Research into *Taxus baccata* L.: genetic diversity in three Baltic Sea countries - part of a regeneration programme of native species in Lithuania

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Abstract

Taxus baccata L. is an extinct species in natural stands of Lithuania and rare in neighbouring states. The paper presented a study of the genetic diversity of *Taxus baccata* growing in natural populations near the Lithuanian border to neighbouring Baltic Sea countries. The main aim of this investigation was to assess genetic based possibilities of regeneration of *Taxus baccata* populations in Lithuanian forests. In this study we used RAPD markers to assess genetic diversity of 58 *T. baccata* samples of provenances from Latvia, Lithuania, Poland and Russian Kaliningrad region. We identified 58 RAPD profiles on the basis of 64 amplified DNA fragments. 73.4% of RAPD bands were polymorphic. Four provenance specific bands were identified. Results demonstrate rather high level of DNA polymorphism and genetic differentiation of studied *T. baccata* provenances.

Key words: *Taxus baccata* L., provenances, RAPDs, genetic diversity, regeneration, natural population

Introduction

English yew (*Taxus baccata* L., Taxaceae) is a species notable for specific ecological demands. Although the area of the species extends from southern Scandinavia to Greece, and from north-west Africa to the Baltic States, this species is entered in the Red Data Books of several countries (Navasaitis, 2004). Only small areas of former large natural population of this species are observed in most Europe countries and in the Baltic States.

The last English yews natural habitats in Lithuania disappeared in the 19th century (Natkevičaitė 1932, Lietuvos TSR flora 1959, Navys 2000). *Taxus baccata* specimens were described in Švėkšna and surrounding area (West part of Lithuania) in 1808-1811 by J. Pabrėža, and in 1823 by Prof. J.Jundzill (Natkevičaitė 1932, Regelis 1936). This species was last found in natural habitants in Lithuania in– 1863 (Navys 2000). Only two oldest examples of English yew growing in Lithuanian manor parks (Švėkšna and Šateikiai), nearly 200-years old, could be the remnants of a former Lithuanian population.

However ecological conditions in certain areas (forests) of Lithuania are suitable for regeneration of natural population of this species (Navys 2000). Moreover, the English yew grows well as a decorative plant in numerous parks of Lithuania, as well as botanical gardens and a lot of private gardens in all regions of this Baltic State.

The main purpose of this work was to perform genetic diversity studies of remnants of natural English yew populations in the neighbouring Baltic Sea countries (Latvia, Poland, Russian Kaliningrad region) in areas allocated nearest to the Lithuanian border. In order that the results of this investigation could be used for elaboration and implementation of the programme for regeneration of natural population of this species in Lithuania.

Material and methods

Plant material

Four provenances of *T. baccata* from Poland, Latvia, Kaliningrad and Lithuania region were analyzed in this study (Table 1; Figure 1). Number of individuals analyzed per provenance ranged from 2 to 28. Fresh leaf material of individual's (plants) was collected and kept on the ice. Then this plant material was stored at -40°C up to extraction of DNA. 58 samples were applied for DNA analysis in total.

Provenance	Site	Numbers of sampled plants	
Latvia	Slitere	11	28
	Embrekš	1	
	Zentene	3	
	Daitiu	13	
Kaliningrad	Zaicev	7	11
	Celau	4	
Poland	Czeszki	1	17
	Cisowy Jar	15	
	Węzewo	1	
Lithuania	Šateikiai	1	2
	Švėkšna	1	

Table 1. Number of investigated Taxus baccata L. samples in different provenances.

Figure 1. Locations of four studied provenances of Taxus baccata *L.*

Provenances designations are following: Latvia (Sl - Slitere, Em - Embrekš, Ze - Zentene, Da - Daitiu), Lithuania (Šv - Švėkšna, Ša - Šateikiai), Kaliningrad (Za - Zaicev, Ce – Celau), Poland (Cz – Czeski, CJ – Cisowy Jar, W – Węzewo).

