ZIEMAS RUDZU RAŽA UN KVALITĀTE IZMĒĢINĀJUMOS JÕGEVA PBI

Tupits I.

Rudzu maize gadsimtiem bijis pamatēdiens Igaunijā, Latvijā un Lietuvā. Pētījuma mērķis bija salīdzināt visās trijās valstīs izveidotu ziemas rudzu šķirņu un hibrīdu ražu un kvalitāti Igaunijas klimatiskajos un augsnes apstākļos Jegevas selekcijas institūtā (Jogeva PBI). Trīs gadu izmēģinājumā (2005.-2007.) konvencionāli saimniekojot tika sētas šādas šķirnes un hibrīdi: 'Elvi' (Igaunija), 'Kaupo' (Latvija), 'Joniai' (Lietuva), J 92-5 (Igaunija), LAT 9504 (Latvija) un LIA 426 (Lietuva). Laika apstākļi izmēģinājumu gados bija līdzīgi. Netika novēroti būtiski ziemošanas bojājumi. Visas šķirnes un hibrīdi uzrādīja augstu ražas potenciālu. Salīdzinot trīs gadu rezultātus, augstākās ražas bija šķirnei Elvi un hibrīdam J 92-5. Tūkstoš graudu masa (TKW) pārsniedza 30 g visos pētījuma gados, vidēji augstākais tika novērots 2005. gadā. Krišanas skaitlis (FN) kopumā bija atbilstošs maizes cepšanai, zemākais FN visos pētījuma gados bija 'Joniai' un LIA 426. Mitros apstākļos šīs šķirnes un hibrīda raža vākšana jāveic ātri.

CHARACTERIZATION OF LATVIAN POTATO GENETIC RESOURCES BY DNA FINGERPRINTING WITH SSR MARKERS

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Abstract

SSR (Simple Sequence Repeats) markers have been broadly applied in plant material identification, genetic diversity evaluation, in various gene banks for collections maintenance and in breeding programs for the monitoring of elite alleles and material exchange, as well as in ploidy level prediction, construction of genetic maps, evolutionary and population studies. The high polymorphism level and the co-dominance of SSR markers allow for efficient cultivar characterization and can discriminate even closely related cultivars. However, several factors must be considered when applying SSR markers to polyploid plant species.

After initial optimization and the pre-screening of 15 SSR markers, all potato (*Solanum tuberosum* L. subsp. *tuberosum*) cultivars listed in the Latvian Plant Genetic Resource database were analyzed using eight SSR markers that were found to be most polymorphic. Cultivar fingerprinting, genetic distance evaluation and cluster analyses were performed. Two pairs of the tested cultivars were identical in all screened loci and couldn't be discriminated; the remaining potato cultivars could be discriminated using a minimum of 4 SSR markers. Similar genetic relationships were observed in the potato cultivar collection when analysed with different phylogenetic methods. An increase in the genetic diversity of the newly bred potato cultivars was identified when compared to the older cultivars.

Key words: genotyping, potato, SSR, cultivar identification

Introduction

Potato cultivars are vegetatively reproduced every year for distribution and breeding purposes. Currently the identification of potato cultivars is based on phenotypic characteristics, which are difficult to distinguish, time-consuming and can be affected by environmental factors all of which results in a high risk of misclassification. The autotetraploid genome of cultivated potato (2n=4x=48) and their outcrossing nature makes them a difficult candidate for genetic studies. A narrow genetic base has lead to a high genetic similarity in European cultivated potatoes (Gebhardt *et al.*, 2004; Simko *et al.*, 2006).

Several molecular markers technologies have been applied to different potato genetic materials and compared to evaluate the most efficient method of potato germplasm identification and the evaluation of genetic variation (Milbourne *et al*., 1996; McGregor *et al*., 2000; Braun and Wenzel, 2005). Simple Sequence Repeat (SSR) markers are highly polymorphic, abundant in the non-coding regions of the genome, co-dominant, and many have been placed on genetic maps. The use of the polymerase chain reaction (PCR) and fragment length detection with fluorescent labels makes the use of SSR genotyping simple, fast and robust, and the obtained data comparable and reproducible. SSR markers have been used to successfully characterize potato germplasm collections in several countries and SSR fingerprints have been proposed as one of the units in the cultivar certification process (Moisan-Thiery *et al*., 2005; Reid and Kerr, 2007). Genetic fingerprints can be used for plant cultivar registration, the protection of the plant breeder's rights and for tests of purity for plant producers.

