Pollen susceptibility to acidification and DNA polymorphism of Scots pine (*Pinus sylvestris* L.) plus trees

Asta Abraitienė[1], Donatas Žvingila[2], Sigutė Kuusienė[1]

[1]Lithuanian Forest Research Institute, Laboratory of Molecular Genetics and Biotechnology, Liepų str. 1, Girionys, LT-4312 Kaunas distr., Lithuania
[2]Vilnius University, Dept. of Botany and Genetics, M. K. Čiurlionio 21, LT-2009, Lithuania

A high frequency of acid rains has been reported in Lithuania. The impact of acid rains on pollen viability is known to exist as well. Therefore, Scots pine pollen susceptibility to acidity of the germination substrate (pH 3.0, 4.0, 5.0, and 6.0) was tested. It was found that pollen germination for all the genotypes tested was greatly reduced when the substrate with pH 3.0 was applied. With the substrates of pH ranging within 4.0–6.0, the amount and pattern of response in pollen germinability varied among plus trees considerably. Of the random part of the total variation, the highest part of variation in pollen sensitivity (53%) was caused by a genotype and environment interaction. Differentiation in pollen germinability among the genotypes was affected by substrate acidity as well.

It has been concluded that acidification of germination substrate has a significant influence on Scots pine pollen viability and the pattern of differentiation among the genotypes with possilbility to alter the genetic composition in the next generation. The association tendencies among plus tree genetic polymorphism revealed by RAPD technique and pollen susceptibility to environmental stress are discussed.

Key words: Scots pine, pollen germination, substrate acidity, DNA polymorphism

INTRODUCTION

After 10 years of analysis of the chemical composition of precipitation, it was concluded that there is a potential hazard for ecosystems because of frequent acid rains (with pH < 5.0) as their frequency reached 68–89% (Girgždys et al., 1999). The mean pH of the precipitation reached 4.26, 4.63 and 4.53 in the Western (Preila), Eastern (Molėtai) and Southern (Žuvintas) parts of Lithuania, respectively. The highest peak reached 3.46.

Pollen grains are known to be extremely sensitive to environmental factors. Pollution causes a number of changes in pollen, such as reduction in starch content, morphological immaturity in pollination time, reduction in enzyme activity, pollen germinability and tube growth (Осколов, 1998). Pollen affected by pollutants exhibits changes in cone and seed traits (Chalupka, 1998; Houston and Dochinger, 1977) as well as enlarges seed abortion (Cox, 1988). There is a reduction in pollen viability found because of treating pollen with simulated acid rain before germination (Sidhu, 1983; Neuvonen et al., 1991) or during germination (Hughes and Cox, 1994), while simulated acid fog showed a controversial effect by causing either a significant reduction (Hughes and Cox, 1994) or no impact on pollen viability (Ryn et al., 1988). Anthropogenic impact alters the genetic structure, natural mating design and distribution of parental tree resources through the pollution effect on pollen (Giannini and Hagnani, 1994). However, there is still lack of information in this field.

The aim of the present study was to assess the effect of germination substrate acidity on germinability of Scots pine plus tree pollen grains and to obtain the knowledge about the impact of genetic background on pollen susceptibility of different Scots pine genotypes to acidification.

MATERIALS AND METHODS

Pollen susceptibility. Pollen grains were collected on 16th of May, 2001 at the clone archive from vegetative progenies of different *Pinus sylvestris* L. plus trees (Table 1). After drying, pollen was stored in a freezer at –12 °C until the germination test. The

Table 1. **Origin places of plus trees tested for pollen germinability and DNA polymorphism**

Coresponding tree number in		Plus tree origin place
DNA analysis	state register	
1	68	Alytus forest enterprise, Punia forestry
2	79	Alytus forest enterprise, Punia forestry
3	204	Kuršių Nerija national park, Juodkrantė forestry
4	166	Švenčionėliai forest enterprise, Laukagalis forestry
5	77	Alytus forest enterprise, Punia forestry
6	83	Alytus forest enterprise, Punia forestry
7	875	Kretinga forest enterprise, Darbėnai forestry
8	187	Dzūkija national park, Marcinkonys forestry
9	855	Jurbarkas forest enterprise, Viešvilė forestry
10	189	Dzūkija national park, Marcinkonys forestry
11	164	Druskininkai forest enterprise, Latežeris forestry
12	266	Jurbarkas forest enterprise, Viešvilė forestry

susceptibility of pollen was characterized by reduction in pollen germinability while germinating in a more acid substrate. Pollen was germinated according to U. Eriksson recommendations (Eriksson, 1993).