DNA extraction

Genomic DNA was extracted using a CTAB protocol (Doyle and Doyle 1990). DNA concentration and quality was measured using Eppendorf BioPhotometer.

RAPD-PCR

Methods of molecular markers are among the most efficient modern ways to assess genetic diversity and determine the genetic structure of a population. One of these methods - the RAPD [random amplified polymorphic DNA] technique was used in this study. It is based on PCR [polymerase chain reaction] - one of its modifications (Williams *et al*. 1990). Due to even random distribution of RAPD markers within a genome and higher polymorphism detected this assay method is superior to the allozyme analysis; moreover, its simplicity and lower expenditures for investigations overcome the microsatellite markers.

Data analysis

Data were organized in a binary matrix with 1 indicating band presence and 0 indicating band absence. This matrix was analyzed using TREECON fro Windows (Van De Peer and De Wachter 1994). Genetic distance matrix was generated according Nei and Li (Nei and Li 1979). Clustering analysis was performed using Unweighted Pair - Group Method of arithmetic Averages (UPGMA) method algorithm. The significance level of dendrogram branches was determined using 1000 bootstrap replicates. Shannon index of diversity (I), Nei (1973) gene diversity (h), coefficient of gene differentiation (G_{ST}) and effective number of alleles (n_e) were calculated using POPGENE v.1.32 (Yeh *et al*. 1999). Principal coordinate analysis (PCA) and

distribution of DNA fragments in *T. baccata* provenances was performed using software package GenAlEx v6. (Peakall and Smouse 2006).
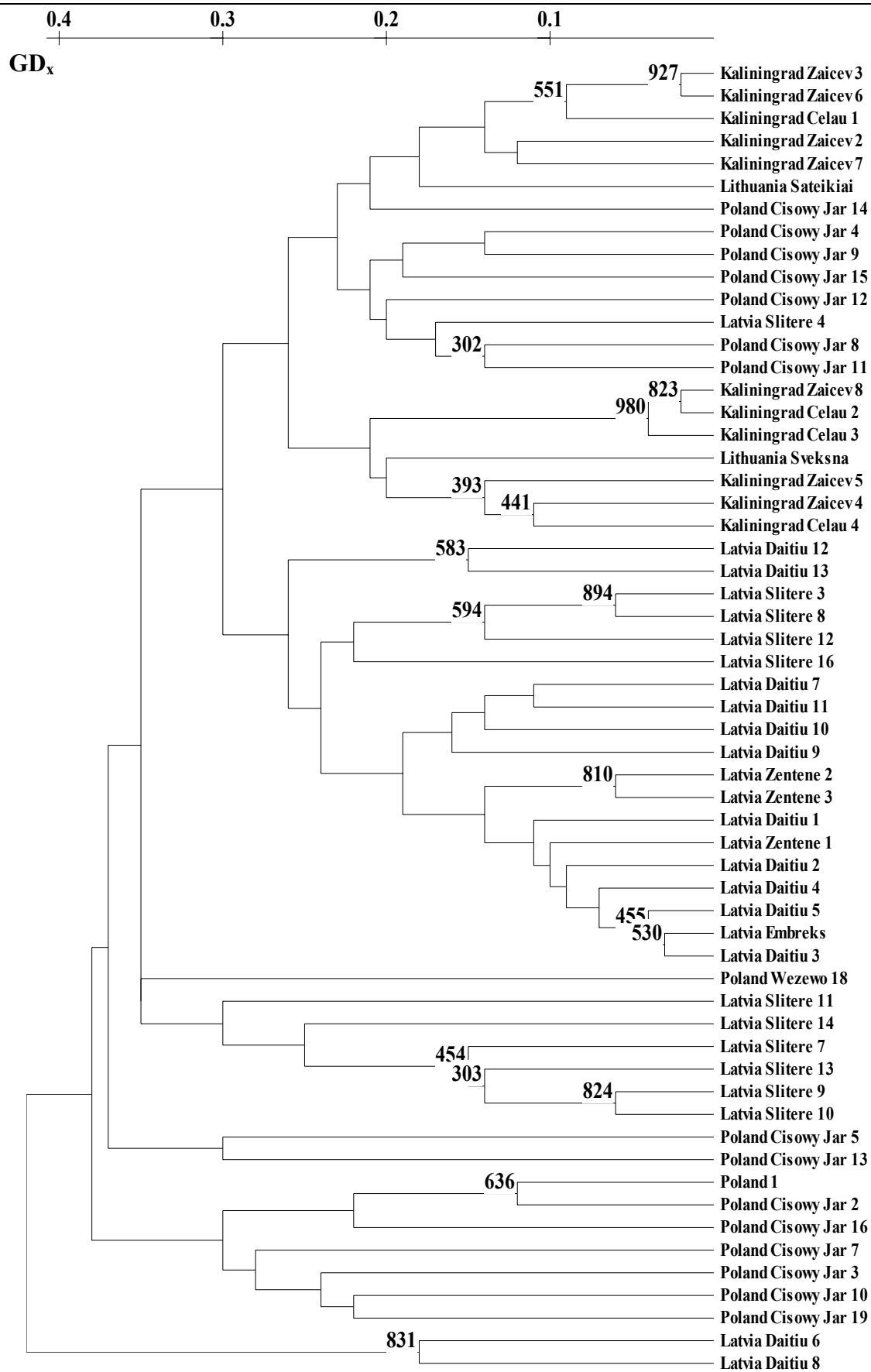
Results and discussions

