The European Lolium perenne *core collection in the Botanical Garden of the Plant Breeding and Acclimatization Institute, Bydgoszcz, Poland*

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Introduction

During the meetings of the ECP/GR Working Group on Forages in 1991 and 1994 it was decided to develop a European core collection for perennial ryegrass (*Lolium perenne* L.) ecotypes (IBPGR 1993; Gass *et al.* 1995). It was developed in 17 European countries holding ryegrass collections. One hundred and fifty-four ecotypes with four control varieties ("main" core collection) were planted, cultivated and evaluated according to the methodology given by Sackville Hamilton *et al.* (1997, 1998).

The main goal of the evaluation of the core collection in Bydgoszcz was to examine the variation of ryegrass ecotypes under Polish conditions, using a wider range of traits than is normally used to assess the "main" core collection.

Materials and methodology

- **"Main" core collection**

Seeds of 154 accessions were provided by curators of *Lolium perenne* collections from 17 European countries. In the spring of 1995 seeds of ecotypes, together with control varieties ('Arion', 'Frances', 'Talbot' and 'Vigor') were sown to produce seedlings which were transplanted into small pots and grown from March to June in a cold greenhouse. In July the young plants were planted in the field in 4 replicates, in 1.5 m-long rows with 7 plants at 25 cm intervals and 75 cm between the rows in a split-plot design. Two replicates were subjected to conservation management, with a cut when the last accession had 50% anthesis. The other two replicates were subjected to frequent cutting management (to simulate periodic grazing) with cuts every 3-5 weeks, as appropriate for the evaluation site.

The evaluation protocol was based on the IPGRI descriptor list with small changes (Sackville Hamilton *et al.* 1997, 1998). The following scores were noted:

- heading tendency: tendency to produce inflorescences in the year of sowing (on a scale of 1 = none to 9 = high);
- winter damage: estimated % of dead tillers (on a scale of 1 = minimum to 9 = maximum),
- heading date: number of days from 1 April to emergence of the inflorescence;
- plant habit: tiller angle (on a scale of 1 = horizontal/prostrate to 9 = vertical/erect);
- leaf blade width at heading (on a scale of 1 = very narrow to 9 = very wide);
- bulks: in conservation management = green matter yields (in kg per plot); in frequent cutting management = visual estimation of yield (on a scale of 1 = very low to 9 = very high);
- rust infestation (on a scale of 1 = no rust to 9 = all plants completely infected);
- aftermath heading: intensity of heading after second and third cut in conservation management (on a scale of 1 = none to 9 = numerous heads).

Under the conservation management regime, cuts were adjusted to the developmental phase (i.e. earliness) and were made at five different dates for the first cut (28.05.96, 03.06.96, 21.06.96, 25.06.96 and 01.07.96), once for the second cut (01.09.96) and once for the third cut (15.10.96). Estimations of yield under frequent cutting management were made ca. 6 times per month from 15.05.96.

Winter 1996-97 damage affected the majority of the collection so strongly that further evaluation would not have been worthwhile. We then decided to redefine the core collection on the basis of the results from 1995 and 1996 and to make a more precise evaluation on a "sub-collection". Cluster analysis (UPGA and Euclidean distance) was performed on 22 characters and a sub-collection of 28 ecotypes from 9 clusters was selected. The methodology of selection of sub-samples for further analysis was similar to that of Charmet *et al.* (1990) and Casler (1995).

- **Sub-collection**

Control varieties were the same as in the "main" core collection and two Polish varieties were added: 'Nadmorski' and 'Arka'.

Seeds from the selected accessions were sown in August 1997. Young seedlings were planted in pots in the hot bed and planted out in the field at the end of September 1997. Six replications were used (3 replicates in the frequent cut and 3 replicates in conservation management) with 10 plants per replicate. Evaluation and measurements were carried out in 1998-1999.

Apart from traits similar to those assessed in the "main" collection, additional evaluations were done on:

- young seedlings in pots: number of leaves (4 weeks after planting in pots), number of tillers and seedling dry matter weight (grams per seedling at 8 weeks after planting) and average dry matter of tiller (total plant dry matter weight/number of tillers);
- plants in the field: aftermath heading in the frequent cut treatment, plant height and length of stem leaf at the beginning of heading in conservation management;
- dry plants (cut at the heading phase): for the three longest stems per plant the following traits were measured: height, length of inflorescence, number of spikelets per inflorescence, number of florets per spikelet, length of spikelet (mm), number of spike branches, intensity of spike branching (on a 1-9 scale where 1 = no branching, 3 = branching only on a few spikes, 5 = branching on some spikes, 7 = numerous branching on few stems, 9 = very numerous branches on numerous stems), distance between florets in spikelet and distance between spikelets in spike.