The data processing was made by a two-way analysis of variance using MIXED procedure of the SAS, Type III sums of squares.

The ecovalence (Wi) of Wricke was calculated to evaluate the contribution of clones to the clone x treatment interaction. The following equation was used:

$$W_i = \sum_{j=1}^{s} \left(\overline{X}_{ij} - \overline{X}_{i\cdot} - \overline{X}_{\cdot j} + \overline{X}_{\cdot\cdot} \right)^2 , \qquad (1)$$

where W_i is the ecovalence estimate, $\bar{X}_{ij}$ is the mean of the clone i under environment j, $\bar{X}_{i\cdot}$ is the mean of the clone i, $X_{\cdot j}$ is the mean of the environment j, $\bar{X}_{\cdot\cdot}$ is the grand mean.

Evaluation of DNA polymorphism. DNA extraction was performed using a genomic DNA purification kit (MBI Fermentas, Lithuania). DNA polymorphism was investigated by the random amplified polymorphic DNA (RAPD) method (Williams et al., 1990). The PCR methodology followed that described earlier (Žvingila et al., 1999). The PCR products were fractionated by electrophoresis using a 1.5% (w/v) agarose gel in Tris-borate (TBE) buffer. Gels were stained with ethidium bromide. DNA fragments were then visualized under UV light and photographed using a gel documentation system (BioDocAnalyse).

RESULTS AND DISCUSSION

A reduction in pollen germinability by 29% was fixed after changing the germination substrate acidity from pH 6.0 to pH 3.0 (germinability was respectively 37 + 0.6% and 8% + + 1.1%). This was in agreement with results obtained after treatment of *Picea glauca* (Moench) Voss. pollen with simulated acid rain pH 3.6 (germinability in agar-based substrate reduced by 30% (Sidhu, 1983). The most pronounced change in pollen viability was caused by substrate acidity fall from pH 4.0 to 3.0 (15%), which was as high as in range of pH change from 6.0 to 4.0. Most of forest tree species pollen is known to be sensitive to pH 3.0 and just a few of them to pH 4.6. Possibly because of a high sensitivity of pollen to the environment and great intensity of stress factor (pH 3.0), the effect of acidity on pollen viability was very significant ($p < 0.001$). The great impact of genotype × environment interaction was found to indicate 53% of total random variation. However, it is known that a significant effect of genotype × environment interaction is associated also with differences among the genotypes (Houston, Dochinger, 1977). A great variation in Scots pine pollen susceptibility to substrate acidification was found (Fig. 1). Nevertheless, average pollen germinability was reduced by substrate acidification. The response of some genotypes may show a slightly differing pattern in the pH range from 6.0 to 4.0. The number of significant differences among the genotypes was dependent upon germination substrate acidity (Table 2). The highest changes in genotypic differentiation and ranks by pollen germinability were found in germination at substrate acidity pH 3.0.

Pollen most sensitive to substrate acidification was found in plus tree No. 189 (change in germinability up to 52%, slope 16) originated in Marcinkonys forestry and plus tree No. 77 (change in germinability

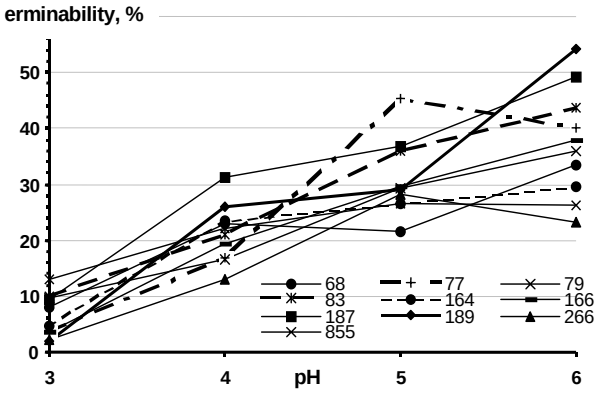

Fig. 1. Pollen germinability of different Scots pine plus trees in the range of substrate acidity (designated by the number in state register)

