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Analysis of the genetic structure of a model Scots pine (*Pinus sylvestris*) seed orchard for development of management strategies

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ABSTRACT: Genetic structure, diversity and clonal homogeneity were determined on the basis of the isozyme gene markers in a model Scots pine seed orchard in the north-eastern part of the Czech Republic that originated from natural forest regions NFR 28 and 29 (Jeseníky Mts.) and the 2nd and 3rd forest altitudinal zones (FAZ). Clone and ramet crop variation for the 2009–2011 period was assessed. Comparing the loci measured also in two pine orchards in central Slovakia, a similar proportion of homogeneous clones but considerably lower expected heterozygosity were found out for most of the measured isozyme loci. Heterozygosity of the investigated orchard did not change considerably after the exclusion of alien and wrongly placed ramets. No difference in average cone production between clones originating from different NFR was observed. Verification of the clone identity of seed orchards managed in a certain way can be suggested with the subsequent removal of alien ramets.

Keywords: fructification; gene markers; isozymes; Monte-Carlo test; Rao coefficient; Shannon index

The main goal of seed orchards is to produce forest tree seeds which are genetically superior to those that are available from forest stands (Gömöry et al. 2003). This is done by selecting plus trees (ortets) based on phenotypic or genotypic classification followed by the mating of clones (ramets) within the orchard. The genetic composition of a seed lot is important and depends upon the clone composition in the seed orchard. Genetic gain, avoidance of inbreeding depression and genetic diversity are essential, and these may be affected negatively or positively by factors such as self-fertilization, inbreeding, relatedness of the parents, pollen contamination, spatial distribution, flowering phenology and synchrony, plus male and female fertility (Prescher 2007).

The genetic contribution to seed crops depends on both male and female fertility and pollen contamination from external sources. A pronounced variation of male and female gametic contributions was found in three Scots pine seed orchards in Slovak Republic (Gömöry et al. 2000).The estimated level of pollen contamination in Scots pine seed orchards varies from 2% to 74% (e.g. El-Kassaby et al. 1989; Paule, Gömöry 1992). Prescher (2007) estimated that contamination levels of about 50% decreased the genetic gain of a seed orchard crop by about 25%. This leaves little doubt that the clone composition has a dominant impact on the genetic composition of a seed lot.

Crop contamination can occur by the planting of alien ramets as well. The occurrence of alien genotypes of unknown identity causes the internal contamination of the gene pool of seed orchard crops (Gömöry et al. 2003). Alien clones can be introduced into the seed orchard usually by human errors when labelling collected scions or when grafting or by the growing of rootstocks instead of grafts after mortality of scions. Ramets, which do not belong to any seed orchard clone, were found for larch (*Larix decidua* L.) and Scots pine (*Pinus sylvestris* L.) seed orchards in Slovak Republic (Gömöry, Paule 1993; Gömöry et al. 2003) and for larch in the Czech Republic (Ivanek, Procházková 2006). However, the alien ramets did not affect allelic or genotypic diver-

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sity of the Scots pine seed orchard crop (Gömöry et al. 2003). The authors emphasized the necessity of greater caution in the use of reproductive material produced from orchard seeds. They suggest taking all measures which can improve the genetic composition of seed orchard crops including a verification of the clone identity at least in younger seed orchards with subsequent removal of alien ramets.

The quality of seeds produced in the orchard depends on several factors such as phenological coincidence of flowering and flowering intensity of orchard clones, background pollination, specific interaction of weather, wind and relief in different years and so on. The genetic structure of the progenies differs to a certain extent from one year to another and will not be identical with the collective of orchard clones. These features are highly complicated, but they should be integrated into any possible model of the quality of produced seeds. It was the reason why such a model was not the object of this paper.

