?
3. Average performances of the different accessions described by Ndikumana and Kamidi were based on an analysis of proportional differences from best local cultivar yields (Table 6 of their paper). Run a least squares analysis on the mean values in Table 4 of their paper. Compare the rankings you get from this analysis and compare the results you get with Table 6. Comment.
4. Having developed through on-station research recommendations to put to farmers on the most suitable accessions for them to plant, sketch the outline of an on-farm study that might be conducted with participation from farmers to confirm that these best-bet accessions perform well both in terms of dry matter yield and when fed to dairy cows.
5. Prepare a strategy for the research you intend to do for an MSc (or other) research project, including decisions on the type of study you need to do and how you intend to manage and analyse the data. It may be helpful to refer to the Research strategy teaching guide.

The following questions refer to the protocol by Hanson and Fernandez-Rivera.

1. Under the paragraph headed ***Animals*** the authors describe how they will assign the lambs to experimental groups in a way that the interval and mean of initial weight will be similar across groups. What do you think the authors mean? Do you think that this will achieve satisfactory animal randomisation to accessions?
2. Under the paragraph headed ***Experimental design and data analysis*** the authors describe their proposed analysis for a completely randomised design with initial weight as a covariate. Do you think that they are

justified in analysing the experiment in this way when they have grouped the lambs before randomising them? Investigate by fitting alternative models to the data in CS5Data3.

3. Under the paragraph headed ***Monitoring of experiment*** the authors describe how they will weigh the sheep three times at the start. Why do you think they want to do this? Describe two ways in which average daily gain might be calculated. Which would you recommend to the researchers?
4. Under the paragraph headed ***Experimental design and data analysis*** the authors refer to the experiment as being an 'exploratory non-replicated experiment'. Do you think that it is right to describe the experiment in this way? Describe how you might design a follow-up study. Which type of livestock would you use?
5. Review the protocol. Make any recommendations for improvement.

Related reading

Hanson, Jean and Fernandez-Rivera, Salvador 2005. *Experimental protocol: 'Genetic variation in in vivo digestibility and voluntary intake by sheep in five accessions of Napier grass'*. International Livestock Research Institute, Addis Ababa, Ethiopia 7 pp. Full text

Ndikumana Jean and Kamidi Roger,E (eds) (unpublished). *Regional evaluation of agronomic performance and nutritive quality of accessions of* Pennisetum purpureum *and its hybrids with* Pennisetum typhoides *in sub-Saharan Africa*. Draft ILRI Research Report. ASARECA Animal Agriculture Research Network (A-AARNET) 26pp. Full text

Van de Wouw, Mark, Hanson, Jean and Luethi, Samuel. 1999. Morphological and agronomic characterisation of a collection of napier grass (*Pennisetum purpureum*) and *P.purpureum x P.glaucum*. Tropical Grasslands **33**:150-158. **Abstract**,

Acknowledgements

We acknowledge the many researchers and field and animal technicians who have participated in the various aspects of this research. We thank Dr Jean Ndikumana and Mr Roger Kamidi for permission to use their unpublished report on the regional trials conducted by A-AARNET.