The evaluation of genetic distances for the tetraploid potato is complex due to the inability to interpret the dosage of alleles based on microsatellite markers data (Provan *et al.*, 1996; Milbourne *et al*., 1996; Braun and Wenzel, 2005). However, the high and stable heterozygosity of the potato cultivars that results from vegetative reproduction by tubers makes them highly discernible and suitable for genetic fingerprinting using SSRs. Recent studies demonstrate that five or six SSR markers pairs were sufficient to distinguish a large amount of potato cultivars (Moisan-Thiery *et al*., 2005; Reid and Kerr, 2007; Ghislain *et al*., 2000; Provan *et al*., 1996; McGregor *et al*., 2000; Coombs *et al*., 2004). Pedigree information about potato cultivars is often scanty, and the use of the hybrids of local cultivars as parents is common in the Latvian potato breeding program. Therefore the second important task of using DNA markers is to help investigate the degree of relatedness in the potato germplasm and to improve parental line selection for breeding programs. In the study of Braun and Wenzel (2005), 26 SSR markers identified 37 German cultivars and 10 advanced clones with known pedigree including sibs and half sibs, but no clear relationships were proven in the case of several clones. Nevertheless, most relationships were confirmed using the pooled binary data of allele presence/absence and the inability to interpret the dosage of allele was considered to be minor as the alleles might be in balance between genotypes. Several methods have been suggested for evaluating the allele dosage in polyploid cultivars. For example, measuring the signal strength for each allele peak obtained (Provan *et al*., 1996; Esselink *et al*., 2004; Nybom *et al*., 2004 – by Moisan-Thiery *et al*., 2005), or using a computational algorithm for estimating the allele frequencies under certain pattern of inheritance (De Silva *et al*., 2005). However, these methods are still under development and have not been widely applied. SSR marker fingerprints are not publicly available, therefore it is impossible to compare our data with other studies and genebanks. In order to be able to directly compare genetic diversity and the relationships between potato collections, and also for breeder rights protection, it would be useful to determine a common SSR fingerprinting marker set and to standardize this fingerprinting method.

The aims of this study were to evaluate the discriminatory power of SSR markers within Latvian potato genetic resources, fingerprint all potato cultivars listed in the Latvian plant genetic resource database and to characterize the amount and partitioning of genetic diversity within this collection.

Materials and Methods

61 potato cultivars were obtained from the State Priekuli Plant Breeding Institute collection. This included 45 Latvian bred cultivars and 14 locally adapted cultivars with an unknown pedigree ("local cultivars") and two foreign cultivars "Felicitas" (Germany) and "Kuras" (Netherlands) (http://tor.ngb.se/sesto/ - accessed on 18.03.2008). Genomic DNA was extracted from fresh leaves or sprout material with the Genomic DNA Purification Kit K0512 (Fermentas). To verify the results, DNA extraction was repeated for cultivars with identical genetic fingerprints.

Fourteen SSR markers were selected from markers previously developed by Milbourne *et al*. (1998) and one marker "STPATP1" by Provan *et al*. (1996). Previous studies found that five or six SSR marker pairs were sufficient to distinguish a large amount of potato cultivars (Moisan-Thiery *et al*., 2005; Reid and Kerr, 2007). Five of these markers were present also in our study ("STM2005", "STM1097", "Lemalx", "STM1024" and "STM2020"). Initially, the 15 marker set was tested on ten Latvian potato cultivars and based on the criteria of good amplification quality, the number of alleles and the chromosomal location eight markers were selected for the final