Especially important for elaboration the programme of regeneration of *Taxus baccata* population in Lithuania is selection of parental plant material. Collecting genetic material from low numbers or improperly chosen individuals can cause low effective population size and inbreeding depression, as well as reduce the adaptive evolutional potential of such population (Barret and Kohn 1991).

Fifty eight unique RAPD banding patterns were observed, i.e. any of the samples have not signified an identical banding pattern and UPGMA cluster analysis based on GDxy values indicated patterns of genetic relatedness among individual plants from different provenances (Figure 2). This demonstrate the existence of genetic variation in the studied plant groups. The Nei's gene diversity (h) within studied plant group provenances of *T. baccata* ranged from 0.1901 Kaliningrad provenances to 0.3751 Latvian provenances (*Table 2*). The coefficient of gene differentiation among studied plant groups is 0.218. This indicated that 21.8% of the total genetic diversity existed among groups, while 78.2% existed within groups. This result indicates the genetic specificity of *T. baccata* germplasm from different countries.

Provenance	Polymorphism (%)	Observed number of alleles, n_e	Nei's gene diversity, h	Shannon's information index, I
Poland	78,72	1,5162	0,2913	0,4288
Kaliningrad	53,19	1,3173	0,1901	0,2849
Latvia	87,23	1,6840	0,3751	0,5392
Mean ± SD	73,05 ± 17,71	1,5058 ± 0,184	0,2855 ± 0,093	0,4176 ± 0,127

Table 2. Calculated values of genetic diversity indices in provenances of T. baccata.

Figure 2 (next page). Bootstrapped (1000 iterations) cluster analysis (UPGMA) of 3 provenances based on the genetic distance among 58 T. baccata examples.

