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SID 5 Research Project Final Report

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Project identification

1. Defra Project code: NF0446

2. Project title

   The Genetic Improvement of Miscanthus as a Sustainable Feedstock for Bioenergy in the UK.

3. Contractor organisation(s)

   Institute of Biological, Environmental and Rural Sciences (IBERS)
   Aberystwyth University
   Plas Gogerddan
   Ceredigion, UK SY23 3EB

   Plant Research International (PRI)
   P.O. Box 16
   6700 AA Wageningen
   visitors address: Building no. 107
   Droevendaalsesteeg 1

   6708 PB Wageningen, Netherlands.

4. Total Defra project costs (agreed fixed price): £ 250000

5. Project: start date .... 01 April 2009

   end date .... 31 March 2010

6. It is Defra's intention to publish this form.
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(a) When preparing SID 5s contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.
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(b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

This bridge project has ensured that the *Miscanthus* breeding programme established in NFO 426 at IBERS and PRI has continued running in 2009 -10.

For this extension project we identified 4 objectives.
1. Initiate field trait assessment of new genetic resources on marginal land.
2. Perform targeted crosses based on existing phenotypic information and improved methods of obtaining triploid interspecific hybrids.
3. Select from ~2000 3-yr old progeny from crosses made in 2006 and promote 100 outstanding selections to plots.
4. Identify and clone two mapping families for the development of molecular markers from crosses made in 2008 between parents with diverse yield traits.

Ten milestones were identified for the bridge proposal.

In this summary we report briefly on activity related to each milestone:

1. *Plant novel germplasm collected from Asia 2006 in the field and design a database (IBERS). Objective/ Approach 1*

Collection data (location and phenotype) from 192 accessions was placed in the IBERS database. 105 genotypes were planted in June 2009. 103 overwintered successfully. Clones of selections are being prepared for replicated trait trialling starting in June 2010.

2. *Perform 50 targeted intraspecific crosses from M. sinensis parents (30 at IBERS and 20 at PRI). Objective/ Approach 2.*

65 targeted intraspecific crosses from *M. sinensis* parents were carried out at IBERS and 239 M. sinensis crosses were carried out at PRI in 2009.

3. *Attempt 20 targeted interspecific crosses (M. sinensis x M. sacchariflorus) at IBERS Objective/ Approach 2*

40 targeted interspecific crosses were carried out at IBERS during 2009. Four of these crosses produced seedlings which are being transferred to field trials (July 10).

4. *Optimise embryo culture including transfer from culture to soil. (IBERS) Objective/ Approach 2*

Various approaches were attempted in order to optimize embryo culture including different media composition and different environmental conditions. The different media and environmental conditions did not show any significant differences in terms of ovule germination. However the transfer from tissue culture to soil was greatly improved.

5. *Confirm ploidy levels. (IBERS)*
   Objective/ Approach 2

Ploidy levels of the seedlings derived from embryo rescue and from viable seedlings from *M. sinensis* x *M. sacchariflorus* crosses were tested using flow cytometry. These were all shown to be diploid.

6. *Maintain and add to the mini plot trial established from selections made in 2008 and 2009 (IBERS)*
   Objective/ Approach 3

Gap filling where possible was performed between March and May 2009. Observations of spring emergence following the winter and tolerance of the newly emerged leaves to a late frost were made in April 2010.

7. *Select from 8S, OD0606 and OD0607 on basis of agronomic records for 2010 trials. (IBERS and PRI).Objective/ Approach 3.*

70 plants were selected from the 8S selection nursery at IBERS and 30 plants were selected from trials at PRI. These have now been planted in miniplots in an augmented (unreplicated) trial design at IBERS. First meaningful yield assessments will be made in 2012.

8. *Take records from 11S and OD0606. (IBERS and PRI).Objective/ Approach 3.*

Spring emergence and frost tolerance measurements have been made on 11S in April 2010 and mineral contents (K, Mg, Ca, Cl, PO_4 and SO_4) were measured on OD0606A in winter 2009.