Analysis of variance (one-way ANOVA) was carried out on 71 characters. To describe the variation of the selected ecotypes under different conditions, the results from the "main" core collection were compared to results from the sub-collection. For sub-collection results, Pearson correlation coefficients (r) were calculated for the same traits evaluated in different years. If r was high (>0.6) mean values from both years were accepted for further calculations. Further, principal component analysis (PCA) with varimax rotation and cluster analysis (UPGA and Euclidean distance) were carried out.

Results

- **"Main" core collection**

For ecotypes from the "main" core collection analyzed on the basis of country of origin, differentiation for all 20 traits was significant for ecotypes from France, Great Britain and Switzerland. The lowest trait differentiation was observed for ecotypes from the Czech Republic (4 traits), Bulgaria (6) and Greece (7).

Cluster analysis selected nine groups of ecotypes. Clusters were characterized as follows:

1. the earliest ecotypes, with a high percentage of heading in the sowing year and high aftermath heading, high rust infestation after the second cut;
2. no heading in the sowing year, low winter damage and good yield, highest value of aftermath heading after the second cut;

3. early heading, narrowest leaves and prostrate habit;
4. high green matter yield estimated in first cut in frequent cutting management, no heading in sowing year, high rust infestation of the third regrowth, good yields especially at spring cuts;
5. low percentage of heading in the sowing year and at the second and third regrowths, good yields of autumn cuts;
6. low winter damage and good yields, wide leaves and erect habit;
7. good yields, latest ecotypes with a low percentage of heading in the sowing year;
8. highest values of winter damage and lowest yields, prostrate habit and highest percentage of heading in the sowing year;
9. no heading in the sowing year, rather late, prostrate habit, high rust infestation of the third cut.

As a result of cluster analysis on country mean values the following groups were selected (Fig. 1): first group: one element = Bulgaria (n=6); second group: Mediterranean countries (Italy, Spain and Greece, n=17); third group: one element = Switzerland (n=4); fourth group: rest of Europe (n=130). In the last group some subgroups also appeared: Czech Republic and Poland; Germany, Belgium and the Netherlands; Great Britain and France.

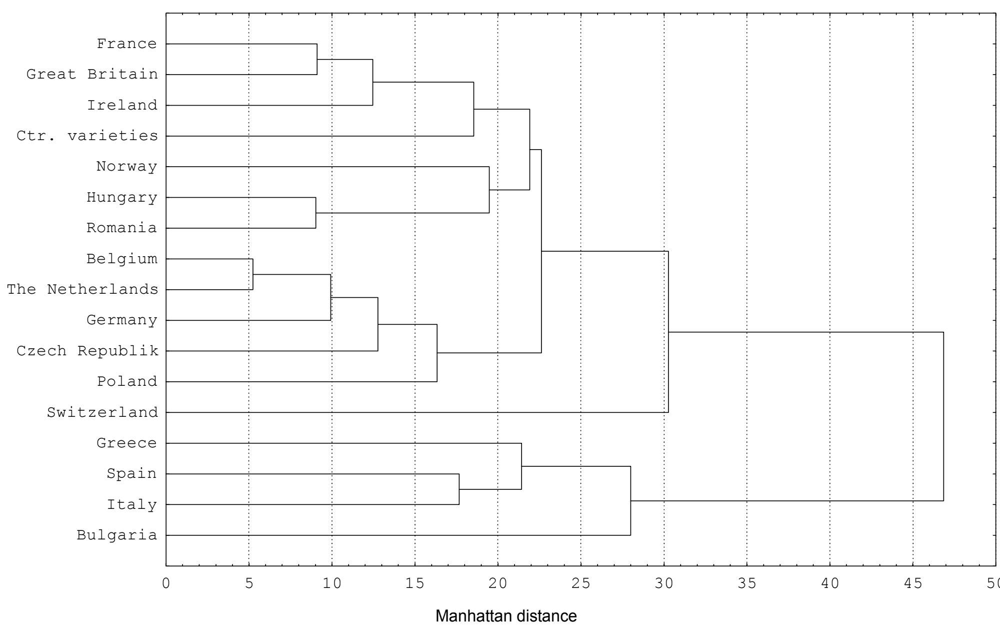

Fig. 1. Results of cluster analysis on country means (main core collection).