0.4   0.3   0.2   0.1

GD_x

927 — Kaliningrad Zaicev 3
551 — Kaliningrad Zaicev 6
Kaliningrad Celau 1
Kaliningrad Zaicev 2
Kaliningrad Zaicev 7
Lithuania Sateikiai
Poland Cisowy Jar 14
Poland Cisowy Jar 4
Poland Cisowy Jar 9
Poland Cisowy Jar 15
Poland Cisowy Jar 12
Latvia Slitere 4
302 — Poland Cisowy Jar 8
Poland Cisowy Jar 11
823 — Kaliningrad Zaicev 8
980 — Kaliningrad Celau 2
Kaliningrad Celau 3
Lithuania Sveksna
393 — Kaliningrad Zaicev 5
441 — Kaliningrad Zaicev 4
Kaliningrad Celau 4
583 — Latvia Daitiu 12
Latvia Daitiu 13
594 — 894 — Latvia Slitere 3
Latvia Slitere 8
Latvia Slitere 12
Latvia Slitere 16
Latvia Daitiu 7
Latvia Daitiu 11
Latvia Daitiu 10
Latvia Daitiu 9
810 — Latvia Zentene 2
Latvia Zentene 3
Latvia Daitiu 1
Latvia Zentene 1
Latvia Daitiu 2
Latvia Daitiu 4
455 — Latvia Daitiu 5
530 — Latvia Embreks
Latvia Daitiu 3
Poland Wezewo 18
Latvia Slitere 11
Latvia Slitere 14
454 — Latvia Slitere 7
303 — Latvia Slitere 13
824 — Latvia Slitere 9
Latvia Slitere 10
Poland Cisowy Jar 5
Poland Cisowy Jar 13
636 — Poland 1
Poland Cisowy Jar 2
Poland Cisowy Jar 16
Poland Cisowy Jar 7
Poland Cisowy Jar 3
Poland Cisowy Jar 10
Poland Cisowy Jar 19
831 — Latvia Daitiu 6
Latvia Daitiu 8

Genetic analysis of Lithuanian material (collected from two oldest *Taxus baccata* specimens growing in Šateikiai and Švėkšna parks, West Lithuania) has been also performed. Comparison of studied material demonstrated that analyzed Lithuanian samples resembled with some of examples from the Kaliningrad provenances, as the dendrogram indicates (Figure 2). This cannot make statistically reliable conclusions about the genetic relatedness of the oldest Lithuanian individuals to those in Latvian and Kaliningrad Region provenances (the material we have tested is not sufficient). Nevertheless, the investigations carried out do not dispose the possibility that the English yew individuals in the parks of Šateikiai and Švėkšna can be remnants of former natural Lithuanian population.

The genetic structure of investigated *T. baccata* provenances illustrates the best PCA (Figure 3). It shows that the most plants from Poland and Kaliningrad provenances tend to group together and are more genetically similar than individuals from Latvian provenances.
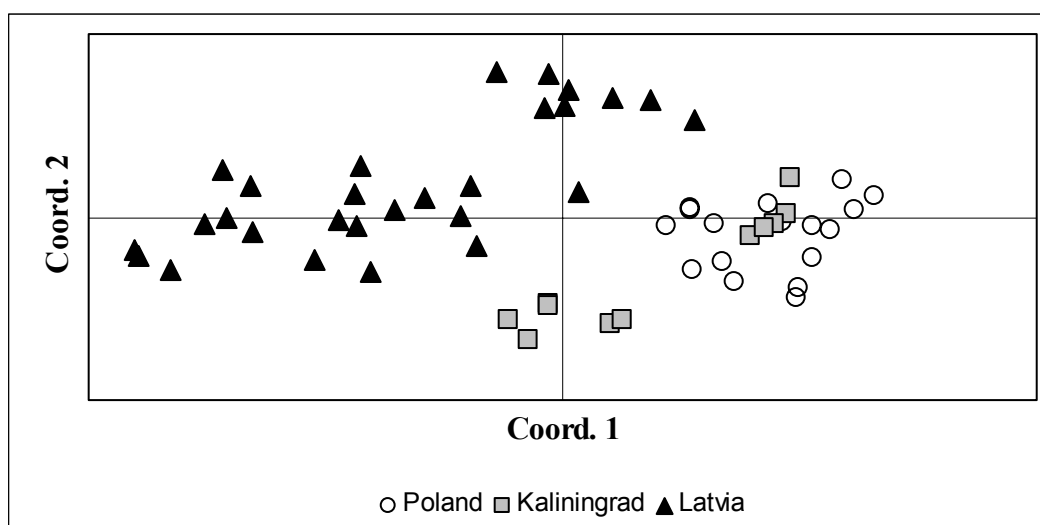

