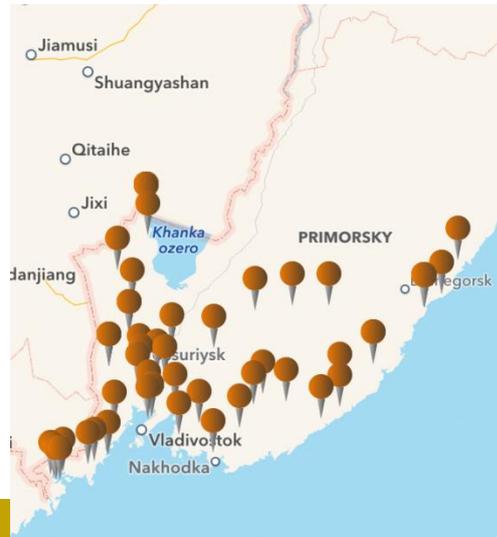


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# Biomass from perennial grasses on marginal lands

## B – Genetics



### Key external stakeholders:

Plant breeders, Academic audience including cooperation partners in other European Projects such as OPTIMA, OPTIMISC, FIBRA and WATBIO; General public and media; policy makers

### Practical implications for stakeholders:

For Miscanthus a much wider genetic base can be used for breeding towards chilling and cold tolerance for improved juvenile growth. There is still unused genetic potential for reed canary grass and cocksfoot.

- Species vary in their physiological behaviour towards waterlogging, drought and salt tolerance and choosing the right species for a location is crucial to optimise biomass yields.

### Main results:

- Novel germplasm collections of reed canary grass, cocksfoot and miscanthus species for pre-breeding were made and genotyped to assess their gene pools for breeding.
- Novel Miscanthus crosses amongst Miscanthus species were made towards better cold adaptation.
- Physiological studies ranked miscanthus, reed canary grass, cocksfoot and festulolium towards abiotic stress resistance and suitability towards growth on a range of marginal conditions.

### Opportunity / Benefit:

For festulolium, festuca and cocksfoot more knowledge towards their suitability and their benefits for marginal land conditions have been unraveled and are commercially available for end-users. For future end-users suitable materials for breeding of reed canary grass and Miscanthus have been identified.

### Collaborating Institutions:

Trinity College Dublin; Aarhus University, Denmark; DLF-Trifolium, Denmark; Tiplant Biotechnik und Pflanzenvermehrung GmbH, Germany; The Institute of Cytology and Genetics SB RAS, Russian Federation; Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, China

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### 1. Project background:

To extend the growing area of miscanthus it is necessary to find genotypes better adapted to different climatic and soil conditions. The high biomass cultivar *M. × giganteus* as an interspecific hybrid has a high degree of heterozygosity. A large contribution to biomass yield can be expected from heterosis in hybrids made from crossing different genetic backgrounds. Collection of new genotypes can broaden the genetic basis of agronomically valuable species, and provides new genotypes for direct testing and breeding of future crops. Investigating the genetic diversity of existing gene pools and novel collected accessions is a prerequisite to identify breeding potential, or possibly gaps in the present collections, and provides valuable information for the planning of future breeding work.

Tolerance to a certain level of stress is of key importance for selection of the most promising grass species and varieties for production on marginal land and for production over a wide climatic gradient. Tolerance to salt, drought, flooding, cold and frost was analysed in the grass species and varieties with a range of methodologies.

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### 2. Questions addressed by the project:

- Is current breeding selection satisfactory for species included in this study and which required characteristics can be manipulated in breeding programmes?
- Can novel germplasm to widen the gene pools for breeding be found in Europe, China and Russia from natural populations?
- How much variation amongst and within species can be found towards tolerance in abiotic stress conditions on marginal lands?

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### 3. The experimental studies:

A mix of experimental approaches was employed. Field collections of germplasm were made in accordance with international standards. These field collections and pre-existing field collections have been genotyped with a range of molecular markers to identify potential breeding pools.