Table 2. **Genotypic differentiation of Scots pine pollen viability while germinating in the substrates of different acidity**

pH=6	pH=5	pH=4	pH=3
189 187 83 77 166 855 68 164 79 266	77 187 83 189 166 855 266 164 79 68	187 77 189 164 68 79 83 166 855 266	68 79 83 855 187 164 166 77 189 266
F 49.21***	22.81***	25.51***	53.21***

Genotypes ordered by ranks of germination percentage and designated according to plus tree state register numbers. Genotypes with not significant (by 95% and higher probability) differences in germinability are indicated by vertical line. The Fisher criterion (F) with the significance of genotype effect levels (***= $p < 0.001$) are given in the bottom of the table

So, the genotypes having pollen most sensitive and most adaptive to acidification were separated into different clusters, suggesting that the dependence of pollen germinability on extremal environmental changes (acidification) is geneticaly determined.

Thus, the acidity of germination substrate had a significant influence on Scots pine pollen viability and as consequence on differences among individual genotypes in their survival. This implies the up to 37%, slope 14) originated in Punia forestry. These genotypes showed the highest genotype and environment interaction (Wi respectively 293 and 218), while change in pollen germinability up to 13% and slope 7 was fixed for the most stable genotype (Punia 79). Thus, a rather high variation in pollen sensitivity was found, providing a buffer for selection pressure during flowering time (Table 3), since it must be taken into consideration that pollen germinability and tube growth appeared to be much more sensitive to atmospheric pollution *in vitro* than *in vivo* (Giannini, Mognani, 1994; Neuvonen et al., 1991).

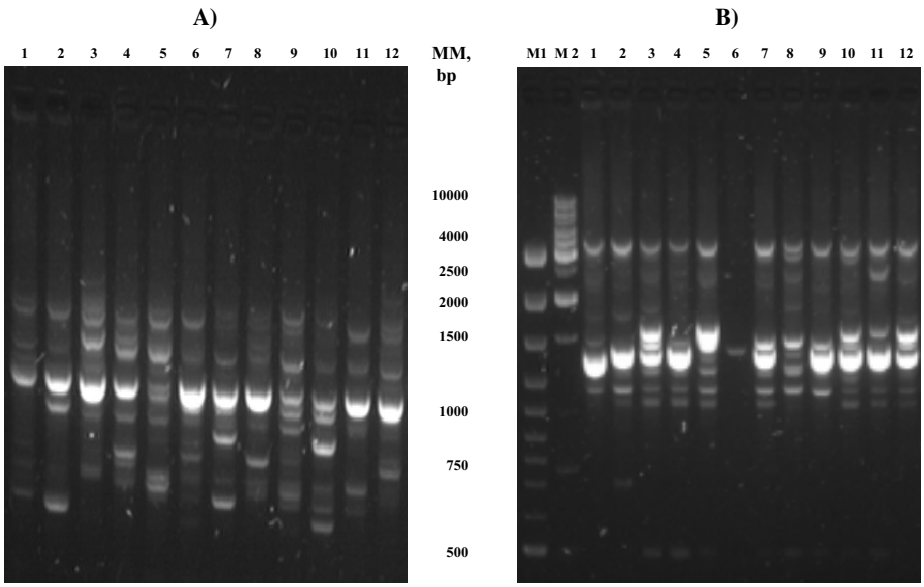

Fig. 2. Scots pine plus trees (ordered respectively by numbers 68, 79, 204, 166, 77, 83, 875, 187, 855, 189, 164, 266) DNA polymorphism evaluated by RAPD using different primers: A6 (A) and A7 (B). M1 and M2 – molecular markers of DNA fragment size by base pairs (bp)

Genomic DNA polymorphism in 12 pine plus tree genotypes was investigated by the RAPD method using six oligonucleotide primers. Five primers generated polymorphic profiles of DNA amplification products. One primer (A4) was uninformative. The greatest differences among the genotypes were found by using primers A6 (5' – GAC CCG TCC C - 3' and A7 (5'-GAA ACG GGT G-3') (Fig. 2). A total of 51 DNA bands were scored for analysis. A dendrogram was constructed using the UPGMA cluster analysis method and clustered the 12 genotypes into two distinct groups (Fig. 3). The genotypes Nos. 68, 79, 266 and 164 made separate group and belonged to the second cluster.