In the Czech Republic, the first Scots pine (*Pinus sylvestris* L.) seed orchards were established in the early 1970s with clones from selected plus trees. To date, 29 first generation orchards (comprised of untested clones) have been officially registered (http://erma.uhul.cz/index.php?mode=list). These seed orchards represent artificial synthetic populations formed from clones of various provenances. Clones in 14 certified seed orchards originate from one natural forest region (thereinafter NFR) while the remaining orchards contain a mixture of clones from two to eight NFR (Jurásek 2011).

The aim of the present study was (*i*) to verify the clone composition in a model Scots pine seed orchard and determine genetic structure and clonal homogeneity on the basis of isozyme gene markers, (*ii*) to assess clone and ramet crop variation, (*iii*) to estimate the genetic diversity in relation to different intended management practices, and (*iv*) to attempt to quantify the effect of possible factors influencing genetic diversity (e.g. origin of clones from different regions).

MATERIAL AND METHODS

Material

Our studies were done in a Scots pine (*Pinus sylvestris*) seed orchard Rudíkovy (CZ-3-3-BO-154-28-4-T) located in NFR 28 (Hrubý Jeseník Piedmont) at an altitude of 480 m a.s.l. (50°11'46"N, 17°34'44"E). The 1.40 ha seed orchard was established in 1999 and contains 379 ramets of 144 clones (Fig. 1) representing an autochthonous Heraltice Scots pine ecotype. Seventy percent of ortets (plus trees) originated from four forest stands in NFR 28 (Hrubý Jeseník Piedmont) and 30% from two forest stands in NFR 29 (Nízký Jeseník) at an altitude of 350–550 m a.s.l.

The origin of 42% clones was localized within the 2nd and 58% clones within the 3rd forest altitudinal zone (thereinafter FAZ) in the sense of the Czech system of forest-site types (Viewegh et al. 2003).

This orchard was chosen as a long-term monitored, representative object from the standpoint of clone and ramet numbers and management practices. The orchard was only six years old when we started the cone crop evaluation (data before the 2009 crop not evaluated here) and it was also possible to obtain samples from some ortets (plus trees) for isozyme analyses.

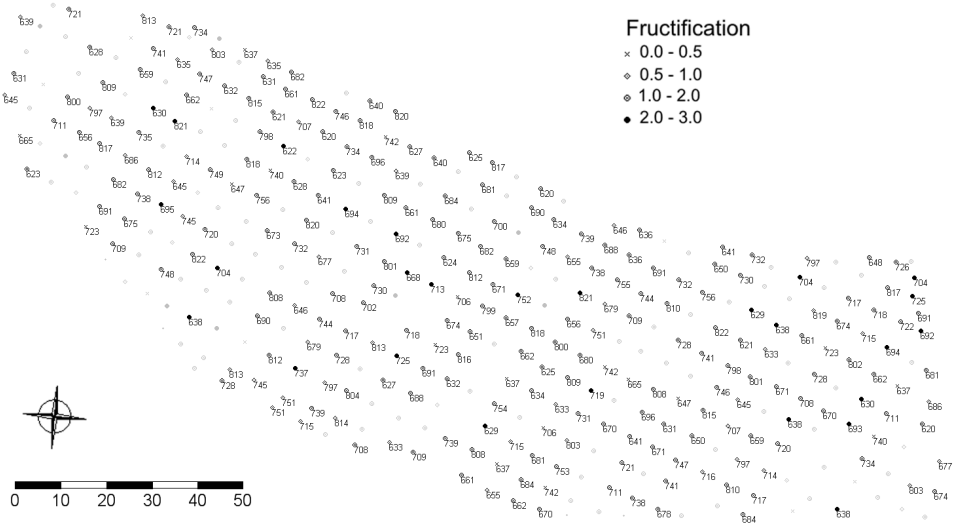

Fig. 1. The map of distribution of *Pinus sylvestris* ramets in the Rudíkovy seed orchard. Selected trees of homogeneous or identified clones are plotted in black with the clone number. The tree symbol is relevant to the average clone-yield index (fructification)

Cone crop

Cone crop of each ramet was scored in three consecutive years of ripening (2009, 2010 and 2011) according to the following criteria:
0 – no crop (< 10 cones per ramet),
1 – poor crop (11 to 50 cones per ramet),
2 – medium crop (51 to 100 cones per ramet),
3 – heavy crop (> 100 cones per ramet).