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### 4. Main results:

- Depending on the target region for hybrids, various genome combinations are conceivable for novel Miscanthus crosses targeted for growing in Northern Europe (*M. sinensis* with *M. sinensis* or *M. sacchariflorus* day-neutral), Middle Europe (*M. sinensis* with *M. sacchariflorus* large-stemmed) or Southern Europe (*M. sacchariflorus* large-stemmed with *M. sinensis*, *M. sacchariflorus* or *M. floridulus*). Seventy two *M. sinensis* × *M. sinensis* crosses were made mainly between genotypes from a gene pool collected in Japan and held by Aarhus University (Denmark) and the gene pool held by Teagasc (Ireland) originating from European collections. Novel hybrids were transferred to the field for further evaluation.
- Three *M. sinensis* × *M. sacchariflorus* (day neutral) crossings were made. The Siberian branch of the Russian Academy of Science selected one *M. sacchariflorus* genotype which had similarity to *M. sacchariflorus* robustus. Novel hybrids were transferred to the field. Their survival of the first winter

varied within each crossing group between 60 and 100%.

- To enlarge the genetic variability in particular with respect to cold resistant genotypes, and to examine the genetic structure of the *Miscanthus* population a collecting trip was undertaken during September 2014 to collect *Miscanthus* in Russia (Primorsky Krai). Rhizome, leaf, seed and herbarium samples were taken from over 20 populations. Ten populations were sampled intensively and the other populations sampled for two individuals. Additionally a *Miscanthus* collecting trip was also undertaken in China (Beijing, Hebei, Liaoning) during mid-September. Rhizome, leaf, seed and herbarium samples were taken from over 20 populations.
- Germplasm collections of Cocksfoot and Reed canary grass were done in Europe (Poland, Germany, Denmark, Sweden, UK and Ireland). Ten Populations of Cocksfoot and of Reed canary grass were sampled in each country for seed, rhizome and leaf. Seed was collected from 30 individuals per population, leaf from 15 individuals per population and rhizomes from 2 individuals per population.
- Genotyping of the newly collected Reed canary grass and Cocksfoot accessions from Poland, Germany, Denmark, Sweden, UK and Ireland was undertaken using plastid DNA SNPs generated via long read PCR and MiSeq sequencing and by genotyping by sequencing (GBS). A GBS experiment was conducted to analyse the living collection of *Miscanthus* held at Teagasc Oak Park, Carlow. In total, 146 genotypes from the Oak Park field (Teagasc, Ireland) collection were analysed, 116 samples from the GrassMargins collection from Far East Russia and the Chinese Hebei province, 14 samples from the collection of the Chinese Academy of Science in Shanghai, and 20 samples from the Japanese collection, held at Aarhus University in Denmark.
- A greenhouse screen of cocksfoot, tall fescue, festulolium and reed canary grass on rockwool blocks was performed. The plants were established without salt stress for 60 days and were later challenged with stepwise increasing salt concentrations from 0.5% to 2.5% salt (w/v). We found variation within and between all the tested species. The ranking of the tested species starting with the most tolerant was:  
tall fescue > festulolium > cocksfoot > reed canary .
- From this salt stress experiment gene expression experiments were performed and differentially expressed genes identified. Similar gene expression experiments were also performed with flooded and droughted miscanthus samples.
- Further field and greenhouse experiments were performed to identify species and varieties with better waterlogging and drought response. For drought tolerance cocksfoot obtained the highest shoot dry weight under both control and drought conditions while reed canary grass showed lowest shoot dry weight. Tall fescue was intermediate between the high-performing cocksfoot and poor-performing reed canary. For flooding tolerance reed canary grass, tall fescue, festulolium and miscanthus all thrived well at the flooded sites, while cocksfoot established well but ended up poorer yielding than the other species.
- From these experiments novel transcriptomes for reed canary grass, cocksfoot and miscanthus have been made to aide molecular selection approaches in the future.

