Safeguarding a unique collection of former Swiss red clover landraces

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Introduction

Red clover (*Trifolium pratense* L.) landraces used to be maintained and propagated on-farm by Swiss farmers in the 19th and 20th centuries. By consciously harvesting the seed in the last year of stand of a 3- to 4-year ley, the farmers improved the persistence of these landraces which became known under the name of 'Mattenklee', meaning "clover of the leys" (Boller 2000). Numerous "farm landraces" developed, some of which were produced and marketed locally on a modest scale. However, most of the farm landraces were maintained only for the purpose of re-sowing on the farm of origin, often for decades. This tradition was abandoned for the most part in the 1970s. In 1971 and 1972, the former red clover breeder of Reckenholz, Bruno Nüesch, collected samples of about 100 landraces which still existed. After a preliminary agronomic evaluation (Nüesch 1976), the original samples, some of which were of doubtful seed quality, were stored at low temperature without regeneration.

An investigation in 1999 revealed that the germination of about two-thirds of the samples had dropped below 10% and it was recognized that many were in urgent need of regeneration (Table 1). Moreover, the landraces had never been described systematically in terms of morphological or agronomic characters. We therefore decided to completely regenerate and evaluate the collection with the help of a federal grant received from the National Plan of Action for maintenance and sustainable use of plant genetic resources for food and agriculture (NAP). The work was carried out in collaboration between a public research station (FAL Reckenholz) and the seed industry represented by the association of Swiss seed and plant traders (VSSJ).

Table 1. Germination in spring 1999 of original seed samples from the 1971-1972 collection of 'Mattenklee' landraces stored at FAL Reckenholz

% germination	No. of landraces
0 to 5	33
5 to 10	36
10 to 20	20
20 to 40	14
>40	2
Overall average: 10.3%	**105**

Materials and methods

- **Regeneration**

Plots of 25 m^2 were established by using the original seed samples of 1971-1972 in a rapeseed field in 1999 and 2000, and in a maize field in 2001 at Neuenegg (Bern Canton). The site was chosen because it lies near the centre of the greatest abundance of farm landraces represented in the collection. The plots were arranged in 1.5 m-wide alleys separated by 15 m of rapeseed/maize crop. Within the alleys, the plots were at least 33 m apart. This arrangement allowed for about 30 landraces to be regenerated per year.

For about one-third of the samples, 20 g viable seeds (between 51 and 182 g total seeds, depending on germination) were available for sowing directly with a plot seeder. For the remaining samples of poorer seed availability, 200 to 220 seedlings were raised in the greenhouse. A seed quantity that was expected to yield 400 seedlings, judging by its germination and thousand-grain-weight, was treated with 4 g/kg Thiram 80. If the percentage of hard seeds exceeded that of germinated seedlings, the seed was scarified before the treatment. Seeds were then evenly distributed in seed boxes (40 x 60 x 5 cm). To avoid direct contact between seeds, no more than 15 g seeds were sown in one box. These precautions were taken to minimize loss of seedlings due to seedborne diseases. In most cases, sufficient seedlings emerged; if not, a second batch of seedlings was raised. When they had developed about three true leaves, seedlings were transplanted to 54 unit quickpot trays and grown on for at least two months. They were then transplanted into the field at 30 x 40 cm distances. A bulk seed harvest was taken from each plot with a plot harvester in late summer of the second year after sowing.

- **Evaluation of morphological and agronomic characters**

A choice of compulsory UPOV characters was observed or measured on 50 to 60 spaced plants per accession at Zürich-Reckenholz. These plants were also evaluated for persistence over three years. Additionally, row trials with 3 replications were observed for 3 years in one location. Yield and persistence were evaluated in standard plot trials with three replications in one to three locations, depending on seed availability. The entire material was split into subsets, each sown or planted in 2001, 2002, 2003 and 2004. First results are available for the subset established in 2001.

Results and discussion

- **Regeneration**

Seed yield varied greatly among the three harvest years 2000, 2001 and 2002, and among the landraces. We aimed to obtain 300 g viable seeds per landrace for storage and evaluation. This objective was achieved for 45 landraces (Table 2). The poor yield in 2002 was due to a greatly delayed harvest because of bad weather conditions. Game deer also caused some damage in all years.

