ICONFORS: Improving Germplasm Conservation Methods for Perennial European Forage Species

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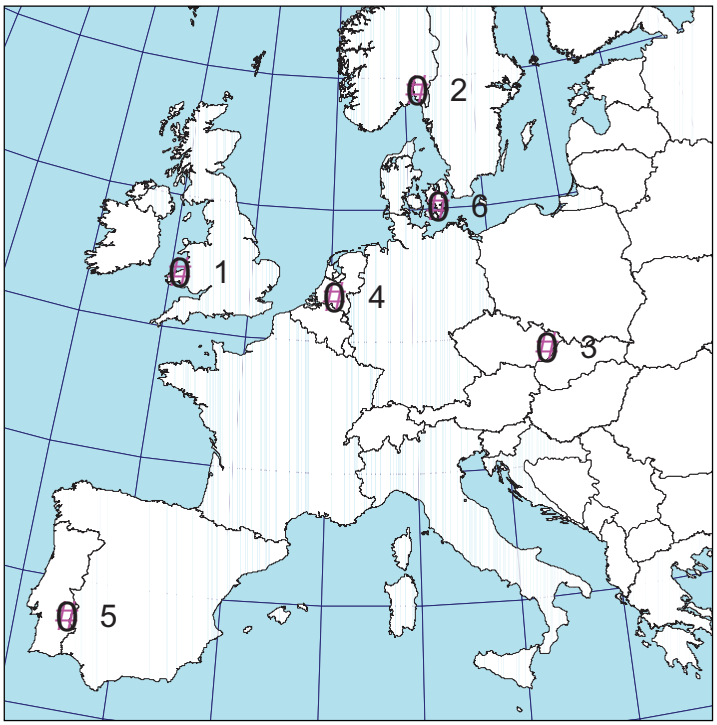
Abstract: Poor conservation of plant germplasm collections has been highlighted as a global problem, both by the Convention on Biological Diversity and the Commission on Genetic Resources for Food and Agriculture (FAO 1999). Within Europe nearly 100 000 populations of forages are conserved as seed samples (accessions) in gene banks for possible use by plant breeders, in reconstituting native pastures and to facilitate research in a broad range of areas such as functional genomics and conservation genetics. Seed cannot be stored indefinitely; even in the best storage conditions they senesce and it is estimated that over 20 000 accessions are now in urgent need of rejuvenation through a cycle of seed multiplication. ICONFORS is a four year EU project funded by the Framework V programme involving six partner countries that aims to acquire the genetic and economic knowledge required to improve seed multiplication methodologies for genebanks maintaining *ex situ* seed collections of perennial European forage species. Within ICONFORS different options for regeneration are considered and the remaining gaps in knowledge about the genetic and economic implications of different approaches to multiplying seed of germplasm collections of perennial forage species in Europe are addressed. The project is focusing on wind pollinated grasses (perennial ryegrass and meadow fescue) and an insect pollinated forage legume (white clover). The main areas of research within ICONFORS are outlined and progress thus far is summarised.

Keywords: accessions; contamination; barrier crop; economic; environment; forage; heritability; genebank; genetic drift(shift)variation; genotype; germplasm; isolation chambers; meadow fescue; multiplication; outbreeders; paternity; perennial ryegrass; pollination; rank order; regeneration; white clover

Perennial forage species are mostly outbreeders. Each sample of seed is a genetically variable mixture of genotypes (Sackville Hamilton & Chorlton 1997). A new generation of young healthy seed is produced by growing a sample of the original and allowing the plants to pollinate each other. The new generation will be genetically different from the original (Sackville Hamilton 1998), for three reasons: genetic drift, genetic shift and contamination.

Such genetic changes are directly counter to the objectives of gene banks, namely to conserve genetic variation intact. We cannot totally eliminate change but how can we minimise it?