9. *Sow seed from 70 targeted crosses from 2008 into spaced plant seedlings trials (IBERS and PRI).*
   Objective/ Approach 3.

Progeny from 35 and 50 targeted crosses at IBERS and PRI respectively were planted in space plant trials in summer 2009. Overwinter survival was scored at IBERS and PRI in June 2010.

10. *Produce clonal material of two yield trait mapping families to establish trial in 2010. (IBERS).*
    Objective/ Approach 4.

Two populations (08-C1575 and 08-C1849) have been identified and overwintered. 08-C1575 has been split and the clones are now being grown up under glasshouse conditions ready for planting. 08-C1849 has not been split yet but could be later this year.

Looking forward

We believe it is important to continue to develop new varieties of *Miscanthus* which maximise the sustainable net energy yield per hectare in a wide range of conditions in the UK particularly on marginal lands. IBERS have proposed a route to take the breeding programme forward. An application was made to the Sustainable Renewables LINK. Scientific approval from a panel was given on the 26 March 2010. PRI in the Netherlands aim to continue variety development to provide the basic material for a sustainable and economically feasible cultivation of *Miscanthus* in NW Europe.

Looking further into the future.

Lignocellulosic 'next generation' perennial crops are carbon-efficient in terms of sequestration, recycle nutrients and can be grown on marginal land. *Miscanthus,* therefore, is an excellent option for the development of large-scale bio-energy. Our vision is that future *Miscanthus* breeding programs will build on previous activities to continue the development of improved *Miscanthus* varieties for the UK. These will be well placed to contribute to the renewable energy mix mitigating against carbon emissions that are causing climate change and cope with the challenge of the environmental stresses caused by climate change.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the scientific objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Transfer).

Introduction

The new work built on the DEFRA NF0426 project at IBERS and PRI with the ultimate aim of developing varieties of *Miscanthus* for sustainable production of biomass for energy production on marginal land.

The technical and scientific aim of the project is to increase the efficiency of primary production in terms of significant increased yield with suitable bio-conversion quality. This will have a major effect on the economics and life cycle analysis of production of bioenergy from *Miscanthus* as well as reducing the risk of genetic vulnerability resulting from only three clones of *M. x giganteus* being used commercially. The objectives, approaches and work plan can be visualised in terms of a breeding pipeline consisting of the assessment of genetic resources, hybridization, and preliminary assessment of selections. Multi-site trials and commercialisation are separate steps which will need to be addressed outside the current project.

Scientific Objectives

1. Initiate field trait assessment of new genetic resources on marginal land.

2. Perform targeted crosses based on existing phenotypic information and improve methods of obtaining triploid interspecific hybrids.

3. Select from 2070 3-yr old progeny from crosses made in 2006 and promote outstanding selections to plots.

4. Identify and clone two mapping families for the development of molecular markers.

Detailed reporting by milestones

Milestone 1. Plant novel germplasm collected from Asia in 2006 in the field and design database. (IBERS). Objective/ Approach 1

Collections made in Asia in 2006 (NF0436) were released from quarantine in Autumn 2008. Phenotypic data recorded at collection has been be added to the IBERS database.
From 194 collected accessions (rhizome or seed), 105 produced live plants. A large proportion of this collection is classified as *M. sacchariflorus.* In June 2009 a genotype of each live accession was planted in the field at IBERS. In May 2010 we recorded that 103 out of the 105 types planted had successfully overwintered.

Cloning of plants with positive yield traits has begun, and a selection of 89 are being promoted to a replicated trait assessment trial in 2010 with part funding from BBSRC ISPG funding. Plants in trials at Aberystwyth are not yet mature enough to make positive genotype selections for use in crosses in the UK. We expect phenotyping of the unreplicated field trials in 2010 will lead to some genotypes being brought into the main crossing programme in 2011.

Milestone 2. Perform 50 targeted intraspecific crosses from M. sinensis parents (30 at IBERS and 20 at PRI). Objective/ Approach 2.