Scoring was done by estimation, i.e. not by exact counting, of cones in July-August 2009–2011. The average cone-yield index was calculated as arithmetical mean of cone-crop scores for all three years (2009, 2010 and 2011) by the clone. The variability of cone-yield index was calculated as standard deviation of cone-yield indices in all three years by the clone.

Sampling and isozyme analyses

Genetic structure and clone homogeneity were studied using isozyme analyses of dormant buds. All ramets (of all clones) within the orchard were sampled in 2005 through 2007 (April and November 2005; November 2006; January and March 2007). As well, 40 ortets related to orchard clones were sampled in April 2008 (Fig. 1 and Table 1). Immediately after collection, the dormant buds were transported to the laboratory and stored in a freezer at –20 ± 3°C until the isozyme analyses were done.

The buds were then homogenized in a modified extraction buffer pH 6.7 for tissues with high levels of interfering substances (Wendel, Weeden 1990). The isozymes were separated by horizontal one-dimensional electrophoresis of the homogenate on starch gel at 3–5°C in the Tris-citrate buffer pH 7.5 using a Multiphor II electrophoretic device. Each sample was randomly placed on two gels. Seven isozyme systems were stained according to Pasteur et al. (1988) at 37°C: glucose-6-phosphate dehydrogenase (G-6-PDH), shikimate dehydrogenase (SDH-A, SDH-B), 6-phospho-glucose dehydrogenase (6-PGDH-B), phosphoglucose mutase (PGM-A), malate dehydrogenase (MDH-A, MDH-C), leucine aminopeptidase (LAP-B) and phosphoglucose isomerase (PGI-B). The gels were scanned and evaluated by the ImageMaster software (Pharmacia Biotech). The enzyme selection and loci indication is in accordance with Hertel (1997) and Bergmann and Hattemer (1995).

Data processing

The data exported from the ImageMaster software were stored in the IsoEnz database (Matějka 2010). Each allele pair determination was done based on relative mobility using the SeqAn programme (Matějka 2009). Each sample (tree) was randomly placed on two or three gels. Alleles were numbered in ascending order from the slowest to the fastest. The classification of the same allele pair in different gels was compared in order to obtain a unique identification of the alleles over the whole data set.

Ramets in the orchard were compared within the following subsets:
– selected trees (homogeneous clones + clones with one ramet + ramets verified by comparison with

Table 1. Cone-yield index (average) in Scots pine seed orchard clones originating from different natural forest regions (NFR), forest altitudinal zones (FAZ) and stands

Cone-yield index	Crop year			Average	Number	
	2009	2010	2011		clones	ramets
Total	1.08	1.10	1.93	1.37	144	379
NFR 28	1.02	1.14	1.96	1.37	102	269
NFR 29	1.24	1.01	1.85	1.36	42	110
NFR 28						
Stand 967 C15	1.12	1.12	1.83	1.36	34	82
Stand 804 B16	1.04	1.06	1.93	1.34	19	54
Stand 15/2c	0.83	1.06	1.98	1.29	22	65
Stand 15/3	1.04	1.28	2.13	1.48	27	68
NFR 29						
Stand 403 B14	1.24	1.00	1.81	1.35	24	64
Stand 403 D14	1.24	1.01	1.90	1.38	18	46
FAZ 2	1.09	1.19	1.96	1.41	83	228
FAZ 3	1.08	1.03	1.90	1.34	61	151

ortets) vs. other trees; selected trees represent selected clones,
- clones originating from the 3rd FAZ vs. clones from the 2nd FAZ (all within the selected trees),
- clones originating from NFR 28 vs. clones from NFR 29 (all within the selected trees),
- clones with the average cone-yield index higher than the limit vs. other clones (all within the selected trees; values 0.5, 1.0, 1.5 and 2.0 have been used as the limits).