- **Sub-collection**

As compared to control varieties, ecotypes from the sub-collection showed higher values of the coefficients of variation for: dry matter weight per stem, winter damage, fresh matter yield of the last conservation cut, yields in frequent cutting, aftermath heading in conservation management in 1996, third and fourth regrowth in frequent cut, rust infestation in 1996 and in frequent cutting in the sub-collection, intensity of spike branching, plant habit, length of spikelet in 1998, average distance between florets in spikelet and width of leaves in 1996 and 1999.

Principal component analysis selected five components:

1. the first component accounted for 19.3% of variation and was mostly related to vegetative development. It was negatively correlated with the following traits: aftermath heading in both treatments and number of florets in spikelet in 1999. Positive correlation was found for the number of days to heading;
2. the second component (yield performance) accounted for 22.4% of variation and was strongly positively correlated with yields from both treatments (especially for first cuts), height of plants at heading, length of leaves and inflorescences. Negative correlation was found between the second component and winter damage, especially in the first year of the experiment;
3. the third component (rust infestation) accounted for 9.8% of total variation;
4. the fourth component was correlated with width of leaves and length of spike, number of florets in spikelet in 1998;
5. the fifth component, accounting for 8.7% of variation, was highly correlated with seedling dry matter weight, average dry matter of tiller and winter damages in 1999.

When plotting them over first and second and first and third components (Fig. 2) it appears that ecotypes nos. 26 and 29 from Belgium, ecotypes no. 83 from Netherlands and 134 from Switzerland had low rust infestation, high yield ability and low ability to produce flowering stems (high vegetative development). The highest yield ability was noted for ecotype 51 from Hungary, and the lowest yield for ecotype 67 from Spain. Variety 'Vigor' displayed the lowest ability to produce flowering stems (highest vegetative development) as opposed to ecotypes from Spain and Hungary (ecotypes 71 and 55, respectively). The ecotype from Switzerland (134) had lower rust infestation as opposed to ecotype 34 from Sweden. Except for 'Vigor', variation of all ecotypes in the sub-collection was wider than that of the control varieties.

For the seven clusters shown in Fig. 3 the mean values for selected traits were calculated. Ecotypes in clusters were characterized as summarized in Table 1.