Phenotype data for use in making selected crosses (IBERS)
Most of the germplasm assembled in the DEFRA funded programme was planted in the phenotyping trial 2TT in 2005. Plants have been intensively phenotyped for the period 2007 to 2009 with funding from BBSRC, EPSRC

and DEFRA. Summaries of the phenotype information undergo continual improvement as more data is collected and analysed.

Creating targeted crosses (IBERS)

Parents were chosen on the basis of superior phenotype or complementary traits (i.e. for two yield components high/low x low/high) or reinforcement of traits (high autumn greenness x high autumn greenness). IBERS isolation chambers were used to make most of the crosses where flowering occurred before early autumn. Crosses using pollination bags were used in late crosses which were made in the glasshouse.

Ripe seed were harvested by hand and stored *in panico*. Panicles were not threshed but laid on moist sand-based compost for germination in spring 2010. Seedlings of 65 paired crosses were transplanted to modular trays and grown until ready for planting in the field in summer 2010 (Table 1).

Table. 1. Intraspecific crosses performed at IBERS during 2009 and there destination into S (spaced plant trials) and MP (miniplot trials).

Cross type		Paired
Selected for S trials		65
Selected for MP trials		6

Creating targeted crosses (PRI)

In 2009, 99 controlled pair-wise pollinations were made in Wageningen using pollination bags with selected plants. Selection was based on phenotypic data (yield and quality) and on general combining ability which were both derived from the recurrent selection programme developed in NF0426. Pair-wise crosses made in a glasshouse produced an average of 24 seeds per plant (Table 2). In total 87 out of 198 plants used to perform pair-wise crosses gave no seeds. The seed yields of the others ranged from 1 to 410. The pedigrees of the crosses became more and more complex; most 2009 crosses made at Wageningen have now three different heterozygous accessions from the Wageningen collection in their ancestry.

Top crosses (seed production on separate self incompatible plants by pollination with the help of a tester) were performed in the field in 2009 with an isolation plot established within NFO 426 in 2004. The tester consisted of the BIOMIS population. Most genotypes tested produced seed (average of 51 seeds per plant, Table 2). In three isolations (trials AV070, AV0702 and AV0703) a few inflorescences of outstanding plants were taken during the autumn of 2009 and pooled per experiment prior to threshing. This resulted in three seed lots with an average of 2783 seeds.

In the trials OD0606A and B seeds from 40 promising plants from outstanding crosses were obtained after open pollination. Most plants yielded over 500 seeds; on average 1727 seeds per plant.

The advantage of open pollination under field conditions is the striking difference in seed yield compared with that in pair- wise crosses made in a greenhouse. This was observed in previous years as well.

Table 2. Mean number of seeds per plant Seed set upon controlled and open pollinations in Wageningen

Type of cross	Number of crosses	Distribution						mean # seeds/cross
		0	1-25	26-100	101-250	250-500	>500	
Controlled Pollinations								
Top crosses	39	4	20	8	3	3	0	51
Pair-wise crosses	99	87	80	19	6	6	0	24
Selfing	2	0	1	1	0	0	0	9
Open pollinations								
Selected single plants	40	0	0	0	3	3	34	1727
Bulks of selected plants	3	0	0	0	0	0	3	2783

Milestone 3. Attempt 20 targeted interspecific crosses (*M. sinensis* x *M. sacchariflorus*) at IBERS. Objective/ Approach 2

In NFO 426 we reported that interspecific crossing is a high risk activity. It is worthwhile because interspecific plants display hybrid vigor, and therefore provide a route to increasing yield. To perform such crosses there are several barriers to overcome. Firstly, flowering has to be synchronised between day neutral *M. sinensis* and photoperiod sensitive *M. sacchariflorus*. In 2009, as a result of work developed in NFO 426 and the BBSRC funded flowering project, we have managed to make 40 pairwise interspecific crosses. Of these forty crosses four produced viable seedlings. These have been transplanted to modules and are due to be planted in the field during summer 2010.