Figure 3. Two-dimensional representation of principal coordinate analysis of T. baccata *provenances.*

The study of mean heterozygosity level within *T. baccata* provenances demonstrate that the highest one – was in Latvia provenance (0.37) and the lowest one was in Kaliningrad provenances (0.19) (Figure 4). Nowadays is taken a view that the higher heterozygosity causes the better adaptation (David 1998). It confirm, at least partly, such a view our investigations too. In our case we have determined the correlation of heterozygosity and morphometry data. In Latvia provenance, with the highest level of mean heterozygosity, were observed numerous individuals of English yew in-fruit, more healthy and significant larger. Whereas those studied in Kaliningrad Region were notable for worse morphology, scrubby and less productive.

In order to regenerate the English yew population in Lithuanian forests, it would be more expedient to take the parental material for propagation from yews growing in Latvia.

Conclusions

- The study of *Taxus baccata* L. provenances reported in this paper gives a more detailed insight about genetic constitution of autochthonous specimens that survived until nowadays in neighbouring With Lithuania countries.

- Results of our investigation demonstrate rather high level of DNA polymorphism and genetic differentiation of *T. baccata* provenances in Latvia, Poland and Russian Kaliningrad region.

- Examples of Polish and Kaliningrad regions *T. baccata* provenances are closer genetic related. The level of mean heterozygosity of examples from Latvian provenance is higher.

- For potential regeneration of *T. baccata* population in Lithuania, the parental material for propagation seems to be better fitting from Latvia. Its higher heterozygosity is related to higher flexibility of plants and their wider potential for adaptation under more diverse ecological conditions
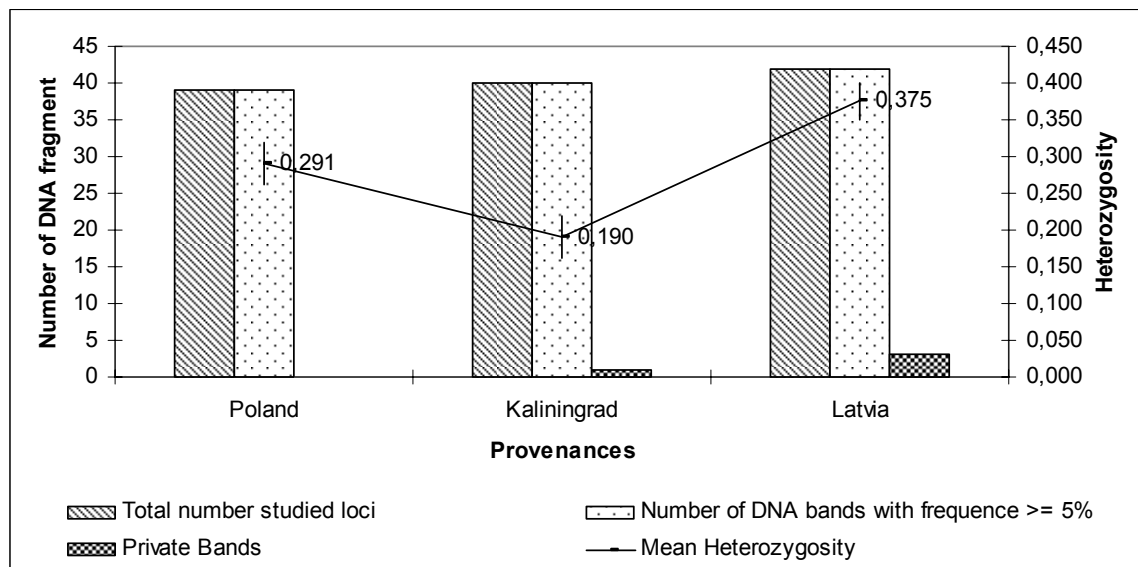

Figure 4. The level of average expected heterozygosity in studied populations of Taxus baccata.

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