A high degree of hardseedness was observed for many samples. However, the sum of germinated seedlings and hard seeds mostly exceeded 80%, which was considered satisfactory for long-term storage. Seed multiplication of landraces with insufficient seed yield or quality is being repeated, starting in 2002 and continuing 2003 to 2004. By the end of 2004, we hope to successfully safeguard 80 to 90% of the original stock of landraces.

Table 2. Seed yield and quality of harvests 2000 to 2002 from regeneration of 'Mattenklee' landraces

Harvest year	Plots established	Seed yield per 25 m^2-plot		Germination			No. of landraces with >300 g viable seeds
		mean	std. dev.	% germinated seedlings	% hard seeds	% total(*)	
2000	32	677	398	65.5	27.2	85.2	26
2001	32	488	399	67.6	12.5	78.5	19
2002	32	56	62	79.4	13.9	92.8	0

(*) % germinated seedlings +% hard seeds, except fractions >20%

- **Evaluation of morphological characters**

The first evaluation year 2002, on 33 landraces, revealed significant differences for flowering date, stem length, leaflet length and width and mildew susceptibility. Cluster analysis of these results separated most landraces clearly from the 'Mattenklee' cultivar 'Milvus' (Fig. 1).

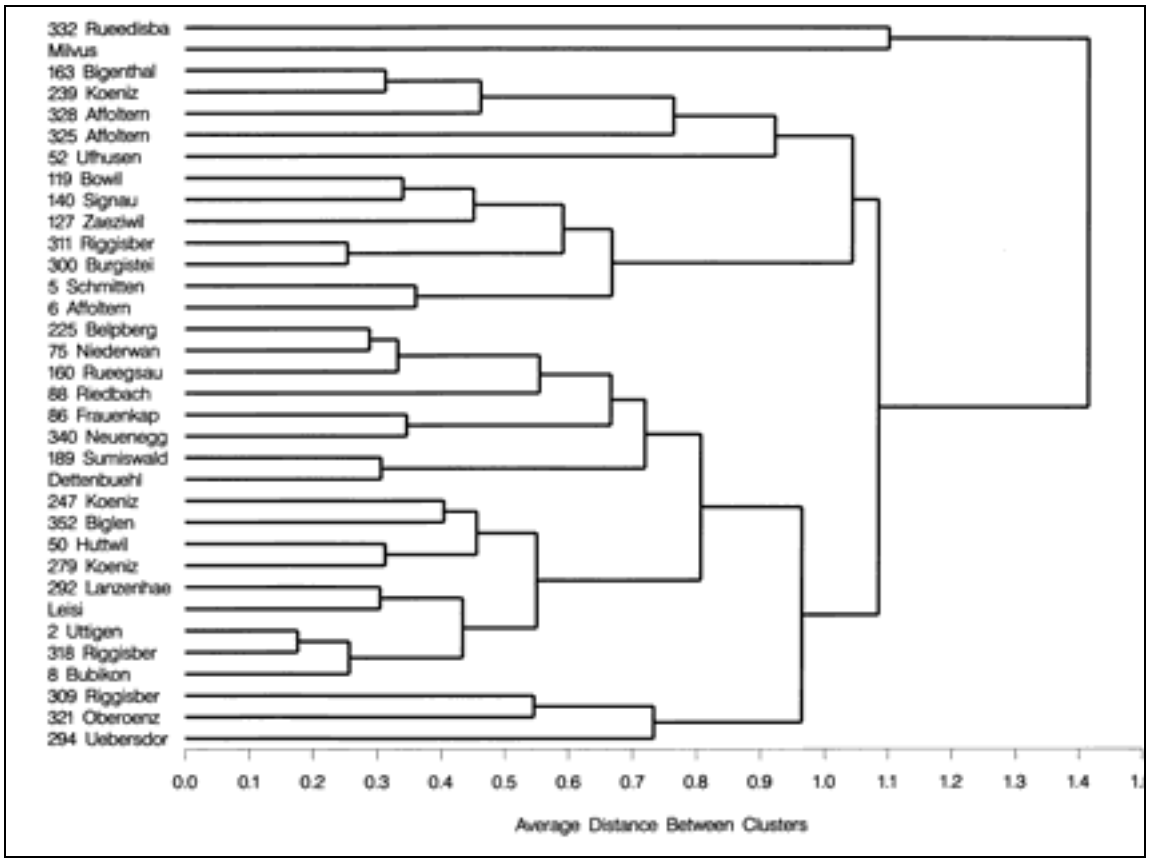

Fig. 1. Cluster analysis of morphological data and mildew resistance observed on spaced plants of 33 landraces and the cultivar 'Milvus'. Deviation from the mean over all accessions was weighted with least significant differences before analysis.