Milestone 4. Optimise embryo culture including transfer from culture to soil. (IBERS) Objective/ Approach 2

We have continued to develop embryo rescue techniques which can be used to overcome incompatibility barriers where normal seed set does not occur. In 2009 we investigated

- different culture media such as higher concentrations of sucrose, the use of Beasley-Ting and Murashige-Skoog media and supplementation with activated charcoal which absorbs toxic compounds released from the aborting maternal tissue
- growth conditions such as warmer temperatures.
- improved procedures and environmental conditions that affect the ability of the seedling to transfer from aseptic culture to soil.

20 ovules were placed onto each of the different media described in the table below. 10 of the tubes from each of the different media conditions were placed at 10°C in the dark to germinate and the other 10 tubes were placed at 20°C in the light to germinate. The results from this are shown in the table 2 below:

Table 3: Results of different media and growth conditions on ovule regeneration using Embryo Rescue.

Environment	**10°C/dark**				**20°C/light**			
Media	**Ovules picked out**	**ovules germinated**	**seedlings transferred to soil**	**Triploid seedlings**	**Ovules picked out**	**ovules germinated**	**seedlings transferred to soil**	**Triploid seedlings**
3% sucrose (B5)	10	3	3	0	10	2	1	0
4.5% sucrose (B5)	10	2	0	0	10	2	0	0
6% sucrose (B5)	10	2	0	0	10	1	0	0
Beasley-Ting	10	1	0	0	10	2	0	0
Murashige-Skoog	10	2	0	0	10	3	0	0
B5 + 0.5% activated charcoal	10	2	0	0	10	2	0	0
Total	60	12	3	0	60	12	1	0
Percentage success		20	5	0		20	1.7	0

No difference in ovule germination success rate was detected on the different media or between the different environmental conditions (Table 3). As expected warm and light conditions produced a larger and more vigorous seedling compared to those grown up in cooler dark conditions. However it is impossible to tell if the ovules which did not survive did so because of the media and growth conditions or due to their genetic make-up. Due to time limitations only one set of cross progeny (09-C2534 – tetraploid *M. sacchariflorus* x diploid *M. sinensis*) was tested and a larger study of this kind could show up significant differences between growth media and environmental conditions.

The method developed by Sue Dalton at IBERS for transferring plantlets from aseptic culture to soil proved to be very successful and all 4 of the seedlings which were transferred survived the process and became good sized plants.

Milestone 5. Confirm ploidy levels. (IBERS) Objective/ Approach 2

The ploidy levels of plants were tested using a Partec PA flow cytometer. The protocol was carried out according to the manufacturer's instructions and known diploid, triploid and tetraploid controls were run alongside the samples to be tested.

Plants from embryo rescue have now been tested for their ploidy and all have been shown to be diploid and not triploid as had been anticipated. The viable seedlings from the *M. sinensis* x *M. sacchariflorus* interspecific crosses were also tested for ploidy using the same method and were also found to be diploid.

Milestone 6. Maintain and add to the mini plot trial established from selections made in 2008 and 2009 (IBERS) Objective/ Approach 3

Superior plants were split into 12 clonal propagules. These were planted (12 plants per plot at 2 plants per $1m^2$) in an unreplicated miniplot trial (9MP). There were 192 experimental plots plus 24 control plots of *M. sinensis* cv Goliath and 24 plots of *M. x giganteus*, resulting in a total of 240 plots. The trial is composed of 17 clones from 2TT, 70 from 4S, 23 from 5S, 67 from 7S and 15 from other sources. A dry spell after planting resulted in poor establishment in ~70% of selections. 23 missing plants were re-established in March 2009. The first assessments have been made in 2010 in terms of spring emergence and frost tolerance. It has been decided not to replace missing plants.

Milestone 7. Select from 8S, OD0606 and OD0607 on basis of agronomic records for 2010 trials. (IBERS and PRI).Objective/ Approach 3.