fingerprinting of the remaining cultivars (Table 1). The forward primer was synthesised with a 6-FAM, HEX or NED fluorescent label to allow for the visualisation of the amplified products. SSR locus amplification was carried out using the following PCR conditions: 95 ^{0}C for 3 min, 35 cycles of 95 ^{0}C for 20 sec, 50 ^{0}C – 20 sec, 72 ^{0}C – 20 sec; 72 ^{0}C - 10 min; in a total volume of reaction 20 μl 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 (labeled) and reverse primers (*Applied Biosystems*).
Amplification fragments were separated on an ABI Prism 3130xl Avant Genetic Analyzer (*Applied Biosystems*) and analysed with the GeneMapper 3.5. The diversity index (DI) was calculated using the formula “$DI=1-\sum pg^2$”, where “pg” is the frequency of an individual genotype. Phylogenetic analyses were conducted using MEGA 4 (Tamura *et al*., 2007) on the combined binary allelic data of all markers. Population analyses were performed with the GenAlEx 6 version (Peakall and Smouse, 2006). Information on the pedigree of potato cultivars was supplied by the State Priekuli Plant Breeding Institute (SPPBI) or obtained from the online potato pedigree database resource (Berloo *et al*., 2007).

Results and Discussion

A total of 63 SSR alleles were observed within the cultivars analyzed, with a maximum of 12 alleles generated by the marker “STM1004” (Table 1). Altogether 168 unique genotypes were generated; from which 15 genotypes were monoallelic, 53 – diallelic, 66- triallelic and 34 – quadriallelic. The marker “STM1021” generated the most quadriallelic genotypes. The remaining markers detected more two or three- allelic variants (average frequency (fq)=0,36), but fully monoallelic or quadriallelic genotypes were rare (average fq=0,14).
The obtained average marker Diversity Index (DI) in Latvian potato cultivars (0,82) is comparable with that estimated for potato cultivars by Milbourne *et al*., 1996 (0,73), Moisan-Thiery *et al*., 2005 (0,84); Braun and Wenzel, 2005 (0,98). The highest discriminative power was observed for markers the “STM1021”, “STM1004” and “STM0037” (Table 1).

Table 1. Information on microsatellite markers used for potato cultivar fingerprinting.

Marker	Repeat	Product size, bp	Location	DI	Number of alleles	Number of genotypes
STM1021	$(C)_{17}$...$(CT)_8$ $(AT)_9$	161 - 213	IX	0.93	12	32
STM0037	$(TC)_5$ (AC) $_6$AA...$(AC)_7$ $(AT)_4$	64 - 82	XI	0.94	8	26
STM2020	$(TAA)_6$	140 - 158	I	0.90	7	19
Lemalx	$(ATT)_6$	119 - 146	V	0.73	5	10
STM1049	$(ATA)_6$	177 - 198	I	0.69	4	9
STM1004	$(AAG)_7$	151 - 259	VII	0.96	12	37
STM0007	$(AC)_9$	187 - 237	XII	0.84	10	20
STM3016	$(GA)_{27}$	97- 109	IV	0.87	5	15

We clearly distinguished 55 cultivars, but two pairs of cultivars showed completely identical fingerprint patterns. All the procedures, including sample acquirement, DNA extraction and fingerprinting were repeated. However, the same fingerprints were obtained for the cultivars “Laima” and “Priekulu Baltie”; and for cultivars the “Spidola” and “SPO-11”. In both cases the identical cultivars have one maintainer. Cultivars “Laima” and “Priekulu Baltie” belong to the old generation of Latvian potato cultivars and have a common ancestor – “Irish Cobbler” (Figure 1).

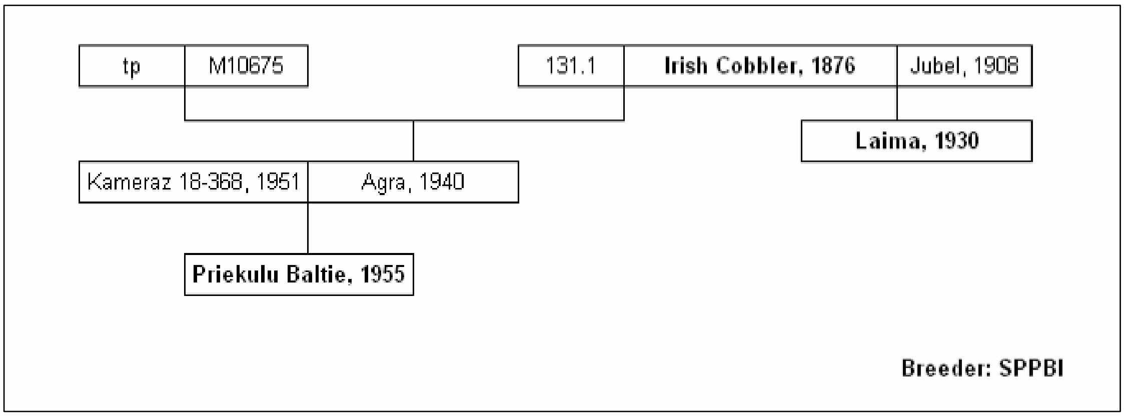

Figure 1. Pedigree of the potato cv. ‘Priekulu Baltie’ and ‘Laima’