Homogeneous clones were clones with the same multilocus genotype within the set of measured loci, other clones were remaining clones and other trees were non-selected trees. Indices of diversity were evaluated using all processed enzymatic systems.

Genetic diversity of the population was evaluated as follows:

Genotype richness (number of different multilocus genotypes) was used as the simplest parameter. Potential maximum genotype richness (c_{pot}) – theoretical number of all possible multilocus genotypes based on all alleles present in the investigated set of trees under free recombinations can be viewed as an indicator of diversity in a theoretical population of unlimited size.

The following two indices were selected as a measure of genetic diversity in a population. They are based on the multilocus genotype of all analysed individuals in the population. They are not used in genetic studies frequently, but they are comparable with other biodiversity analyses. Hereinafter, observed values are evaluated in a combination, compared on the basis of the Monte-Carlo testing.

Shannon's index:

$$H = -\sum_{i=1}^{c} p_i \times \ln_2 p_i \quad (1)$$

where:

c – number of classes,

p_i – relative proportion of trees within the i^{th} class (Shannon, Weawer 1949).

This index was applied in the population genetics in several cases earlier (e.g. Afif et al. 2008; Lia et al. 2008). Equitability (Eq. 2) completes the set of information indices:

$$e = \frac{H}{\ln_2 c_{pot}} \quad (2)$$

The last index is a generalized quadratic entropy measure, known as the Rao coefficient (Eq. 3) (Rao 1982):

$$R = \sum_{i=1}^{c}\sum_{j=1}^{c} d_{ij} \times p_i \times p_j \quad (3)$$

where:

c – number of classes,

d_{ij} – distance between the i^{th} and j^{th} genotype class has been calculated according to the equation $d_{ij} = a_{ij}/2N$, where: a_{ij} – number of alleles to be not contained in both multilocus genotypes, N – number of loci,

p_i – relative proportion of trees within the i^{th} class, (for p_j analogously).

The population was subdivided into two or more subpopulations according to a selected feature. The basic question is "Does the subpopulation have the same feature as the whole population?" or "Does the subpopulation represent a random selection from the whole population?" These questions correspond to the null hypothesis H_0: the mean of a selected variable (e.g. Shannon index, Rao index or expected heterozygosity) over the subset of trees (subpopulation) is equal to the mean over the whole set of all trees (population). The statistical significance of differences in selected variables for a subpopulation and the whole population was tested using the Monte-Carlo method as fully randomized permutation test (2000 permutations were used in all cases).

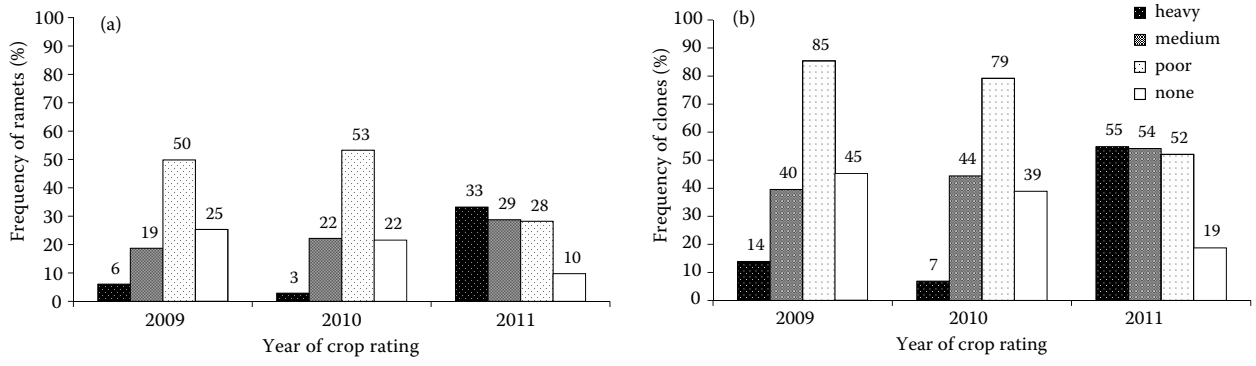

Fig. 2. Ramets with heavy, medium, poor or no cone crop in percentages of the total number of ramets (a) and clones with ramets having either heavy, medium, poor or no crop in percentages of the total number of clones in 2009–2011 (b)