Crosses made in 2006 were planted in a selection nursery (8S) in 2007 at Aberystwyth. 8S plants with outstanding or interesting phenotypes at assessment in autumn 2009 (in terms of biomass yield, canopy height and spread, leafiness, stem diameter and density) were selected for promotion to miniplots. In a similar way, promising plants were selected by Oene Dolstra at Wageningen from a range of trials supported by NFO 426. Seventy selected plants from Aberystwyth and thirty from Wageningen were cloned by rhizome splitting and were planted in miniplots in an augmented (unreplicated) trial design (13MP) at Aberystwyth on the 1-4th June 2010. The design of this trial is the same as 9MP (as described in Milestone 6). The remaining 122 experimental plots have been filled with plants from other sources. Irrigation has been applied to ensure transplant survival. The first assessments of over winter survival will be made in May 2011.

Milestone 8. Take records from 11S and OD0606. (IBERS and PRI).Objective/ Approach 3.

The 11S selection nursery was planted at IBERS in 2008. Following a colder than average winter there was a 32% winter-kill rate. Spring emergence and frost tolerance measurements were made in April 2010 and these measurements showed a good winter survival rate with only a 4% winter kill despite the late frosts and much colder than average winter. Further measurements will be made on the 11S trail during the growing season.

At Wageningen single plants were selected in winter 2010 from the trials OD0401/OD0505, OD0606A and B, OD0607, and three isolations from 2007; in total 170 genotypes. These genotypes together with 25 accessions and 12 selected genotypes from IBERS were divided into eight propagules for planting in a randomized block design trial with two reps of one-row ‘mini-plots’. Our aim is to obtain a better impression of the agronomic value of the selected genotypes from multi-year observations. In addition one plant per genotype was put in a special field for maintenance and hybridization.

Low contents of Cl, K, and Ca are desirable since they reduce slagging, fouling and corrosion when the biomass is combusted. Early March harvested biomass from selected plants in the trial OD0606A had low mineral contents as expected (Table 4). The variation in mineral content was found to be partly heritable.

Table 4. Variability in contents of six minerals found in biomass of 50 FS and HS progenies (OD0606A). Statistics for each mineral are the mean (mg/g DM) , heritability of progeny means (h^2_m), standard deviation (SD), as well as maximum and minimum values.

	Statistic				
Mineral	Mean	h^2_m	SD	Max	Min
Cl	0.026	0.05	0.025	0.117	0.000
K	1.359	0.59	0.460	2.453	0.492
PO4	0.830	0.30	0.415	1.788	0.122
SO4	0.338	0.21	0.137	0.663	0.140
Mg	0.648	0.61	0.136	1.135	0.406
Ca	1.358	0.48	0.267	1.878	0.742

Milestone 9. Sow seed from 70 targeted crosses from 2008 into spaced plant seedlings trials (IBERS and PRI). Objective/ Approach 3.

At IBERS, progeny from 35 targeted crosses carried out in 2008 were planted into a seedling trial (12S) on 20th July 2009. Overwinter survival scores have been taken on 12S on 14th June 2010 and showed that 53% of the plants had survived.

At PRI 53 crosses from 2008 were planted out in August 2009 in a field trial at Wageningen in a replicated trial (OD0907). The experimental units were one-row plots with 10 plants. The winter survival was 88% and about 80% of all progenies showed no or almost no missing plants although the winter of 2010 was quite severe. Visual ratings for plant vigour in autumn 2009 and June 2010 showed a significant positive correlation (r=0.36). Seedlings from seeds from 2008 crosses with low seed numbers were used to establish simple screening trials (OD0908A and B).

A further two years growth will required before selections can be made for more extensive trialling in plots (as in milestone 7.

Milestone 10. Produce clonal material of two yield trait mapping families to establish trial in 2010. (IBERS). Objective/ Approach 4.

Two populations (08-C1575) (sin x sin) and 08-C1849 (sin x hybrid) consisting of 205 and 179 progeny respectively were identified in 2009 for cloning.

08-C1575 is a *M. sinensis* x *M. sinensis* cross where there are strong differences in canopy architecture (a combination of stem density, leaf length and leaf width) resulting in a difference in yield of nearly 40% in the spaced plant trial. Moisture content at harvest of the two parents ranged from 17 to 33%, and this is also reflected in differences in senescence. 08-C1575 was overwintered (2009-2010) in a heated glasshouse and split to form up to 6 clonal replicates of each genotype within the population. Some genotypes grow more slowly than others. This variation is potentially very useful for mapping, but it does limit the speed at which the population can be advanced to field testing. We are aiming to plant a trial with 3 replicates in August 2010, but this will depend on our judgement as to whether the plants are robust enough to establish well in the field.

