

Chapter

10

Vegetative Collection of Forage Grasses and Legumes, and Method of Regeneration for Seed



**Ken H. Chorlton, N. Ruairaidh Sackville Hamilton,
Ian D. Thomas and Maurice H. Jones**
Institute of Grassland and Environmental Research,
Plas Gogerddan, Aberystwyth, Ceredigion
SY23 3EB, UK

Summary

Collecting vegetative grass and clover ecotypes from non-seed-producing habitats and growing them on under optimal conditions to maximise seed yield, enables more variation to be sampled and conserved.

Introduction

The Institute of Grassland and Environmental Research (IGER) undertakes an integrated, cross-disciplinary programme of basic, strategic and applied research for a range of customers, emphasising grassland-related and extensive agricultural systems. The main research station is at Aberystwyth in Mid Wales with out-stations nearby (Trawsgoed), in Devon (North Wyke) and in South Wales (Bronydd Mawr). The Genetic Resources Unit of IGER is based in Aberystwyth and is primarily concerned with the collection, storage, characterisation and documentation of temperate forage grasses and legumes. It holds some 10,000 accessions and makes these available to breeding and research programmes within IGER and to other institutes both nationally and internationally. Target genera for agricultural improvement are *Lolium*, *Festuca*, *Dactylis* and *Trifolium*. Those for amenity use are *Festuca* (fine-leaved species), *Poa* and *Agrostis*. Material is collected from throughout the UK and across Europe. Collecting is sometimes carried out due to perceived risk of genetic erosion (for instance, in the UK, threats include agricultural improvement and urbanisation) or because populations have not been studied and used in breeding programmes. Examples of collecting programmes include:

- Collection of samples from isolated forage populations on Welsh islands in 1998/9.
- Sampling of Portuguese grasslands as a joint effort with the Universidade de Tras-os-Montes e Alto Douro, UTAD in 1995 (Chorlton *et al.*, 2000).
- Exploration of North Eastern Italy in 1998.

The primary collection target species are *Lolium perenne* L., *Festuca pratensis* Huds., *Trifolium repens* L. and *Trifolium pratense* L. which are perennial out-breeders. They frequently occur in habitats that are cut, grazed or trodden and so have limited opportunities to produce flowers and set seed. Consequently, seed collecting (see Way, 2003 – Chapter 9) is ineffective at capturing the genetic variation present and it has been necessary to pioneer an alternative approach. Vegetative material is collected and brought to flower within controlled glasshouse conditions. Then, seed is harvested in a way that maximises genetic representation.

Collecting

The preferred method of collection is to gather 25–50 separate vegetative units per ecotypic population at each site visited. Where possible, the units are taken at distances of 5 m from one another. Apart from often being the only option, collection of vegetative material avoids bias towards sexually reproductive genotypes and is not limited to periods of seed harvest. Identification can however be a problem with vegetative material because assigning correct identity often relies upon access to floral morphology. Furthermore, difficulty can sometimes be encountered in keeping vegetative material alive.

Collection details include location, site description, relief, general and species habitats, management at the site (e.g., high or low input agricultural systems), soil details, etc. Analysis of such data in the GRU database in turn helps identify gaps for future collecting.

Seed Harvest and Storage

On their return to Aberystwyth, the vegetative units are cleaned and reduced to one tiller, or stolon, per unit. The populations are ‘grown-on’ in a quarantine glasshouse under optimum conditions of light, heat, nutrients and water. The aim is to encourage active plant growth and minimise losses of individual genotypes. Supplementary heat and light is withdrawn by mid-December to allow the low temperatures and short day lengths required for floral induction of this temperate material.

The quarantine glasshouse consists of separate isolation chambers. Each chamber is pollen and insect proof. Fan-blown, filtered air is ducted into each chamber and across the plants. The air movement facilitates the distribution of pollen in the case of wind-pollinated species. Hand pollination is not used. Water is supplied by capillary action. Populations of the same species are isolated in separate chambers and insect pollinators can be introduced for legume species. The pollinators used are leaf-cutter bees imported under licence from Canada.

The population samples within the chambers are monitored for peak anthesis (often quite difficult to judge in the case of legumes) and are harvested about 28 days later when maximum seed yields can be expected. Harvested seed heads from each labelled mother plant are kept separately and hung up in 355 mm × 455 mm ‘Kraft’ paper bags for air-drying in the glasshouse. The air-dried seed heads are threshed, sieved and blown to

provide clean, high quality seed from each mother plant. The seed is then dried down to about 5% moisture content over silica gel. Subsequently, a germination test is carried out on 2×50 samples drawn from the 'balanced bulk' (see below) in 'repli-dishes', and for most species, at constant 20°C in the dark. If the germination test result is $\geq 80\%$, and the amount of seed produced for the balanced bulk is ≥ 5 g, then the seed is packaged inside laminated aluminium foil pouches. If the seed lot does not meet the minimum standards of germination and weight, then the regeneration process is repeated the following season. A population sample of 30 plants (genotypes) gives rise to 30 labelled 'mother plant' seed samples each ranging from one seed to about 0.5 g, depending on quantity available. These are stored as conservation material in the long-term gene bank store at -18°C where a storage life of many decades might be expected. Additionally, a balanced bulk sample is made up of equal aliquots from each mother plant. The weight depends on the weight of the lightest mother plant seed harvest. This bulk usually weighs at least 1g and is used for genetic analyses at IGER and, where appropriate, for fulfilling seed requests from breeders. Finally, the remainder of the seed is put together to form an 'unbalanced bulk' sample. This is used for the majority of seed requests. These two bulk samples are placed in the medium-term store at 0-2°C where a storage life of several decades might be anticipated.

References

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- Way, M. (2003). Collecting seed from non-domesticated plants for long-term conservation, pp. 163–201. In: R.D. Smith, J.B. Dickie, S.H. Linington, H.W. Pritchard and R.J. Probert (eds). *Seed conservation: turning science into practice*. Royal Botanic Gardens, Kew. UK.