Table 2. Comparison of expected heterozygosity (H_e). number of alleles (n) and number of alleles per locus in the whole seed orchard Rudíkovy, in the subset of selected trees and in comparable orchards Háj and Sýkorová in the Slovak Republic (see Gömöry et al. 2000, 2003)

Seed orchard	Rudíkovy			Háj	Sýkorová	Rudíkovy		Háj	Sýkorová
Set of trees	all	selected	P	H_e		all	selected	n	
Parameter	H_e								
G-6-PDH	0.034	0.005	< 0.001*						
SDH-A	0.234	0.215	0.137	0.3412	0.4166	4	4	3	4
SDH-B	0.028	0.019	0.143	0.0844	0.1591	2	2	3	2
6-PGDH-B	0.380	0.379	0.462	0.4362	0.4882	4	3	2	2
PGM-A	0.103	0.127	0.960*						
MDH-A	0.059	0.096	0.999*	0.0797	0.1862	2	2	2	2
MDH-C	0.382	0.377	0.368	0.4479	0.5972	3	3	3	3
LAP-B	0.092	0.056	0.001*	0.0834	0.0775	4	3	2	2
PGI-B	0.075	0.078	0.594						
Number of alleles per locus						3.17	2.83	2.50	2.50

P – probability of the Monte Carlo test, the null hypothesis is equality of the parameter in the whole set and in the subset ($P < \alpha$ – parameter in the subset is significantly lower compared to the whole set, $P > (1 - \alpha)$ – parameter in the subset is significantly higher compared to the whole set. α – selected limit error probability; *indicates significant values

All diversity indices and statistics were calculated using the IsoEnz software (Matějka 2010).

RESULTS

Cone crop

In each of the three years (2009, 2010 and 2011), cone crops occurred on almost all clones (94–99%) while the cone production of all ramets varied from 75 to 90% depending on the year. The best crop was in 2011 (the first good crop 12 years after the seed orchard establishment), when only 10% of all ramets were without cones and more than half of all ramets belonging to 84% of clones produced either heavy (33%) or medium (29%) crops (Fig. 2a).

By contrast, in 2009 and 2010 at least one ramet of the prevailing part of clones (85–79%) produced only poor crop and ramets in less than 15% of clones had no cones (Fig. 2b). The prevalent cone-yield index of selected clones ranged from 1.0 to 2.0 (Figs 1 and 3) with the variability (inter-year standard deviation) of 0.25 to 0.50 (Fig. 4). While in 2009–2010 only 36 to 40% of all clones showed above-average annual cone production, in 2011 above-average fructification was observed in 60% of clones.

No difference in average cone production between clones originating from different NFR and FAZ was observed even though in years with weaker crop (2009 and 2010) cone production varied somewhat depending on the original natural forest region and stands (Table 1). There was no clone without fructification in the years of investigation. Distribution of fructifications was quite regular with the exception of a few ramets growing at the margin of the orchard (Fig. 2).

Selected trees vs. the entire seed orchard

Isozyme systems of all the 376 ramets of 144 clones were successfully analysed. The set of selected trees was represented by 269 trees (71.5% of all ramets) belonging to 123 clones (85.4% of all clones). Two alleles (one in G-6-PHD and one in 6-PGDH-B) were eliminated in the set of selected trees.