The other two cultivars – “Spidola” and “SPO-11”- have an unknown parentage. These cultivars were developed by the breeder Alberts Saulitis; and it is possible that these two cultivars are irradiation induced somaclonal variants, where the cultivars have different phenotypic traits, but little genetic changes. In these cases SSR markers may not distinguish the two variants as in the study of Reid and Kerr (2007). Several reasons could be suggested for this result, such as the low genetic variability of the material, errors in plant reproduction or in marker selection. Careful comparison of the cultivars’ phenotypic traits will be undertaken by the maintainer (SPPBI).

Phylogenetic analyses were performed with the binary data of eight SSR markers scored as presence/absence of allele. The data sets were analyzed with the UPGMA (Unweighted Pair Group Method with Arithmetic mean) - Sneath and Sokal, 1973 – by Tamura *et al.*, 2007) combined with the bootstrapping analysis (Felsenstein, 1985 – by Tamura *et al.*, 2007), the Maximum Parsimony method that is based on the minimal information principle with consensus tree uilding and the bootstrapping test and the Neighbour-Joining (NJ) method.

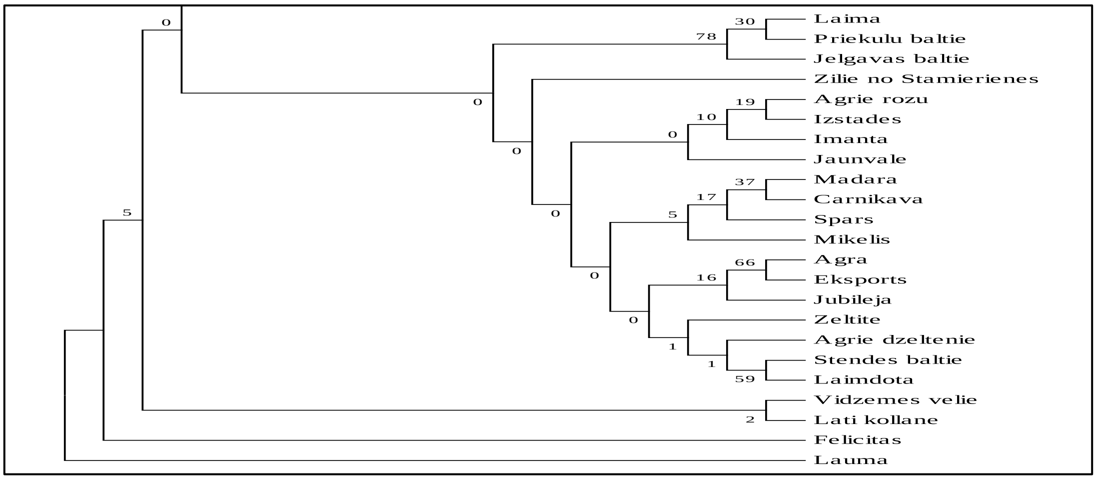

Figure 2. Cluster of old and locally cultivated potato cultivars divided in Maximum Parsimony analysis with bootstrapping based on allele presence/absence

UPGMA phylogenetic clustering was not robust as demonstrated by the generally low bootstrap inference values (data not shown). However, some of the cultivars clustered together with high inference values in likelihood, parsimony and NJ analyses: “Stendes baltie” clustered with “Laimdota”, “Jelgavas baltie” with “Priekulu baltie” and “Laima”; “Agra” with “Eksports”; “Mutagenagrie” with “Bertas”, “Astra” with “Sniegoga”; “Vita” and “KPAX-1”..

