Máchová P.*, Cvrčková H., Trčková O., Vítová K.

Forestry and Game Management Research Institute, Strnady 136, 252 02 Jíloviště, Czech Republic

Corresponding author: Ing. Pavlína Máchová, Ph.D., Forestry and Game Management Research Institute, Strnady 136, 252 02 Jíloviště, Czech Republic; machova@vulhm.cz

Use of DNA analyses for verifying the declared origin of forest reproductive material

Introduction

Identity of forest tree reproductive material is essential in artificial forest regeneration. The Czech Republic as a member state of the European Union and in accordance with international legislation (Council Directive 1999/105/EC on the marketing of forest reproductive material on the market) has the obligation to create a functioning control system for determination of forest reproductive material. The purpose of our study was to investigate the possibilities of using objective methods of DNA analysis to verify the declared origin of reproductive material of selected forest species (Norway spruce, European beech) in terms of the Czech Republic.

Methods

Monitoring of the identity of reproductive material was carried out during three years, i.e. from seed collection to transplanted plants production. Sampling of reference samples was performed from the sets and units of reproductive material listed in Table 1, 2. Total genomic DNA was extracted using a DNeasy Plant Mini Kit (Qiagen, Hilden, Germany). Analyses of microsatellite (SSR) markers were performed on 1920 samples of the 32 sets of Norway spruce reproductive material from 8 selected sources of forest reproductive material (units of forest reproductive material) and on 1242 samples of the 21 sets of European beech reproductive material from 7 selected sources of forest reproductive material (units of forest reproductive material). Seven for Norway spruce (PAAC23, SpAG2, WS00111.K13, WS00716.F13, WS0022.B15, WS0073.H08, WS0023.B03) and nine for European beech (FS1-03, FS1-15, sfc0036, mfc 5, mfc 7, csolfagus_31, Fagsyl-000905, Fagsyl-001018, Fagsyl-002929) optimally polymorphic markers with sufficient informative values were used for the subsequent evaluation of the genetic structure by Bayesion clustering. PCR and fragment analysis procedures were optimized for selected markers. PCR products were separated by capillary electrophoresis using the Applied Biosystems 3500 genetic analyser. The program GenAlEx 6.503 (Peakall, Smouse 2006, 2012) and the Bayesian clustering method implemented in the software STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003, 2007; Hubisz et al. 2009) were used to analyze the genetic data. The analysis was based on the admixture model with Lock prior. Optimal numbers of clusters (K number) were detected by Structure Selector (Li, Liu 2018). The obtained genetic compositions of sets were compared after statistical processing.

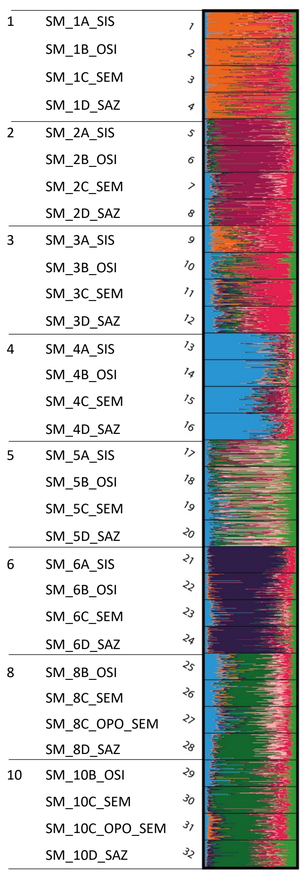

Figure 1: Evaluation of genetic structure by Bayesian method for K = 8 in 32 Norway spruce sample sets

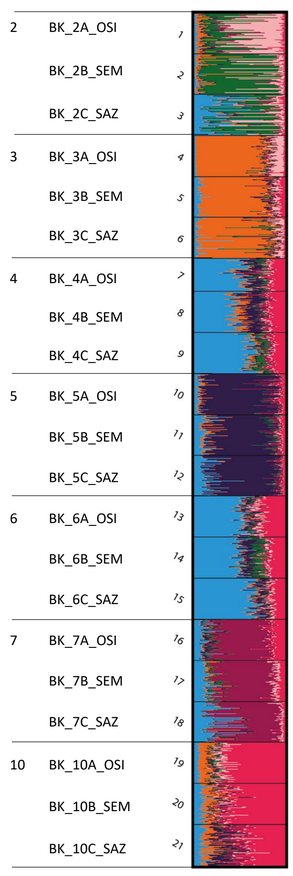

Figure 2: Evaluation of genetic structure by Bayesian method for K = 7 in 21 European beech sample sets

Results

Selected seven and nine optimally polymorphic markers reported sufficient informative value for the subsequent evaluation of the genetic structure of the monitored sets of Norway spruce and European beech reproductive material by Bayesion clustering. The most optimal number of clusters were K = 8 for Norway spruce sample sets and K = 7 for European beech sample sets. The structuring of investigated Norway spruce and European beech sample sets were confirmed by various proportions of genetic profiles according to the Bayesian clustering method results (Fig. 1–2). According to the performed evaluations of forest reproductive materials sets with different sample numbers (60, 30, 20, 10) in the compared sets, it can be declared that the recommended number of samples to confirm agreement in population structures is 60.