The decrease in the G-6-PDH expected heterozygosities was statistically significant in the set of selected trees. Heterozygosities by SDH-B and MDH-C were slightly decreased. The significant increase of

Table 3. Comparison of genetic diversity in the whole seed orchard and in a subset of selected trees in the Rudíkovy seed orchard

Set	All trees	Selected trees	P
n	376	227	
H	5.22	4.62	< 0.001*
R	0.137	0.134	0.178

P – probability of the Monte Carlo test, *significant values, n – number of trees (ramets), H – Shannon index, R – Rao coefficient

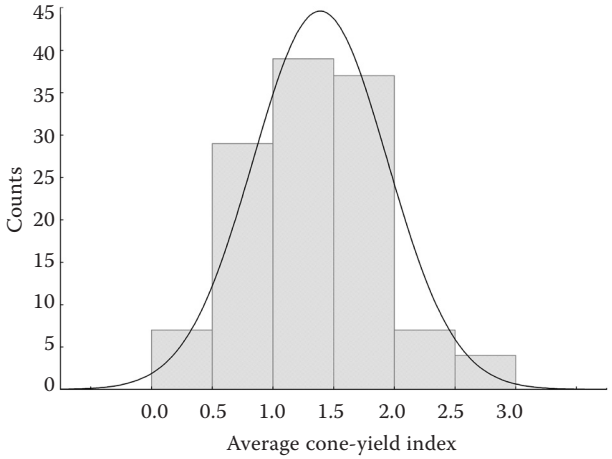

Fig. 3. Histogram of the selected clone counts in the Rudíkovy orchard according to average the cone-yield index. Expected normal distribution curve is fitted

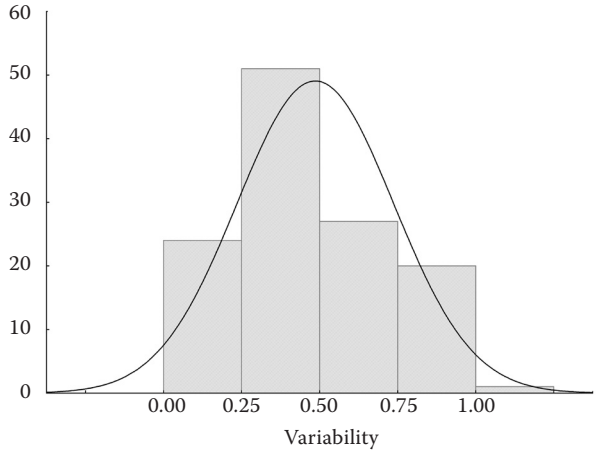

Fig. 4. Histogram of the selected clone counts in the Rudíkovy orchard according to variability of the cone-yield index. Expected normal distribution curve is fitted

such heterozygosities was observed by 6-PGDH-B and MDH-A. A certain increase in the PGM-A heterozygosity was also observed (Table 2).

The selected trees represent a group with narrower genetic diversity compared to the whole seed orchard. The Shannon index of diversity (*H*) was lower in the set of selected trees ($P < 0.1\%$). The change in the Rao index (*R*) was not statistically significant (Table 3). Due to the sensitivity of the index *H* to the number of individuals and robustness of the Rao index in this context, this decrease is not considerable. On the contrary, selection of the homogeneous clones (from the ramet set of known origin) represents a significant decrease of genetic diversity because there is a significant reduction of both *H* and *R* indices.

Clones originating from different natural forest regions (NFR) and forest altitudinal zones (FAZ)

No statistically significant difference in genetic diversity of clones originating from different NFR (28 and 29) of or from different FAZ (2 and 3) was determined. Total genetic diversity was insignificantly higher in the clones originated in the 2nd FAZ

Table 4. Genetic diversity of selected Scots pine clones from different natural forest regions and forest altitudinal zones compared to the whole set of selected clones