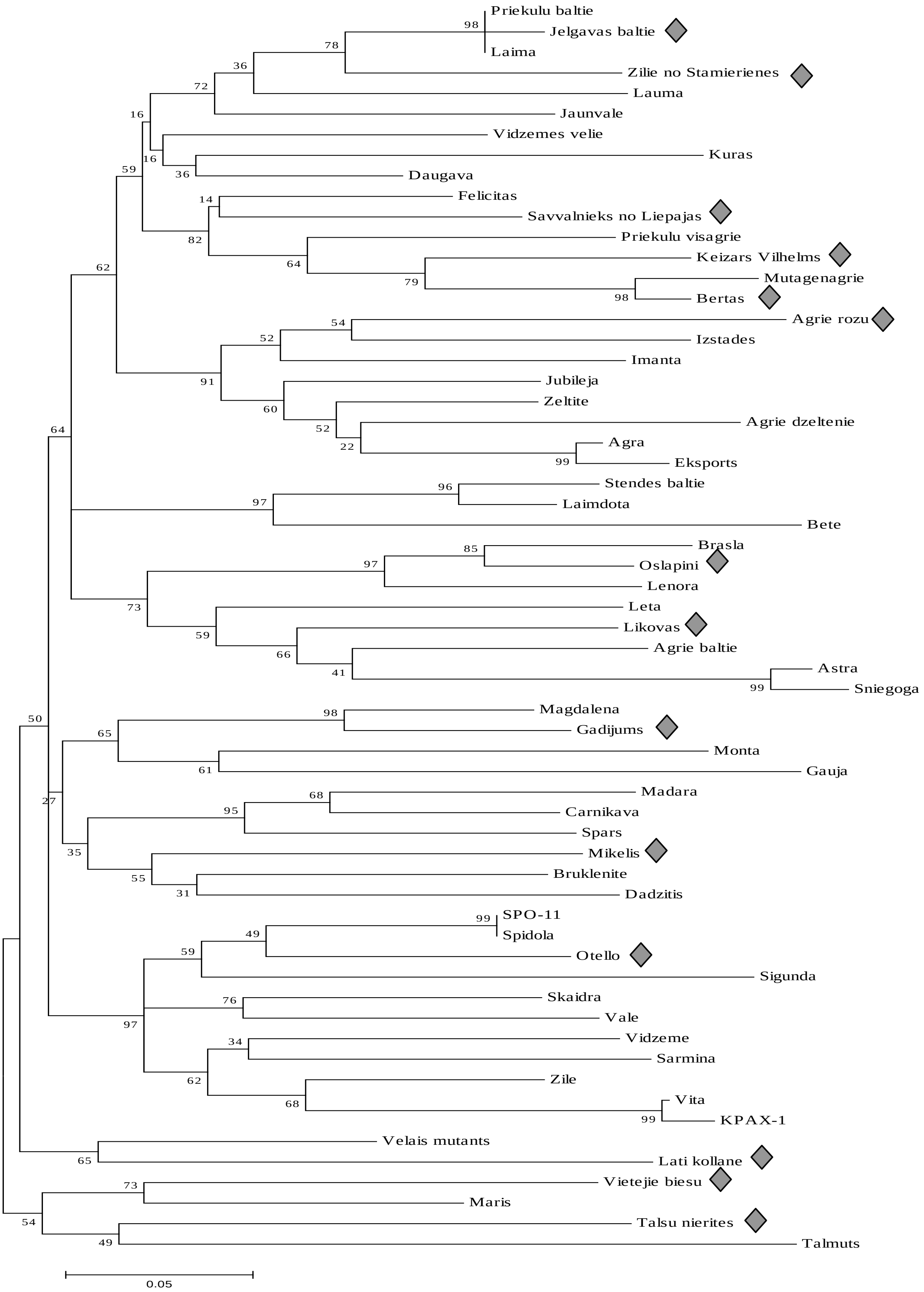

Figure 3. Genetic relationships of the potato cultivars studied. The phylogenetic tree is constructed based on the computation of allele presence/absence using Neighbour-Joining cluster analysis followed with the Interior-Branch test. Local cultivars are marked

We noticed that Maximum Parsimony divided cultivars bred in the beginning the 20th century and some of the local cultivars formed into a separate group (Figure 2) with a few exceptions such as the cultivars “Imanta” (2008) and “Madara” (1984). The German cultivar “Felicitas” and the Latvian cultivar “Lauma” clustered separately from other cultivars. NJ phylogenetic tree branching shows higher confidence values using the Interior-Branch test (Figure 3).