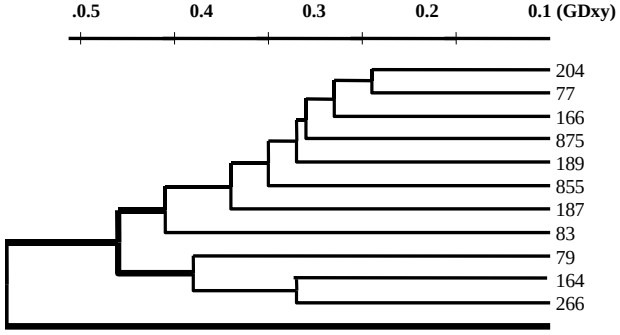

Fig. 3. The dendrogram of plus trees compiled with computer program using the TREECON UPGMA cluster making method. GDxy – genetic distance by Nei and Li (1979)

Table 3. **Changes in the mean values of pollen germinability (%) within different germination acidity intervals (pH 3 to 4 and pH 3 to 6), Wrickle ecovalence (*Wi*), and parameters (slope (b_1), and regression coefficient (R^2)) that fit the linear effect model of the clones studied**

Clone No.	Change in germinability within pH interval		*Wi*	Linear regression coefficients	
	3–4	3–6		slope	R^2
68	15	26	81	7.5	0.86
77	13	37	218	13.8	0.82
79	9	13	159	4.4	0.81
83	11	34	24	11.6	0.99
164	19	25	53	7.8	0.80
166	16	34	10	11.4	0.98
187	22	40	58	12.4	0.94
189	24	52	293	16.0	0.93
266	11	21	68	7.9	0.77
855	7	26	26	9.1	0.98

possibility to alter the gene pool of the next generation.

CONCLUSIONS

The acidity of germination substrate showed a significant influence on Scots pine pollen viability and the pattern of differentiation among the genotypes with the possibility to alter genetic composition in the next generation.

The genotypes tested were unstable under treatment by differing acidity of the germination substrate (genotype × environment interaction caused 53% and the genotype 46% of random variations).

The among-plus-tree differences in DNA polymorphism revealed by the RAPD method were similar to those shown by pollen germination *in vitro* in non-stress conditions (substrate pH 6.0 and 5.0).

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Asta Abraitienė, Donatas Žvingila, Sigutė Kuusienė

PAPRASTOSIOS PUŠIES (*PINUS SYLVESTRIS* L.) PLIUSINIŲ MEDŽIŲ ŽIEDADULKIŲ JAUTRUMAS RŪGŠTINGUMUI IR DNR POLIMORFIZMAS

S a n t r a u k a

Lietuvoje dažnai pranešama apie rūgštų lietų. Rūgštaus lietaus poveikis žiedadulkių gyvybingumui taip pat žinomas. Todėl buvo tirtas paprastosios pušies (*Pinus sylvestris* L.) žiedadulkių jautrumas substrato rūgštingumui (pH 3,0, 4,0, 5,0, 6,0). Nustatyta, kad žiedadulkių daigumas visų tirtų genotipų labai sumažėjo, esant substrato pH 3,0. Esant substrato pH 4,0–6,0, žiedadulkių daigumas įvairavo priklausomai nuo genotipo. Bendra atsitiktinė variacija tarp genotipo ir aplinkos sąveikos sudarė 53%.

Nustačius substrato rūgštingumo poveikį paprastosios pušies klonų žiedadulkių gyvybingumui, buvo atliktas klonų genetinio polimorfizmo įvertinimas taikant atsitiktinai pagausintos polimorfinės DNR metodą.

Raktažodžiai: paprastoji pušis, žiedadulkių daigumas, substrato rūgštingumas, DNR polimorfizmas