Genetic resources activities on forages in the Netherlands

Loek J. M. van Soest, Noor Bas and Rob van Treuren

Centre for Genetic Resources, the Netherlands (CGN), Foundation DLO, Wageningen, The Netherlands

Introduction

Much emphasis has been given to the broadening of the CGN forage collection during the last three years. Old Dutch varieties of a number of grasses and ecotypes of both grasses and forage legumes have been introduced, regenerated and included in the collection.

At present, the forage collection of CGN includes 886 accessions, comprising 628 accessions of 11 different grass species, and 258 accessions of two *Trifolium* species. Since the last meeting of the ECP/GR Working Group on Forages in 1999, the collection has been increased by 245 new accessions of both grasses and legumes.

In 2000 a project was started to investigate the genetic diversity present in traditional Dutch pastures. For this purpose vegetative material of *Lolium perenne* and *Trifolium repens* was sampled in 2000 from 16 selected old grasslands situated in the different cattle-raising areas of the Netherlands. The collected material was characterized for morphological traits and molecular markers. This project will be briefly discussed and some preliminary results presented.

The collections

- **Grasses**

An overview of the CGN collection of grasses is presented in Table 1. The collection includes 628 accessions. Since the last meeting of the ECP/GR Working Group on Forages in 1999, 189 new accessions of different grasses have been added to the collection. In particular the collections of *Lolium perenne*, *Festuca rubra* and *Poa pratensis* were substantially increased with 98, 42 and 28 accessions respectively. Accessions of *Agrostis* spp. were newly introduced into the collection. Most of the 189 newly introduced accessions were of Dutch origin and in particular, old varieties were included in the collection. The new accessions of *Lolium perenne* included both old varieties and collected ecotypes. The latter group was received from a private Dutch breeding company which collected this material in the period 1970-1976 in mainly old permanent grasslands in the south of the Netherlands. It is the policy of CGN to broaden its forage collection with original Dutch material. Table 1 shows that 77% of the grasses are from the Netherlands.

- **Forage legumes**

At present, the *Trifolium* collection of CGN consists of 258 accessions (Table 2). It includes 162 accessions of red clover (*T. pratense*) and 96 accessions of white clover (*T. repens*). 78% of the collection is of Dutch origin and was largely obtained from CGN collecting missions in the Netherlands and Central Asia (van Soest and Dijkstra 1986, 1998; van Soest and Bas 2000). During two expeditions in Central Asia (Uzbekistan and Kyrgyzstan), organized in 1997 and 1999, ecotypes of both red and white clover were collected at altitudes ranging from 940 to 1790 m asl (van Soest 1998; van Soest *et al.* 1998; van Soest and Bas 2000).

Table 1. Collection of grasses maintained by CGN in 2003 (including material regenerated in 2002 but not yet stored in the genebank)

Species	No. of samples		Origin of the material
	Total	NLD*	
Dactylis glomerata	36	16	Ecotypes from NLD and Hungary
Festuca rubra	55	44	Old varieties from NLD
Festuca pratensis	1	1	Old varieties from NLD
Lolium multiflorum	59	56	Old varieties from NLD
Lolium hybridum	1		Variety from Austria
Lolium perenne	287	203	Ecotypes NLD, old varieties NLD and Europe
Phleum pratense	91	77	Old varieties from NLD and varieties from Europe
Phleum bertolonii	7	7	Old varieties from NLD
Phleum spp.	5		Ecotypes from Uzbekistan
Poa pratensis	80	73	Old varieties from NLD
Agrostis spp.	10	10	Old varieties from NLD
Total	**628**	**487**	

* NLD = samples originating from the Netherlands

Table 2. Collection of forage legumes maintained by CGN

Species	No. of samples		Origin of the material
	Total	NLD*	
Trifolium pratense	162	132	Ecotypes and a number of varieties
Trifolium repens	96	69	Ecotypes and a few varieties
Total	**258**	**201**	

* NLD = samples originating from the Netherlands

Regeneration

Since the last meeting in 1999 approximately 250 accessions of both grasses and forage legumes have been regenerated. With the exception of the apomict *Poa pratensis* all forage species are regenerated in field plots spatially isolated by triticale. The distance between the plots is 50 m. In general the forages are sown in late summer and thereafter kept in an unheated greenhouse during the winter in order to stimulate vernalization. In April of the following year the grasses and clovers are transplanted into isolation plots established in a large triticale field. Approximately 60 plants of each accession are planted, together with other crops, in a large isolation plot of 25-30 m^2. Seeds are harvested in July/August and after cleaning and drying samples are taken for germination tests. Accessions with an initial viability of 80% are included in the collection.

Documentation, storage and utilization

The collections are documented for passport data in the data management system of CGN, called GENIS, based on Oracle software. These data can also be found on CGN's Web site (http://www.genebank.nl).

The material is maintained under long-term storage conditions (-20°C) in the genebank facilities of CGN and is freely available to *bona fide* users. However, before receiving CGN material the user is requested to sign a "Material Transfer Agreement" (MTA). An example of the MTA used by CGN can be found on the Web site.

Table 3 presents an overview of the distribution of forage genetic resources to users over the last 15 years. It shows that, as a result of the additions to the collection during the last three years, the requests and distribution of material have increased significantly. Requests for red and white clovers in particular have increased tremendously. This is because of the organic farmers' desire to include forage legumes in pastures and to make use of the nitrogen-binding effect of these legumes in order to reduce nitrogen fertilization.