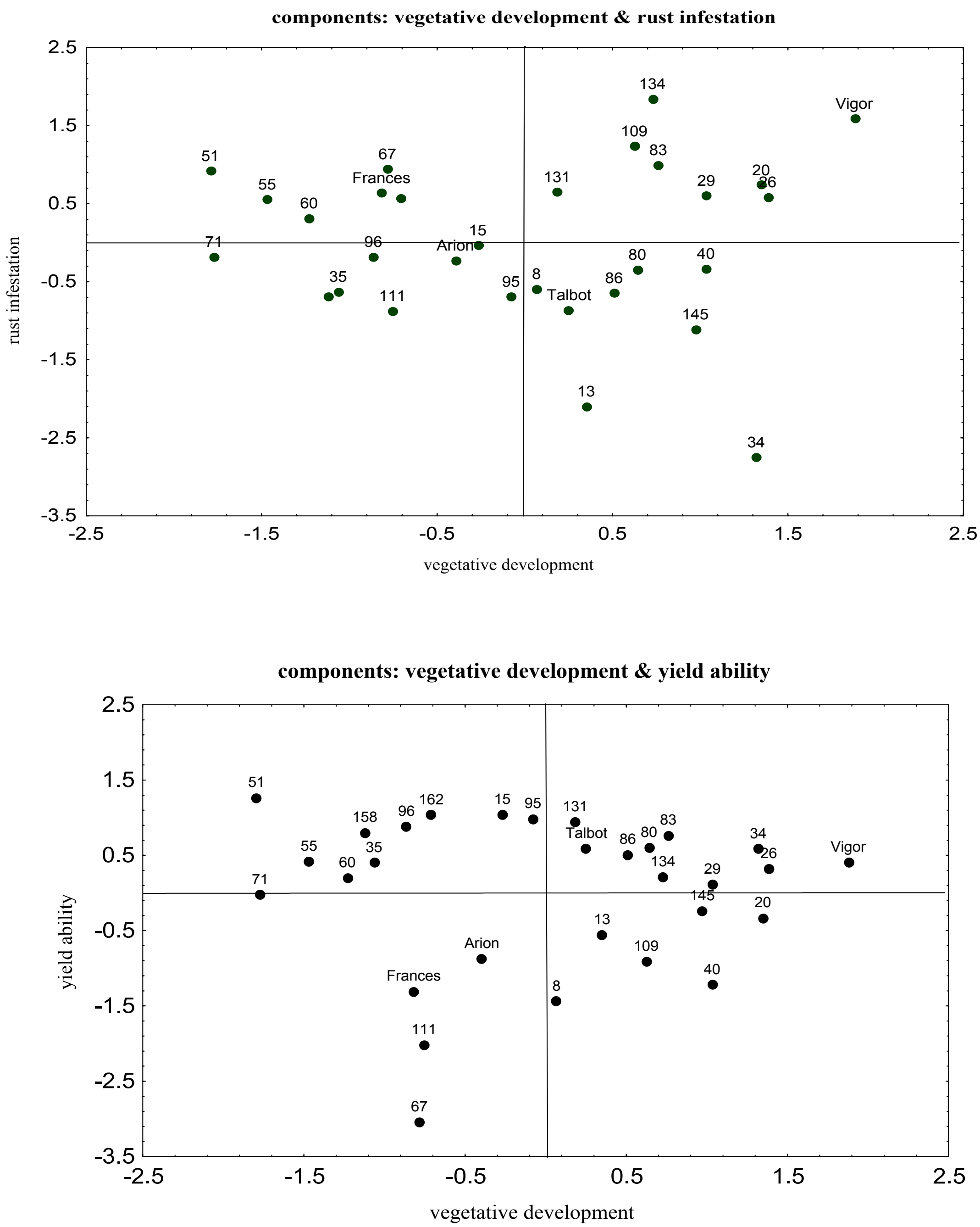

Fig. 2. Principal component analysis (sub-collection).
Figures and names near dots on graphs = no. of ecotypes and names of varieties.

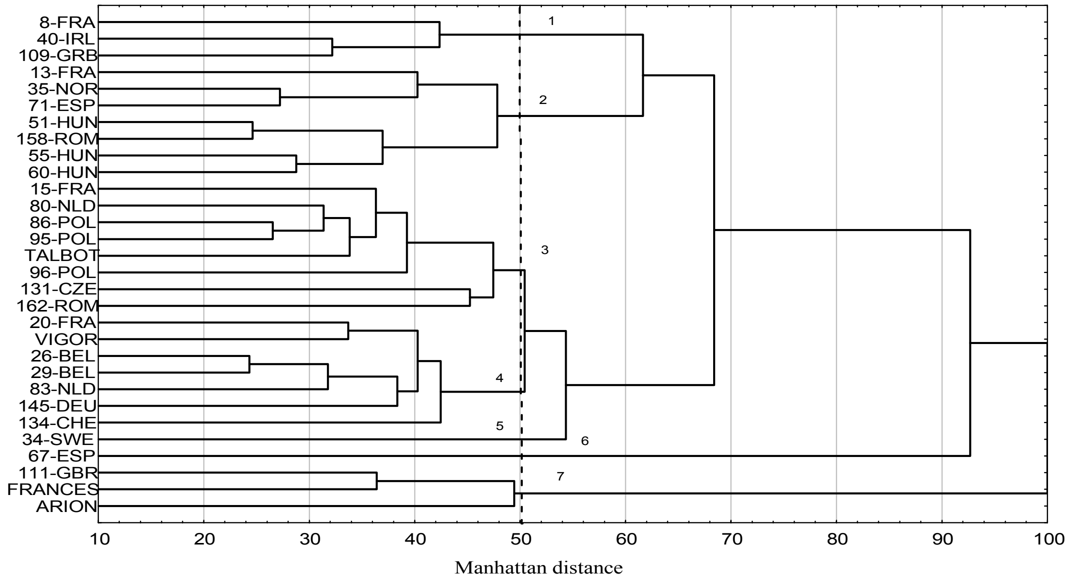

Fig. 3. Results of cluster analysis (sub-collection).
Figures inside graph (1-7) = cluster number (see also Table 1)

Table 1. Composition of clusters and characteristics of the ecotypes in the sub-collection

Cluster no.	Ecotypes	Characteristics
1	ecotypes from western Europe	low yield ability, high winter damage and low dry matter of seedlings
2	ecotypes from Hungary and one from Romania, Norway and France	low dry matter yield of seedling, low winter damage, high yield ability, highest yield of fresh matter in early spring cut, high tendency to aftermath heading at second regrowth
3	variety 'Talbot', three ecotypes from Poland and one from France, the Netherlands, Czech Republic and Romania	high yield ability and low winter damage
4	variety 'Vigor', two ecotypes from Belgium and one from France, the Netherlands and Germany	high dry matter yield of seedlings, good yielding, late heading and highest plant at heading
5	one ecotype (no. 34) from Sweden	high yield ability in frequent cut management, wide and long leaves, highest number of florets per spikelet, high intensity of spike branching and low dry matter of seedlings
6	one ecotype (no. 67) from Spain	high yield ability in frequent cut management, very low plants during heading, short and wide leaves
7	varieties 'Arion' and 'Frances', one ecotype (no. 111) from Great Britain	very early heading, high winter damage, high aftermath heading in regrowth after the second cut, short and narrow leaves, short inflorescence, low intensity of spike branching and high seedling dry matter

