pielietošana selekcijā arī paplašina mainības ietvarus, paaugstina augu plastiskumu un izturību pret nelabvēlīgiem biotiskiem un abiotiskiem apstākļiem. Pētījuma mērķis ir izpētīt un optimizēt poliploīdijas pielietošanas iespējas liliju selekcijā, apstrādājot liliju sīpolu zvīņlapas ar 0.1, 0.5 un 1 g kg^{-1} kolhicīna šķīdumu un 0.05, 0.1 un 0.5 g kg^{-1} orizalīna šķīdumu.

GENETIC FINGERPRINTING OF LATVIAN RED CLOVER (*TRIFOLIUM PRATENSE* L.) VARIETIES USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS: COMPARISONS OVER TIME AND SPACE

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Abstract
We have established Simple Sequence Repeat (SSR) marker genetic fingerprinting protocols for red clover (*Trifolium pratense* L.) varieties found in the Latvian Gene Bank (LGB). As red clover is an obligate outcrosser, and the varieties are grown and renewed in the field without any particular isolation techniques, a high degree of intra-varietal heterogeneity is to be expected.
We analysed 7 diploid varieties, which were developed in three different breeding stations. We tested seeds from these varieties that were placed into the LGB in 1999 and 2000. In addition, we analysed a range of source material for one variety ('Priekuļi 66'). For this variety, we tested seeds that were repatriated from the VIR institute (placed into the VIR collection in 1982, seeds reproduced in 2005), the samples from the LGB (seed reproduction - 1997), and also plant material grown in the field this year (2007).
By analysing samples from various sources, we can examine the effect of space (varieties developed at different breeding stations), as well as time (repatriated seeds, LGB seeds, and current crop), in an obligate outcrossing crop species, where intra-varietal heterogeneity is high.

Key words: red clover, *Trifolium pratense L.*, genetic fingerprinting, Simple Sequence Repeat, Latvian Gene Bank, plant genetic resources

Introduction
Red clover is an important forage legume, widely grown in temperate regions and used in crop rotations. It is an obligate outcrosser, with a gametophytic self-incompatibility system (Taylor and Quesenberry 1996). Red clover is a diploid ($2n = 2x = 14$) species, however artificial tetraploid varieties have been created in breeding programs. Generally breeding programs are based on mass selection, and therefore the varieties produced are heterogeneous with highly heterozygous individuals. Initial molecular analyses of this species were undertaken using dominant marker systems such as RAPDs and AFLPs (Ulloa *et al*, 2003; Herrmann *et al*, 2005). The development of Simple Sequence Repeat (SSR) markers for red clover has allowed the analysis of these highly heterozygous varieties using highly informative co-dominant markers (Kolliker *et al*, 2006). At the Latvian Gene Bank, we are in the process of establishing protocols for genetic fingerprinting of all species in our collection. We have focused our efforts on the use of SSR markers for as many species as possible, due to their high information content (alleles per marker), co-dominant nature (which allows more sensitive detection of heterozygosity and variation within cultivars and lines), and their ease of use (provided appropriate SSR marker primers have been developed).
Our aims were to establish SSR fingerprinting protocols for the red clover varieties placed in the Latvian Gene Bank and to examine the inter- and intravarietal variation of Latvian clover varieties. Clover breeding has been undertaken in at least three breeding institutes in Latvia, and the Latvian Gene Bank holds seeds of accessions developed at all of these institutes. Currently, the main institute involved with clover breeding and maintaining the genetic resources is Skriveri Breeding Institute, but varieties have also been developed at the Priekuli Breeding Institute and Stende. Prior to the establishment of the Latvian Gene Bank, some of these cultivars developed at these breeding

institutes were placed into the genetic resources collection at the Vavilov institute (VIR) in St. Petersburg, Russia. We examined one variety ('Priekuļi 66') in more detail in order to compare the SSR polymorphism of three separate collections of this variety. 'Priekuļi 66' was transferred into the VIR institute collection in 1982, and renewed there in 1988. This material was repatriated to Latvia in 2004, at which time the seed material was renewed. The 'Priekuļi 66' seeds were placed into the Latvian Gene Bank collection in 1997. We also took samples of this cultivar from the field collections at Priekuļi, where it is resown annually.