As information on the parentage of the cultivated potato is scarce and hybrid lines occur in the parentage of many cultivars, in most cases we were unable to compare the constructed phylogenetic trees with cultivar pedigree information

Clustering analysis performed with the same data pools using different methods (UPGMA, Maximum Parsimony, NJ) demonstrate similarity of clustering in terminal branches and some groups of cultivars. Often, each cluster contains a local cultivar along with the bred cultivars, which could reflect the origin of the breeding material. However because the pedigree records of local cultivars are not available, this can not be confirmed. The suggestion that the allele dosage estimation is not required (Braun and Wenzel, 2005) was partly supported by our data as many stable clusters of particular cultivars are present in all phylogenetic trees obtained. Maximum Parsimony separates potato cultivars with higher bootstrap inference values than UPGMA and groups older cultivars into one cluster (Figure 2). NJ phylogenetic trees show higher confidence values, therefore these were used for more detailed comparisons. Still, differences between analytic methods are present and combined with the lack of pedigree information about cultivars; results in the situation that definitive conclusions about of phylogenic relationships are difficult. To better elucidate the genetic relationships between Latvian potato cultivars, additional genotype data is required.To explore, changes in the genetic diversity of Latvian potato germplasm over time, the data was divided into three populations: old cultivars, which were released before 1978; modern cultivars, which were bred in 1978 or later; and local cultivars, which have been continuously cultivated in different regions of Latvia and for which there is no pedigree information.

The foreign cultivars “Felicitas” and “Kuras” were excluded from this analysis. Cultivars with an unknown year of release (bred by A.Saulitis), were classified as modern cultivars.

The number of alleles in each population was 54 (modern), 52 (local) and 48 (old). New cultivars have six unique alleles, local cultivars – five unique alleles, but old cultivars only two unique alleles. In modern cultivars private alleles were generated by the markers: “STM1021” (213 bp), “STM0037” (66 bp), “STM1004” (154 bp), “STM0007” (215 bp, 235 bp), “STM3016” (97 bp). The most frequent of these were 154 bp (0,154) and 97 bp alleles (0,115). In local cultivars private alleles were found with the same frequency (0,071) and were generated with the markers “STM0037” (64 bp), “STM1049” (198 bp), “STM1004” (187 bp, 190 bp, 226 bp). In old cultivars private alleles were generated by the markers “STM2020” (140 bp, fq=0,167) and “STM0007” (237 bp, fq=0,053). The percentage of polymorphic loci in old cultivars is lower (74,6%) than in new cultivars (85,7 %), and local cultivars (82,5 %). Nei's Genetic Distance between new and local cultivars was 0,030; between new and old cultivars 0,061, and between local cultivars and old cultivars - 0,027. AMOVA (Analysis of Molecular Variance) analysis found 6 % ($p<0,001$) polymorphism among these populations. This data shows a higher level of genetic diversity in the modern cultivars.

The distribution of alleles with a frequency higher than 0,34 in each population was compared. 16 alleles were common to all the populations. The most distinct high frequency alleles were found in the modern cultivars (163, 70, 191 and 82); in old cultivars only two such alleles were found (125 and 109), but all alleles found in local cultivars were shared either with modern or old cultivars. A high similarity of microsatellite alleles between all Latvian potato cultivars was found. Local cultivars contain common alleles with both old cultivars and modern cultivars and are more polymorphic than the old cultivars. That may reflect a tendency to use local cultivars as material for breeding with the aim of increasing genetic diversity, while maintaining local adaptive traits. Some new alleles were introduced into the modern potato cultivars bred after 1978. This increase of genetic diversity could be a result of the use of new gene pools in modern potato breeding. The potato breeding program in Latvia was expanded with the development of the infrastructure after 1973 and the ability to acquire germplasm with new meristematic methods after 1978 (Bebre, 2003).