	Natural forest region				Forest altitudinal zone			
	28		29		2		3	
	value	*P*	value	*P*	value	*P*	value	*P*
n	83		40		72		51	
H	4.86	0.650	4.20	0.393	4.75	0.586	4.31	0.236
R	0.147	0.796	0.132	0.239	0.149	0.839	0.132	0.134
Isozyme	expected heterozygosity							
G-6-PDH	0.025	**0.913**	0.000	0.455	0.014	0.253	0.020	0.574
SDH-A	0.257	0.620	0.240	0.403	0.311	**0.974***	0.161	**0.018***
SDH-B	0.012	0.165	0.050	**0.943**	0.014	0.279	0.038	0.737
6-PGDH-B	0.434	0.890	0.368	0.123	0.377	**0.085**	0.457	**0.924**
PGM-A	0.104	0.241	0.142	0.760	0.133	0.702	0.094	0.279
MDH-A	0.058	0.114	0.095	0.862	0.117	**0.992***	0.000	**0.009***
MDH-C	0.346	0.261	0.381	0.717	0.364	0.634	0.349	0.442
LAP-B	0.126	0.490	0.119	0.515	0.131	0.561	0.112	0.386
PGI-B	0.095	0.616	0.075	0.337	0.096	0.692	0.078	0.353

n – number of clones, *H* – Shannon index, *R* – Rao coefficient, *P* – probability of the Monte Carlo test, in bold – error probability less than 10%, *error probability less than 5%

Table 5. Genetic diversity in the subset of selected Scots pine trees distinguished according to average fructification expressed by the cone-yield index

	Cone-yield index							
	> 0.5		> 1.0		> 1.5		> 2.0	
	value	*P*	value	*P*	value	*P*	value	*P*
n	116		87		48		11	
H	5.07	0.765	4.90	0.656	4.08	**0.066**	2.85	0.311
R	0.144	0.621	0.144	0.525	0.111	**0.001***	0.092	**0.050**
Isozyme	expected heterozygosity							
G-6-PDH	0.009	**0.026***	0.000	**0.077**	0.000	0.394	0.000	0.500
SDH-A	0.252	0.433	0.255	0.548	0.173	**0.064**	0.169	0.408
SDH-B	0.026	**0.999***	0.034	0.671	0.042	**0.909**	0.000	0.500
6-PGDH-B	0.422	0.832	0.430	0.829	0.400	0.345	0.397	0.362
PGM-A	0.124	0.594	0.131	0.666	0.061	**0.069**	0.000	0.288
MDH-A	0.075	0.410	0.067	0.531	0.061	0.500	0.000	0.416
MDH-C	0.359	0.679	0.344	0.249	0.334	0.238	0.351	0.588
LAP-B	0.115	0.177	0.100	0.104	0.061	**0.066**	0.000	0.219
PGI-B	0.094	**0.999***	0.102	0.721	0.021	**0.015***	0.095	0.726

n – number of clones, *H* – Shannon index, *R* – Rao coefficient, *P* – probability of the Monte Carlo test, in bold – error probability less than 10%, *error probability less than 5%

and NFR 28 according to the Rao coefficient. Significant differences were found in SDH-A and MDH-A (and 6-PGDH-B with increased error probability) heterozygosities for clones from different FAZ (Table 4).

Genetic structure and fructification of selected clones

Genetic diversity among clones with different fructification (cone-yield index) is slightly different. Clones with higher cone production whose cone-yield indices (3-year average) were higher than 1.5 show the most distant genetic features. Genetic diversity of this subset was narrower and significantly different according to the Rao index. Expected heterozygosities were decreased by SDH-A, PGM-A, LAP-B and PGI-B loci, and increased by SDH-B locus. A certain change was observed in five of the nine analysed loci (Table 5). However, also clones with the lowest cone production (≤ 0.5; such a difference is equivalent to the difference of a subset of clones with cone-yield index > 0.5 compared with the whole set of all clones) showed a significant difference in heterozygosity in some loci (G-6-PDH, SDH-B and PGI-B).