Discussion and conclusions

Winter damage affected all characters examined. It was confirmed by observations made on the perennial ryegrass core collection in Norway and in the Czech Republic (Sackville Hamilton *et al.* 1997). A different reaction was observed in Great Britain and Germany where practically no winter damage was observed and even winter yields were measured, but ecotypes from the Czech Republic and Norway yielded low. Winter damage is therefore related to the origin of the ecotype and place of examination, with lowest damage in the region closest to the original collecting site of the ecotype.

Variation of winter damage in the second year was lower for conservation management than for frequent cutting. It may have been due to a weaker condition of the plants resulting from cuts made in the later phases compared to those under a frequent cut management regime.

Yield analysis indicated that ecotypes from northeastern and eastern Europe (especially from Romania) were of higher yield potential in spring cuts. Similar results were obtained for Romanian ecotypes by Tyler *et al.* (1984) and Charmet and Balfourier (1991). Differences in yield were observed for different cuts and management regimes. But, as mentioned before, winter damage most affected yield. High, negative correlation was calculated between first cuts and winter damage for the "main" core collection (Schmidt and Kaszuba 1997). However, the decreasing value of the correlation coefficient in later cuts could be the effect of the high regeneration ability of ecotypes.

Observations made by Sadowski *et al.* (1997) on rust infestation in the "main" core collection indicated *Puccinia coronata* Corda as the causal agent of 60% of rust visible symptoms and *Puccinia graminis* Pers for the rest of the symptoms. The highest differentiation of ecotypes by their degree of rust infestation symptoms was observed in summer, at the initial stage of the disease. Chorlton and Thomas (1987) obtained similar results when studying the susceptibility of ryegrass ecotypes to *Puccinia coronata* infestation. It is worth noting that in our experiment none of the tested ecotypes showed lower infestation than the Polish variety 'Arka'.

Aftermath heading was negatively correlated with the number of days to heading and it was also found that ecotypes originating from northern Europe produced lower numbers of heads. This is probably due to the different life strategies of ecotypes originating from different regions (Lorenzetti *et al.* 1971; Breese and Tyler, after Breese 1989). Ecotypes from Romania showed high values of aftermath heading after the first cut, as also found by Charmet and Balfourier (1991).

The key role of origin in overall ecotype performance, as found in our study, was also suggested by Lorenzetti *et al.* (1971), Balfourier and Charmet (1991), Charmet *et al.* (1993), Loos (1994), Casler (1995) and Amin and Thomas (1996).

Preliminary observations on mean heading dates and winter damage recorded in other institutions cooperating in the perennial ryegrass core collection (Sackville Hamilton *et al.* 1998) as well as conclusions from other researchers (Charmet *et al.* 1990; Elgersma 1990a, 1990b; Charmet and Balfourier 1991; Solberg *et al.* 1994) strongly support the multi-site evaluation of collections. It is especially recommended for traits of low heritability, with high dependence on environmental conditions. Therefore, to obtain more complete information on the nature of variation of European perennial ryegrass ecotypes and its interaction with environment, it is necessary to discuss similar results from other countries.

The above results strongly support the development of core collections for other species stored in European genebanks.

Further conclusions from the evaluation of the perennial ryegrass core collection in Bydgoszcz, Poland are as follows:

- it is possible to indicate the following regions of origin of the ecotypes of the tested species in Europe: southern Europe, western Europe, northern-central Europe and Romania and Hungary;
- none of the tested ecotypes showed better yields than control varieties; however a few ecotypes appeared to have advantages for particular traits (better winter performance, faster spring regrowth, etc.);
- the high differentiation of ecotypes examined indicates the existence of many botanical varieties and intermediate ecotypes;
- traits associated with inflorescence morphology (distance between florets in spikelet and distance between spikelets in spike, length of spikelet) seem to be the most stable traits, in contrast the most variable traits were aftermath heading, tendency to produce inflorescences in the year of sowing and green matter yield of the first cut.

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