### 5. Opportunity/Benefit:

For festulolium, festuca and cocksfoot more knowledge of their suitability and their benefits for marginal land conditions have been unraveled and are commercially available for end-users by commercial project partners. For future end-uses suitable material for breeding of reed canary grass and *Miscanthus* have been identified. For research purposes novel transcriptomes for reed canary grass and cocksfoot were constructed as well as molecular markers for reed canary grass.

### 6. Dissemination:

#### Main publications:

##### Book:

Barth S, Murphy-Bokern D, Kalinina O, Taylor G & Jones MB (eds.) (2016) Perennial Biomass Crops for a Resource Constrained World, Springer, Dordrecht (ISBN 978-3-319-44529-8) DOI: 10.1007/978-3-319-44530-4.

##### Book chapters with involvement from Teagasc staff:

Hodkinson TR, Petrunenko E, Klaas M, Münnich C, Barth S, Shekhovtsov SV, Peltek SE (2016) New breeding collections of *Miscanthus sinensis*, *M. sacchariflorus* and hybrids from Primorsky Krai, Far Eastern Russia. In: Perennial Biomass Crops for a Resource Constrained World (eds: Barth S, Murphy-Bokern D, Kalinina O, Taylor G & Jones MB), Springer, DOI: 10.1007/978-3-319-44530-4

Muennich C, Kørup K, Klaas MJ, Barth S, Bonderup Kjeldsen J, Finnan J, Fonteyne S, Jankowska M, Jørgensen U (2016) Creation of novel cold tolerant *Miscanthus* hybrids. In: Perennial Biomass Crops for a Resource Constrained World (eds: Barth S, Murphy-Bokern D, Kalinina O, Taylor G & Jones MB), Springer, DOI: 10.1007/978-3-319-44530-4

Barth, S. Jones MB, Hodkinson TR, Finnan J, Klaas M, Wang Z-Y (2014) Grasslands for forage and bioenergy use: traits and biotechnological implications. In: Grassland Science in Europe 19 (edited by Hopkins A, Collins RP, Fraser MS, King VR, Lloyd DC, Moorby JM, Robson PRH); Gomer Press Ltd, Wales/UK; p 438-449

Peer-refereed journal articles with involvement from Teagasc staff:

Perdereau A, Klaas M, Barth, S & Hodkinson TR (2016) Plastid genome sequencing reveals biogeographic structure and extensive population genetic variation in wild populations of *Cocksfoot arundinacea* L. in north western Europe. Global Change Biology – Bioenergy, DOI: 10.1111/gcbb.12362

Barth S, Jankowska JM, Hodkinson TR, Vellani T & Klaas M (2016) Variation in sequences containing microsatellite motifs in the perennial biomass and forage grass, *Cocksfoot arundinacea* (Poaceae). BMC Research Notes 9:184 DOI: 10.1186/s13104-016-1994-6

Haiminen N, Klaas M, Zhou Z, Utró F, Cormican P, Didion T, Jensen CS, Mason C, Barth S, Parida L (2014) Comparative exomics of *Cocksfoot* cultivars under salt stress. BMC Genomics 15 (Suppl 6):S18 (doi:10.1186/1471-2164-15-S6-S18)

#### Popular publications:

Barth S, Klaas M, Burke B, Doyle D & Finnan J (2013) Perennial grasses for marginal soils. TRResearch 8(3), p 42-43

Television interview: euronews, first broadcasted on Tuesday 28 July 2015

Television interview: Irish TV, broadcasted on 14.06.2014 as part of episode 4 of the County Carlow matters.

Interview by Antony King on GrassMargins project January 2014:  
<http://www.renewableenergyworld.com/articles/2014/01/can-grass-be-a-new-biofuel.html>

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7. **Compiled by:** Susanne Barth

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