Materials and Methods

Seed material was obtained from the Latvian Gene Bank collection. Seeds were germinated and genomic DNA was extracted from seedlings with the Genomic DNA Purification Kit K0512 (Fermentas, Lithuania). The red clover cultivars analysed are listed in Table 1. DNA from 24 individuals was extracted and analysed separately. In addition, seed material from the VIR institute was obtained and germinated. Plants from these germinated seeds were planted and seed material collected. These seed were germinated and DNA extracted from them. A total of 92 individuals derived from 34 repatriated seeds were analysed. For the field collections, leaf samples of 35 individuals were collected 2007, and the DNA extracted as above. To allow for a more detailed comparison with the VIR and field populations, 48 'Priekuļu 66' seeds from the gene bank collection were germinated and DNA extracted.

Table 1. Red clover cultivars analysed, breeding institute where each cultivar was developed, year of cultivar release and year of seed reproduction

Cultivar	Breeding institute	Year of cultivar release	Year of seed reproduction
Priekuļu 66 (Raunis)	Priekuļi	1968	
- Field			2007
- Gene Bank			1997
- VIR			2005
Stendes Agrais	Stende	1968	1994
Stendes Vēlais II	Stende	1951	1997
Dižstende	Stende	1999	1997
Agra	Skrīveri	1996	1997
Ārija	Skrīveri	1999	1999
Skrīveru Agrais	Skrīveri	1976	1997

Eight SSR markers were used to genotype these cultivars (TPSSR13, TPSSR17, TPSSR16, TPSSR34, TPSSR44 and TPSSR50) (Kolliker *et al*, 2005). The forward primer was synthesised with a 6-FAM, HEX or NED fluorescent label to allow visualisation of amplification products on a fluorescent sequencer.

SSR locus amplification was carried out using the following PCR conditions: 95 ^{0}C for 3 min, 38 cycles of 95 ^{0}C for 30 sec, 55 ^{0}C – 30 sec, 72 ^{0}C – 30 sec; 72 ^{0}C - 10 min; in a total volume of reaction 20µl containing 50 ng template DNA, 1x PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTP mix, 0.5 U *Taq* polymerase (*Fermentas*), 0.5 mM of forward (labelled) and reverse primers (*Applied Biosystems*). Amplification fragments were separated on an ABI Prism 3130xl Avant Genetic Analyzer (*Applied Biosystems*) and analyzed with GeneMapper 3.5. Population analyses were performed with GenAlEx 6 version (Peakall and Smouse, 2006), and dendrograms constructed using NTSYSpc2.1.

Results and Discussion

The six SSR markers revealed a high level of genetic polymorphism, with 22-44 alleles detected per marker. Within populations, the majority of markers were in Hardy-Weinberg equilibrium, with only two populations having statistically significant departures ('Priekuļu 66' – Field – TPSSR16 and TPSSR17 and 'Priekuļu 66' – VIR – TPSSR44 and TPSSR50). Only three markers

significantly departed from equilibrium in more than one population (TPSSR16, TPSSR17 and TPSSR34).

The mean number of alleles found in each population ranged from 11.3 (‘Ārija’) to 19.2 ‘Priekuļi 66’ - Gene Bank. The mean number of alleles per population with a frequency of over 5% ranged from 5.5-7.83. The mean number of effective alleles per population was similar between populations, ranging from 7.79-10.65. However, the mean number of unique alleles found per population was variable, ranging from 0.17 (‘Stendes Vēlais II’) to 2.33 (‘Priekuļi 66’ – VIR) (Table 2, Figure 1).