08-C1849 is a *M. sinensis* x hybrid derived from an earlier *M. sinensis* x *M. sacchariflorus* cross. Both the parents are erect and tall with mid season flowering (July), but differ in basal spread and yield. It has a different genetic background to 08-C1575 and thus will test the robustness of QTLs for important traits. The 08-C1849 population was not overwintered in a heated glasshouse in 2009-10, and is therefore developmentally behind 08-C1575. Currently we have 179 live individuals which are being grown under glasshouse conditions. They will be ready to split in winter 2010.

Discussion and looking forward

The Bridge funding has been essential to continue the work started in NFO 426. Good progress has been made during this year. We have successfully a) established 103 plants from the Asia 06 collection in marginal land in the UK (higher level objective 1), b) exceeded our targets for successful intraspecific crosses resulting in progeny to field test (objective 2), c) made interspecific crosses, which here-to-for have proven very unsuccessful (objective 2), d) selected over 100 plants from crosses made at IBERS and PRI for promotion to plot trials (objective 3) , and e) gathered phenotype data for furthering informed crosses (objective 3), and f) started the development of two paired crosses as mapping populations (objective 4).

We believe it is important to continue to develop new varieties of *Miscanthus* which maximise the sustainable net energy yield per hectare in a wide range of conditions in the UK particularly marginal lands. IBERS have proposed a route to take the breeding programme forward. An application was made to the Sustainable Renewables LINK. Scientific approval from a panel was given on the 26 March 2010. We are negotiating an acceptable collaboration agreement to combine NFO 426, this bridge and commercial breeding activities funded at IBERS since 2007. PRI in the Netherlands aim to continue variety development to provide the basic material for a sustainable and economically feasible cultivation of *Miscanthus* in NW Europe. PRI has pursued further financing by the Dutch government and is engaging with the private sector to secure the future of this promising bio-based crop. PRI and IBERS are currently responding to an EU FP7 call, and plan to work together in a project with other groups across Europe to deliver the science necessary to underpin breeding.

Looking to the future. A key question is how to deliver bioenergy without jeopardising food security. This can only be achieved by moving from the use of carbon-inefficient non-sustainable annual food crops such as wheat and oilseed rape and from raw materials such as palm oil which have devastating effects in terms of the loss of major carbon sinks and on biodiversity. Lignocellulosic 'next generation' perennial crops are carbon-efficient in terms of sequestration, recycle nutrients and can be grown on marginal land. *Miscanthus,* therefore, is an excellent option for the development of large-scale bio-energy. Our vision is that future *Miscanthus* breeding programs will build on previous activities to continue the development of improved *Miscanthus* varieties for the UK which are well placed to contribute to the renewable energy mix needed to ensure fuel security and help to mitigate against climate change.

References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Trindade, L. M., O. Dolstra, E.N. van Loo & R.G.F. Visser (2010). 5. Plant Breeding and its Role in a Biobased Economy. *In: Langeveld H., J. Sanders & M. Meeusen (Eds) The Biobased Economy, Biofuels, Materials and Chemicals in the Post-oil Era. Earthscan, London, Washington D.C, p67-82*.

John Clifton Brown[1], Steve Renvoize[2], Yu-Chung Chiang[3], Yasushi Ibaragi[4], Richard Flavell[5], Joerg Greef[6], Lin Huang[1], Tsai Wen Hsu[7], Do-Soon Kim[8], Astley Hastings[9], Kai Schwarz[6], Paul Stampfl[10], John Valentine[1], Toshihiko Yamada[11], Qingguo Xi[12] and Iain Donnison[1]. (2010) Developing *Miscanthus* for bioenergy. *In* Energy Crops Halford and Karp eds. (to be published by the Royal Society of Chemistry)