The situation is complicated by economics. We work with limited budgets. If we try too hard to



Project partners

1. Institute of Grassland and Environmental Research, UK
2. The Norwegian Crop Research Institute, Norway
3. OSEVA PRO Ltd., Grassland Research Station, Czech Republic
4. Centre for Genetic Resources, the Netherlands
5. Estacao Nacional de Melhoramento de Plantes, Portugal
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maintain genetic stasis, we reduce the number of accessions that we can process, and so we may reduce the effectiveness of the gene bank. Conversely, if we relax regeneration standards too much in order increase the number of accessions processed, we also reduce the effectiveness of the gene bank. We need to seek and adopt the optimum compromise between **quality** of regeneration for each accession and the **quantity** of accessions regenerated (Sackville Hamilton 1998; Sackville Hamilton & Chorlton 1997).

Objectives

The work in ICONFORS [http://www.iger-gru.bbsrc.ac.uk/iconfors/index.htm] is subdivided into 6 workpackages (WP) using representative forage species (details of species in brackets).

WP1 is based on a common seed multiplication protocol that is being followed by four partners representing all the major climatic zones within the partnership. It aims to assess the effect of environment of multiplication on the potential magnitude and direction of genetic changes resulting from differential seed production, as a function of species and the origin of the population being multiplied. Measuring the effect of the site of multiplication on the direction of genetic change requires vegetative replication, so that every plot of each population uses an identical set of genotypes. This allows assessment of the variation in rank order of genotypes with site of multiplication (*Lolium perenne, Trifolium repens*).

WP2 focuses on the effects of different management options on the potential magnitude of genetic changes resulting from differential seed production. Comparisons of open field plots with isolation chambers (permanent and temporary), size of pots and date of sowing plants are the options being tested at different sites (*Lolium perenne*).

WP3 forms the second phase of experiments on variation in seed production which aims to determine the heritability of variation in seed yield within populations. A second cycle of seed multiplication will be undertaken using vegetative cuttings from the parent plants and progeny seed produced during the first cycle in WP2, then we will calculate the within-populations parent-offspring regression for seed yield. An assessment of heritability is crucial to partitioning potential genetic changes into random genetic drift and non-random drift towards genotypes with consistently high seed production whilst also providing an assessment of variation with year (*Lolium perenne*).

WP4 constitutes the first phase of the analysis of variation in pollination between plants within populations and comprises two tasks. The first

task concerns the development of the technology required for paternity identification, and secondly, the collection of field data that will be required for analyses aimed at predicting paternity in WP5 (*Lolium perenne*).

WP5 forms phase 2 of the study of variation in cross-pollination rates. Determinations will be made on the distribution of paternity and how acurately the distribution of paternity can be predicted from data on the spatio-temporal distribution of pollen sources within the plot (*Lolium perenne*).

WP6 deals with contamination of field plots with pollen from other plots or other sources of pollen, quantifying the extent of contamination between field plots, from feral populations and the effects of varying location, species, distance between plots, position within the plot, and direction from source (*Festuca pratensis* – wind pollinated, *Trifolium repens* – insect pollinated).

Expected achievements

The project will develop the technology required to eliminate many of the major remaining gaps in knowledge about the genetic and economic implications of different approaches to multiplying seed of germ-plasm collections of perennial forage species in Europe.

Progress to date

Data from WP1 and WP2 is being analysed and will include the economic implications of different regeneration methodologies and processes. Preliminary analysis has shown significant variation in reproductive potential when populations are multiplied in different environments. Further analysis will determine the extent to which this variation varies with species and origin of population.

WP3 constitutes the second phase of work carried out in WP2 and will be harvested in the summer of 2004. Work on WP4 continues with developing the technology required for paternity identification in WP5.

In WP6, screening of *Trifolium repens* progeny has been completed. Levels of contamination in the receptor plots were low and no significant genotypic or directional effects were detected. Significant levels of contamination were found from feral populations (Hinton-Jones *et al*. 2003). The receptor plots of *Festuca pratensis* were harvested in 2003 and progeny assays will be carried out in 2004.

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