Table 2. Numbers of alleles (Na), alleles with a frequency over 5%, effective alleles (Ne), unique alleles, and expected heterozygosity (He) across populations

Number of alleles	Population								
	P66 Field	P66 Gene Bank	P66 VIR	Stendes Vēlais II	Stendes Agrais	Dižstende	Agra	Ārija	Skrīveru Agrais
Na	17.67	19.17	15.00	13.33	15.83	12.33	12.67	11.33	14.83
Na (freq. ≥ 5%)	6.17	7.00	6.67	6.17	8.33	6.67	5.50	6.00	7.83
Ne	10.10	10.65	9.90	7.79	10.01	7.91	9.29	8.09	10.52
No. unique alleles	1.50	1.33	2.33	0.17	0.50	0.33	0.33	0.50	1.00
He	0.84	0.85	0.87	0.84	0.85	0.82	0.84	0.85	0.88

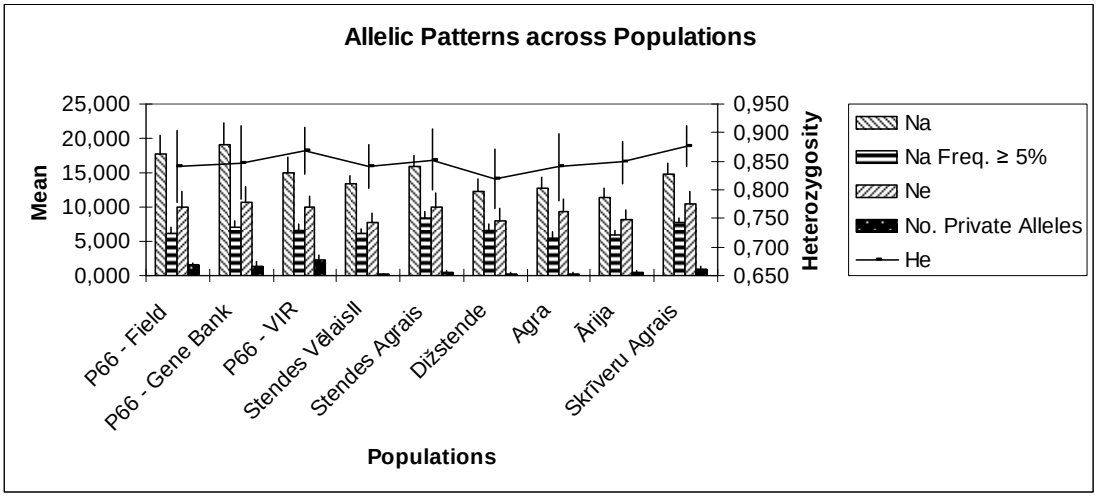

Figure 1. Allelic patterns across populations using 6 SSR markers (see also Table 2)

Pairwise Fst values revealed low population differentiation (values ranged from 0.006-0.043) (Table 3). AMOVA analysis revealed only 2% of the genetic polymorphism was found between populations (p=0.01). Frequency based likelihood population assignment tests (Paetkau *et al*, 2004) also highlighted the low differentiation, with only 51% of individuals assigned to the correct population. The frequency of unique alleles found in the populations was under 0.1, with the exception of 2 alleles (tpSSR17/134bp /f=0.136 in ‘Priekuļi 66’ – VIR and tpSSR13/199bp/f=0.125 in ‘Ārija’).

Table 3. Pairwise Fst values between cultivars and populations

Variety									
P66 - Field	0.000								
P66 - Gene Bank	0.006	0.000							
P66 - VIR	0.014	0.012	0.000						
Stendes Vēlais II	0.024	0.019	0.023	0.000					
Stendes Agrais	0.017	0.015	0.020	0.018	0.000				
Dižstende	0.032	0.026	0.028	0.030	0.020	0.000			
Agra	0.025	0.024	0.026	0.023	0.023	0.042	0.000		
Ārija	0.030	0.030	0.030	0.038	0.030	0.043	0.028	0.000	
Skrīveru Agrais	0.025	0.019	0.022	0.026	0.021	0.029	0.029	0.028	0.000

The cultivars were divided into populations according to the breeding institute where they were developed (Skrīveri, Stende or Priekuļi), and this enabled slightly better differentiation of these cultivars according to their provenance. AMOVA analysis revealed that the 2% variance found between populations was actually partitioned between breeding institutes. Population assignment calculations were also more successful, with 87% of individuals assigned to the correct breeding institute (Table 4). The dendrogram (constructed using Fst values and the Neighbour Joining clustering algorithm) confirms this division by provenance, with each of the cultivars developed in one particular breeding institute clustering together (Figure 2).