Table 1: Overview of forest reproductive material sources and designation of Norway spruce sample sets

Designation of forest reproductive material source	Source of forest reproductive material	Proof maturation year	Natural Forest Areas	Forest vegetation zone	sampling phase	designation of sample set
1	CZ-2-2A-SM-0004-36-3-Z	2017	3. LVS	36 – Středomoravské Karpaty	collected cones seed after processing nursery seedlings nursery transplanted plants	SM_1A_SIS SM_1B_OSI SM_1C_SEM SM_1D_SAZ
2	CZ-2-2A-SM-00010-17-3-E	2017	3. LVS	17 – Polabí	collected cones seed after processing nursery seedlings nursery transplanted plants	SM_2A_SIS SM_2B_OSI SM_2C_SEM SM_2D_SAZ
3	CZ-2-2A-SM-00002-8-3-S	2017	3. LVS	8 – Křivoklátsko	collected cones seed after processing nursery seedlings nursery transplanted plants	SM_3A_SIS SM_3B_OSI SM_3C_SEM SM_3D_SAZ
4	CZ-2-2A-SM-03411-38-5-Z	2017	5. LVS	38 – Bílé Karpaty a Vizovické vrchy	collected cones seed after processing nursery seedlings nursery transplanted plants	SM_4A_SIS SM_4B_OSI SM_4C_SEM SM_4D_SAZ
5	CZ-2-2A-SM-03546-31-4-E	2017	4. LVS	31 – Českomoravské mezihoří	collected cones seed after processing nursery seedlings nursery transplanted plants	SM_5A_SIS SM_5B_OSI SM_5C_SEM SM_5D_SAZ
6	CZ-2-2A-SM-03379-40-5-T-G185	2017	5. LVS	40 – Moravskoslezské Beskydy	collected cones seed after processing nursery seedlings nursery transplanted plants	SM_6A_SIS SM_6B_OSI SM_6C_SEM SM_6D_SAZ
8	B-SM-56+4-25-6-RK	1995	6. LVS	25 – Orlické hory	collected cones seed after processing nursery seedlings nursery transplanted plants	SM_8B_OSI SM_8C_SEM SM_8C_OPO_SEM SM_8D_SAZ
10	CZ-2-2A-SM-3134-25-7-H	2006	7. LVS	25 – Orlické hory	collected cones seed after processing nursery seedlings nursery transplanted plants	SM_10B_OSI SM_10C_SEM SM_10C_OPO_SEM SM_10D_SAZ

Table 2: Overview of forest reproductive material sources and designation of European beech sample sets

Designation of forest reproductive material source	Source of forest reproductive material	Proof maturation year	Natural Forest Areas	Forest vegetation zone	sampling phase	designation of sample set
2	CZ-2-2A-BK-00030-19-5-U-G082-1	2018	5. LVS	19 – Lužická pískovcová vrchovina	seed nursery seedlings nursery transplanted plants	BK_2A_OSI BK_2B_SEM BK_2C_SAZ
3	CZ-1-2C-BK-00009-38-4-B	2018	4. LVS	38 – Bílé Karpaty a Vizovické vrchy	seed nursery seedlings nursery transplanted plants	BK_3A_OSI BK_3B_SEM BK_3C_SAZ
4	CZ-2-2A-BK-06035-10-4-C-G42	2018	4. LVS	10 – Středočeská pahorkatina	seed nursery seedlings nursery transplanted plants	BK_4A_OSI BK_4B_SEM BK_4C_SAZ
5	CZ-2-2B-BK-03454-26-4-H-G101-1	2016	4. LVS	26 – Předhoří Orlických hor	seed nursery seedlings nursery transplanted plants	BK_5A_OSI BK_5B_SEM BK_5C_SAZ
6	CZ-2-2B-BK-06065-10-4-C-G42	2018	4. LVS	10 – Středočeská pahorkatina	seed nursery seedlings nursery transplanted plants	BK_6A_OSI BK_6B_SEM BK_6C_SAZ
7	CZ-1-2C-BK-01004-1-6-U	2018	6. LVS	1 – Krušné hory	seed nursery seedlings nursery transplanted plants	BK_7A_OSI BK_7B_SEM BK_7C_SAZ
10	CZ-2-2B-BK-00009-36-3-Z	2018	3. LVS	36 – Středomoravské Karpaty	seed nursery seedlings nursery transplanted plants	BK_7A_OSI BK_7B_SEM BK_7C_SAZ

Conclusion

Using the performed Structure analysis, the obtained profiles of monitored units of Norway spruce and beech reproductive material (4 and 3 sample sets from one units) of different origin were distinguishable from each other. Optimized methodological procedures could be used in the state control systems of verifying declared origin of Norway spruce and beech reproductive material and in order to increase consumer protection of forest owners and nursery producers in the Czech Republic.

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References

Falush D., Stephens M., Pritchard J.K. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics, 164 (4): 1567–1587. DOI: 10.1093/genetics/164.4.1567

Falush D., Stephens M., Pritchard J.K. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. Molecular Ecology, 7(4): 574–578. DOI: 10.1111/j.1471-8286.2007.01758.x

Hubisz M.J., Falush D., Stephens M., Pritchard J.K. 2009. Inferring weak population structure with the assistance of sample group information. Molecular Ecology Resources, 9 (5): 1322–1332. DOI: 10.1111/j.1755-0998.2009.02591.x

Li Y.L, Liu J.X 2018. StructureSelector: A web based software to select and visualize the optimal number of clusters using multiple methods. Molecular Ecology Resources, 18: 176–177.

Peakall R., Smouse P.E. 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6: 288–295. DOI: 10.1111/j.1471-8286.2005.01155.x

Peakall R., Smouse P.E. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research – an update. Bioinformatics, 28: 2537–2539. DOI: 10.1093/bioinformatics/bts460

Pritchard J.K., Stephens M., Donnelly P. 2000. Inference of population structure using multilocus genotype data. Genetics, 155 (2): 945–959. DOI: 10.1093/genetics/155.2.945