With few exceptions, the landraces were earlier flowering, shorter and more susceptible to mildew than 'Milvus'. Grouping by morphological characters was not related to the geographic origin of the landraces. For example, landraces 309, 311 and 318, all originating from the same small community Riggisberg, were each allocated to a different main cluster. Conversely, one of them (318) was grouped very closely with two landraces that originated from farms 7 and 236 km apart, respectively. These preliminary findings need to be corroborated by the evaluation of a larger number of landraces. A study on the basis of molecular marker diversity confirmed that 'Mattenklee' landraces form a diverse group of accessions clearly distinct from current 'Mattenklee' cultivars (Kölliker *et al.* 2003). In this study, it was also found that clustering on the basis of AFLP markers was not related to the geographic origin of the landraces.

- **Relationship between original seed quality, success of regeneration and morphological characters**

Germination of the original seed sample was positively correlated with seed yield in those plots that had been sown with a plot seeder in 1999 (harvest 2000, Table 3). Apparently, a linear increase in sowing density was insufficient in this case to compensate for poor germination. No correlation between germination and seed yield was observed in the other years.

Table 3. Correlation between seed yield and germination of original seed sample in sown and planted plots harvested 2000 to 2002

Harvest year	Method of plot establishment	Seed yield (g/plot)	Coefficient of correlation (r) between seed yield and germination of original sample (*p* for r≠0)
2000	Planting	650	n.a.
	Sowing	678	0.35 (*0.05*)
2001	Planting	503	0.10 (*0.64*)
	Sowing	450	-0.11 (*0.76*)
2002	Planting	56	-0.19 (*0.31*)

Failure to raise sufficient seedlings for transplanting resulted from extremely poor germination only. From all except one sample which had shown at least 2% germinated seedlings in an official ISTA test, it was possible to raise the desired number of 200 plants. Even seven samples with 0 to 1% germination yielded enough plants for transplanting. Once established, these planted plots yielded as much seed as those sown with the plot seeder, irrespective of their original germination (Table 3). Seed quality (germination and purity) tended to be better for the planted vs. the field sown plots.

Poor germination might lead to a shift in the population structure because long-term seed survival could be linked with other plant characters. Indeed, there was a trend for germination of the original seed sample to be correlated negatively with flowering time (Table 4). This would indicate that genotypes that survived long seed storage would tend to flower later than the population mean. More data are needed to confirm and interpret this trend.

Table 4. Correlation between original seed germination or seed yield during regeneration and morphological characters of 32 'Mattenklee' landraces

Character	Coefficient of correlation, r (with *p* for r≠0) between	
	Character and original germination	Character and seed yield
Date of flowering	0.32 (*0.07*)	-0.12 (*0.51*)
Stem length	0.14 (*0.43*)	-0.32 (*0.07*)
Leaflet length	0.04 (*0.83*)	-0.18 (*0.31*)

- **Evaluation of agronomic characters**

Results for the first full harvest year of the first series of trials indicate that most farm landraces have an inferior yield potential as compared to current cultivars of the 'Mattenklee' type. Annual dry matter yield of the 20 farm landraces averaged 91% of that of the 'Mattenklee' cultivar 'Milvus', ranging from 86 to 101%. 'Dettenbühl' was the only landrace that outyielded 'Milvus' slightly: formerly, it used to be produced on a modest scale for the local market, and had been recommended officially in the 1960s and 1970s. These preliminary results must be complemented by the second full harvest year, decisive for the expression of the 'Mattenklee' character of persistence.

Outlook

The primary goal of safeguarding an important part of the remaining pool of Swiss red clover landraces can probably be achieved within the next two years. This unique material will be made publicly available. We plan to complete morphological description and agronomic evaluation of the collection to assess its value as a potential genetic resource for further breeding. The results will be complemented further by molecular marker studies, and by a study of performance of some landraces at their sites of origin. These studies will be useful for making a choice of landraces to be included in a breeding programme, and may result in the identification of obsolete duplicates in the collection.

References

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