Table 3. Distribution of forage accessions by CGN

Forage species	Period 1988-98	Period 1999-2003*	Total
Grasses			
L. perenne	164	21	185
Other grass species	13	61	74
Clovers	15	284	299
Total	**192**	**366**	**558**
% of total distribution	34	66	100

* up to 1 March 2003

Biodiversity in old grasslands in the Netherlands

- **Background**

The Netherlands are situated in the northwestern part of the European-Siberian region of diversity. This region holds only limited biodiversity of our cultivated crops. However, a number of temperate grasses and legumes, important for animal feeding, have their centres of diversity in this region (Zeven and de Wet 1982). Species commonly found in this centre of diversity are the important forage grass *Lolium perenne* L. and the pasture legume *Trifolium repens* L.

In the last decades, practices to optimize fodder production, e.g. reseeding with uniform commercial cultivars and high nitrogen fertilization, have reduced the diversity of most Dutch grasslands. Traditional grasslands are becoming rare in the Netherlands. However, about 50 traditional grassland sites were identified by CGN in 1997 and 1998. These traditional grasslands are located in nearly all provinces where cattle-raising takes place. Some are under organic management. Unique genetic diversity may still exist within these grasslands. As the farmers who manage them cannot guarantee the maintenance of these traditional grasslands, *in situ* conservation is regarded as an option to safeguard the diversity existing within these ecosystems. However, the extent to which traditional grasslands have been affected by the widespread use of uniform commercial cultivars is currently unknown. To determine which grasslands are worth saving and should have priority for *in situ* conservation, data are needed about the genetic diversity present within and among traditional grasslands. In addition to *in situ* measures conserving the entire ecosystem, the relevant species found in these grasslands will be conserved *ex situ* in the genebank.

- **Experimental set-up**

In 2000, vegetative material (splits) of *L. perenne* and *T. repens* was collected in 16 traditional grasslands selected from the previously identified 50 traditional grasslands (Fig. 1). From each of the 16 grasslands 36 splits were selected and planted in the autumn of 2000 in a three-replicate trial at an experimental field in Wageningen. In addition tissue material was collected from each of the 36 plants for DNA analysis. Reference cultivars of both species, particularly varieties which had been widely used in Dutch pastures during the period 1940-1990, were included in the experiments in order to establish the diversity of the old grasslands using comparisons with these reference cultivars. The material in the field was evaluated in 2001 and 2002 for a set of morphological characters, which are also used in the registration of new cultivars. These so-called DUS descriptors are considered to be "distinct, uniform and stable". The molecular characterization was carried out with AFLP markers (Vos *et al.* 1995). The analyses for *L. perenne* started in 2000 and in 2003 all analyses will be completed.

Fig. 1. Location of 16 traditional grasslands in the Netherlands selected for the diversity study.

- **Preliminary results**

Preliminary results obtained for *L. perenne* did not indicate introgression from the reference cultivars into the traditional grasslands (van Treuren *et al.* 2005). Moreover, marked differences were observed between some of the grasslands for both the agromorphological and the ALFP data. For the morphological characters this was particularly true for heading date. The results for *L. perenne* suggested that the Netherlands may be considered a more or less continuous population with some grasslands being affected by selection (van Treuren *et al.* 2005).

Preliminary results of the morphological data in *T. repens* also showed a clear distinction between the reference cultivars and the grassland samples, notably in earliness and characters related to plant vigour. A remarkable result was that the most divergent populations of *T. repens* were collected from the same grasslands as the most divergent populations of *L. perenne.* This may be due to variation of ecological conditions among the traditional grasslands studied until now. So far the combined results do not support large-scale conservation measures for these Dutch grasslands, but rather seem to point to the conservation of a selected group which represents most of the variation.

References

Soest, L.J.M. van. 1998. Report of the expedition to Uzbekistan - Itinerary, collected materials and data. Centre for Plant Breeding and Reproduction Research (CPRO-DLO), Centre for Genetic Resources, The Netherlands (CGN), Wageningen, The Netherlands. 34pp.

Soest, L.J.M. van, K.I. Baimatov and V.F. Chapurin. 1998. Multi-crop collecting mission to Uzbekistan. Plant Genetic Resources Newsletter 116:32-35.

Soest, L.J.M. van and N. Bas. 2000. Genetic resources of *Trifolium* in the Netherlands. Pp. 315-318 *in* Proceedings of the final conference of COST Action 814 Crop development for the cool and wet regions of Europe. Achievements and future prospects. 10-13 May 2000, Pordeone, Italy (G. Parente and J. Frame, eds). European Commission, Directorate-General for Research, Brussels.

Soest, L.J.M. van and H. Dijkstra. 1986. Red clover collecting in The Netherlands. Internal report CGN/SVP. Wageningen, The Netherlands. 3pp.

Soest, L.J.M. van and H. Dijkstra. 1998. Current status of CGN forages collection. Pp. 78-80 *in* Report of a Working Group on Forages (L. Maggioni, P. Marum, R. Sackville Hamilton, I. Thomas, T. Gass and E. Lipman, compilers). Sixth meeting, 6-8 March 1997, Beitostølen, Norway. International Plant Genetic Resources Institute, Rome, Italy.

Treuren, R. van, N. Bas, P.J. Goossens, J. Jansen and L.J.M. van Soest. 2005. Genetic diversity in perennial ryegrass and white clover among old Dutch grasslands as compared to cultivars and nature reserves. Molecular Ecology 14:39-52.

Vos, P., R. Hogers, M. Bleeker, M. Reijans, T. van de Lee, M. Hornes, A. Frijters, J. Pot, J. Peleman, M. Kuiper and M. Zabeau. 1995. AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

Zeven, A.C. and J.M.J. de Wet. 1982. Dictionary of cultivated plants and their regions of diversity. Pudoc, Wageningen, The Netherlands. 263pp.