Table 4 - Summary of Population Assignment Outcomes (Number if individuals assigned to "self" or "other" population)

Pop	Self Pop	Other Pop
Stende	50	9
Skrīveri	33	8
Priekuļi	76	7
Total	159	24
Percent	87%	13%

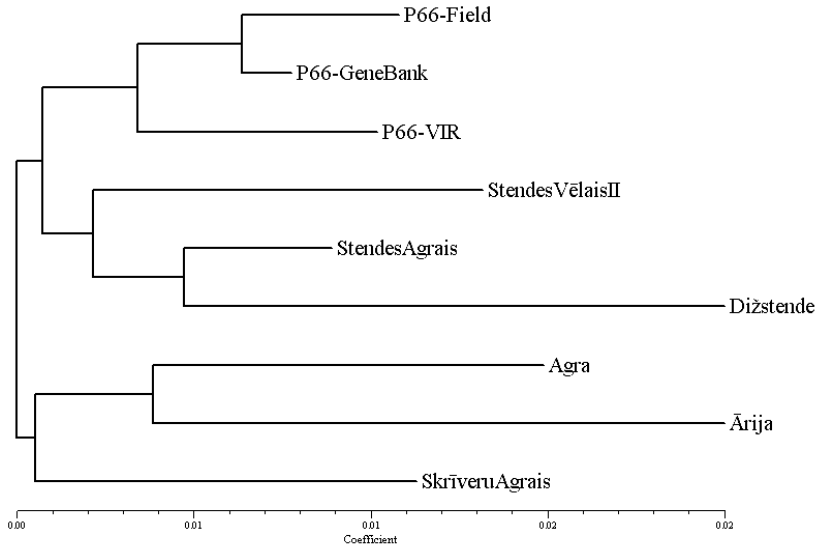

Figure 2 Dendrogram of red clover cultivars constructed using the Neighbour-Joining algorithm and Fst values

The SSR markers were highly polymorphic, confirming that these markers are useful for genetic studies in red clover. The majority of markers in the majority of populations did not deviate from Hardy-Weinberg equilibrium, indicating that there were no significant problems with null alleles, or the markers amplifying multiple loci.

A high level of polymorphism was revealed using these SSR markers, which is not surprising given the obligate outcrossing nature of red clover, and the mass selection breeding methods employed in this species. The number of alleles, particularly those over a frequency of 5% and the effective allele number was similar across all populations. However, the number of unique alleles differed, with the 'Priekuļu 66' – VIR population containing the most, with all 6 SSR markers identifying unique alleles in this population. This concentration of unique alleles in the repatriated population could indicate genetic drift occurring in the Latvian populations. Given that these cultivars are not reproduced in isolation, this would tend to spread the effect of genetic drift across these populations. Fst between the 'Priekuļu 66' – Field and Gene Bank populations was only 0.006, half of the Fst values between the Field and Gene Bank populations and the VIR population (0.014 and 0.012 respectively), which confirms this increased differentiation of the VIR population. These Fst values are approaching the values found between Latvian cultivars such as 'Priekuļu 66' – Field/Gene Bank populations and 'Stendes Agrais' (0.017 and 0.015 respectively). This indicates the effects of genetic drift and gene flow in these cultivars over the 25 year time span that these results survey.

The grouping of the cultivars into populations based on the breeding institute at which they developed yielded a much clearer division between them, as shown by the population assignment results. Using these 6 SSR markers, the probability of assigning a red clover individual to the correct breeding institute is 87%. By increasing the number of markers used for genetic fingerprinting, it should be possible to improve on this assignment probability, in particular if these markers are used in conjunction with phenotypic characteristics.