Conclusions

All 59 potato cultivars listed in the Latvian genetic resources database were fingerprinted and discriminated from each other with eight SSR markers, with the exception of two pairs of cultivars. If these non-discriminated cultivars are excluded, all remaining potato cultivars could be discriminated using a minimum of four SSR markers: STM1021, STM1004, STM3016 or STM0037 (to the separate cultivars "Lenora" and "Oslapini"), and STM1049 (to the separate cultivars "Vita" and "KPAX-1"). The obtained fingerprints will be routinely used in the future for potato cultivar identification for various purposes.

There are no distinct groupings or divisions within Latvian potato cultivars, however the genetic diversity of potato cultivars bred after the 1978 has increased. These modern cultivars contain newly introduced unique alleles as well as alleles from local cultivars and from old cultivars. This reflects the combination of older, locally adapted material with the increased genetic diversity of modern European potato cultivars. For a more detailed investigation into the phylogeny and genetic relationships of Latvian potato cultivars additional SSR or different DNA marker data is required.

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LATVIJAS KARTUPEĻU ĢENĒTISKO RESURSU RAKSTUROJUMS AR DNS MOLEKULĀRO MARĶIERU PASPORTIZĀCIJAS METODI

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Molekulārus marķierus, tai skaitā vienkāršus DNS sekvences atkārtojumus (SSR, no angļ. val. - Simple Sequence Repeat), plaši izmanto dažāda augu materiāla identifikācijai, ģenētiskās daudzveidības noskaidrošanai, gēnu banku kolekciju uzturēšanai, augu selekcijas programmās elitāro alēļu skrīningam un materiāla apmaiņas kontrolēšanai, kā arī ploiditātes pakāpes paredzēšanai, ģenētisko karšu konstruēšanai, evolucionāros un populāciju pētījumos. SSR marķieru augsts polimorfisma līmenis ļauj atšķirt pat tuvi radniecīgas šķirnes.

Kartupeļu (*Solanum tuberosum* L. subsp. *tuberosum*) šķirnes (59) reģistrētās Latvijas Augu Ģenētisko Resursu katalogā tika izpētītas ar SSR molekulāriem marķieriem. Tika identificēti astoņi īpaši polimorfi marķieri no 15 marķieru grupas un tie izmantoti visas kartupeļu šķirņu kolekcijas raksturošanai. Tika veikta katras šķirnes raksturojošo molekulāro nospiedumu noteikšana (molekulārā pasportizācija), ģenētisko distanču aprēķināšana un filoģenētiskās analīzes. Divi šķirņu pāri tika identificēti vienādi pēc visiem lokusiem, pārējās šķirnes var atšķirt ar četru marķieru molekulāriem nospiedumiem. Tika analizēta līdzīgu šķirņu klasteru veidošanās, izmantojot dažādus datu apvienošanas principus un analīzes metodes. Ģenētiskās daudzveidības palielināšanās atrasta nesen selekcionēto kartupeļu šķirņu grupā, salīdzinot tās ar pagājuša gadsimta sākumā veidotajām šķirnēm. Efektīvo molekulāro marķieru metožu izmantošana Latvijas šķirņu identifikācijai un radniecības pakāpes noskaidrošanai uzlabos turpmāku kartupeļu šķirņu kolekciju uzturēšanu, izplatīšanas kontroli, kā arī selekcijas materiāla atlasi.

POSSIBILITIES TO GROW OATS FOR FOOD IN LATVIA

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Abstract

In order to improve the nutrient content of oat food products, there is a need for good quality grains. In our study, 15 oat varieties grown in a field trial (2005-2007) at the State Stende Cereals breeding Institute and 78 oat samples collected from different farms in Latvia were analysed for volume weight, 1000 grain weight, husk content, crude protein and fat. The grain processing enterprise *Rigas Dzirnavnieks* Ltd has determined the criteria of oats for food. The grains have to have a volume weight higher than 480 g l^{-1} and husk content below 250 g kg^{-1}. Results of the experimental trial showed that these demands are accessible. The grain volume weight ranged from 480 to 518 g l^{-1}, 1000 grain weight from 33.7 to 38.4 g, the husk amount from 206 to 312 g kg^{-1}. However, only four of 15 analysed oat varieties met the standards requested for food quality. The analyses of oat samples collected from farms showed generally lower grain quality than the analysed grain samples from experimental fields. Thus, to obtain oat quality under farming conditions is more difficult than under experimental conditions.

Key words: oats, grain quality, volume weight, husk amount