DISCUSSION

Comparing the loci measured also in two pine orchards Sýkorová and Háj (Gömöry et al. 2000, 2003), considerably lower expected heterozygosity was found in the Rudíkovy seed orchard for SDH-A, SDH-B, 6-PGDH-B, MDH-A and MDH-C. Conversely, the highest number of alleles per locus was found in the Rudíkovy sets, both for the whole seed orchard and in the subset of selected trees (Table 2). The proportion of selected trees in the Rudíkovy orchard is comparable with data reported by Gömöry et al. (2000, 2003) concerning the relative number of Scots pine ramets after the exclusion of alien and wrongly placed ramets in seed orchards in central Slovak Republic which varied in the range of 53–73%. Comparison of the loci, measured both in the Rudíkovy orchard and Sýkorová and Háj orchards in central Slovakia, shows that the expected heterozygosity of most of them is considerably higher in the Slovak orchards, although the number of alleles per locus is lower there. The difference in heterozygosity for these loci among different seed orchards is much larger than that between all clones and the subset of selected trees of the Rudíkovy orchard (Table 2). This indicates that the selection of homogeneous clones and verified ramets has a relatively small effect on genetic diversity for the orchard under study.

Kang et al. (2001b) reviewed the number of clones in 255 conifer seed orchards in Sweden, Finland and Korea; typically, the number was about 90. In the USA, on average, there are 24 clones in loblolly-pine seed orchards and 42 clones in slash seed orchards, but the number of clones varies tremendously (McKeand et al. 2003). The optimum is rather broad, thus it is not essential to deploy the exact optimum, and an approximate optimum will do. The advantage of a few clones is mainly a higher genetic gain. Seed orchards may also have functions in breeding such as clonal archives, or for crossing or collecting progenies. This may be an argument to increase the number of clones (Lindgren, Prescher 2005). Wasielewska et al. (2005) found high genetic diversity (expected heterozygosity H_e = 0.427) and low inbreeding (Wright's index F = –0.028) of plus trees of Scots pine which is comparable to Scots pine trees observed in natural populations and seed orchards. According to Wasielewska et al. (2005), genetic diversity of Scots pine seed orchards is comparable with that in natural populations or plus tree sets. She found out that expected heterozygosity H_e for some enzymes (SDH-A, 6-PGDH-B, MDH-C) in the mating system of Scots pine plus trees from the Tuchola Forests (Poland) is comparable or slightly higher than that we found in the Rudíkovy seed orchard.

Verification of clone identity with the subsequent removal of alien ramets would not dramatically affect genetic diversity of the seed orchard. However, such management strategy will increase the operational cost, so the final gain should be considered carefully. This management strategy should be applied only to promising orchards or to newly established ones. In the latter case, such verification could be demanded before the official approval of the seed orchard. If the alien ramets are not removed, it would contribute adversely to the genetic uncertainty of reproductive material from seed orchards. Although the isozyme analysis is limited by a low number of markers it provides, this method still belongs to the quickest and cheapest and remains an appropriate choice for tasks that only need to identify low levels of genetic differences or variations.

CONCLUSIONS

No difference in average cone production between clones originating from different NFR and FAZ was observed even if in years with weaker cone production they varied somewhat depending on the original natural forest region and stands. There was no clone without fructification in the years of investigation. Distribution of fructifications was quite regular with the exception of a few ramets growing at the margin of the orchard.

Genetic diversity of clones with different fructification was slightly different. Clones with higher cone production (with cone-yield indexes above 1.5) show the most distant genetic features. It points to the fact that the population arising from the seed production would be slightly different from the population in the seed orchard from the genetic aspect.

The selected trees represent a group with slightly lower genetic diversity compared to the whole seed orchard. No statistically significant difference in genetic diversity of clones originating from different NFR (28 and 29) or FAZ (2 and 3) was determined.

Comparing the loci measured also in two pine orchards in central Slovak Republic, a similar proportion of homogeneous clones but considerably lower expected heterozygosity for most of the measured isozyme loci was found. Heterozygosity of the investigated orchard did not change considerably after the exclusion of alien and wrongly placed ramets.

Verification of clone identity with the subsequent removal of alien ramets would not dramatically affect either genetic diversity in current Scots pine seed orchards in the Czech Republic. If the alien ramets are not removed, it would contribute adversely to the genetic uncertainty of reproductive material from seed orchards.

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