These results indicate that while the overall differentiation of Latvian red clover cultivars is low, the partition of genetic variance between breeding stations is much more marked. One obvious conclusion that can be drawn from these results is that when renewing the seed stocks to be placed into the Latvian Gene Bank, this should be done at the original breeding institute, rather than at a single location. The comparison of the genetic changes in the cultivar 'Priekuļu 66' demonstrates that the genetic composition of Latvian red clover cultivars is far from static. In order to maximise the genetic diversity of red clover accessions stored in the Latvian Gene Bank, it is necessary to carefully maintain and renew the seeds which have now been stored in the Gene Bank for approximately a decade. Furthermore, it would be desirable not only to renew seed stocks at the breeding institute of origin, but also to utilise appropriate isolation techniques to minimise gene flow into the Gene Bank material.

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LATVIJAS SARKANĀ ĀBOLIŅA (*TRIFOLIUM PRATENSE* L.) ŠĶIRŅU ĢENĒTISKĀ PASPORTIZĀCIJA: VIETAS UN LAIKA IETEKME UZ ĢENĒTISKO DAUDZVEIDĪBU

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Ģenētisko resursu centrā ir izstrādāta ģenētiskas pasportizācijas metodika Latvijas gēnu bankā (LGB) uzglabātām sarkanā āboliņa šķirnēm. Sarkanais āboliņš ir svešapputes augs un ņemot vērā, ka šķirnes ir audzētas un atjaunotas bez īpašām izolācijas metodēm, šķirnes robežās ir sagaidāma augsta ģenētiskā daudzveidība.

Izpētītas septiņas diploīdā sarkanā āboliņa šķirnes, kuras izveidotas trīs Latvijas selekcijas institūtos. Tika analizētas sēklas, kuras ievietotas LGB 1999.g. un 2000.g. Šķirnei 'Priekuļu 66' tika analizētas no VIR repatriētās sēklas (ievietotas VIR kolekcijā 1982.g.), Latvijas gēnu bankas sēklas (1999.g.), kā arī augi no Priekuļu lauka kolekcijas 2007. gadā.

Šāda dažādu avotu svešapputes augu ar augstu ģenētiskā daudzveidību analīze ļauj izpētīt ģeogrāfisko ietekmi uz ģenētisko daudzveidību (šķirnes izveidotas dažādos selekcijas institūtos), kā arī to izmaiņas laikā (repatriētās sēklas, LGB sēklas un lauku augi).

GENETIC AND ENVIRONMENTAL EFFECT ON THE GRAIN QUALITY OF SPRING BARLEY

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Abstract

The grain quality of spring barley (*Hordeum vulgare* L.) determines the further opportunities of their utilization. This study investigated the effects of genotype and environment on the grain quality of spring barley. Fifty two spring barley genotypes were used for this investigation. Test weight, 1000 grain weight and grain composition (starch, crude protein, crude fat, β-glucans, crude fibre, crude ash) were evaluated. Field experiments were carried out at the State Stende cereal breeding institute from 2004 to 2006. The results were analyzed to synthesize the relative proportion of the influence varios factors (η %) such as variety or year as well as the influence of climatic conditions (mean air temperature and the amount of rainfall) during the period of grain filling. The analysis of variance suggested that the fluidity of grain quality indices were more strongly ($p<0.01$) affected by their genotype. While measuring the significance of each single factor, the influence of genotype factor was found to be over 70% in starch, crude protein, β-glucans, crude fibre, 1000 grain weight and test weight. A significant environmental influence on grain quality was found for all characteristics except β-glucan and test weight. This was attributed to the varied meteorological conditions during the years of investigation in the the first part of grain filling period.

Key words: spring barley, grain quality, analysis of variance, meteorological conditions.

Introduction

Barley is used for a wide range of uses. It is an important crop used for feed for different livestock species in many areas of the world and also in Latvia.

The most commonly used characteristics to describe the quality of barley in breeding are the physical traits and chemical composition of the grain. The major chemical components of barley grain are starch, protein and β-glucans. The other constituents of barley include ash and fiber components. Fiber components include pentosans, cellulose, and lignin. They are important to measure because of their contribution toward the total dietary fiber. Ash represents the mineral component of barley. The amount of energy available to an animal from grain depends on the relative proportion of each chemical constituent (Shewry and Morell, 2001). Variation in these proportions is genetically controlled (Eagles *et al*., 1995), but it is also influenced by environmental