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Genetic variability of phenological forms in selected provenances of Norway spruce of IPTNS-IUFRO 1964/68 experiment test in Poland

Genetische Variation der phänologischen Formen in ausgewählten Herkünften von Fichten des Provenienzversuchs IPTNS-IUFRO 1964/68 auf der Versuchsfläche in Polen

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Key words: *Picea abies*, IUFRO, isozyme, genetic polymorphism, flushing

Stichworte: *Picea abies, IUFRO, Isoenzyme, genetische Polymorphismus, Spülung*

Summary

Here, we present an analysis of genetic variability of early and late phenological forms of Norway spruce growing on international inventory test IPTNS-IUFRO 1964/68 in Krynica, Poland. The polymorphism of 7 isozyme systems encoded in 11 loci was characterized. Early and late flushing spruce had a similar genetic variability of the following parameters: the mean and effective number of alleles per locus and the observed and expected heterozygosity. In each year of the observation, there was a significant correlation between the time of vegetation start and the allele frequency of the locus B of leucine aminopeptidase (*Lap-B*) and the locus A of glutamate dehydrogenase (*Gdh-A*). The geographic variability in the loci *Gdh-A, Mdh-B*, and *Shdh-A* was also observed.

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Zusammenfassung

Der Artikel enthält eine Analyse der genetische Variation der Fichten mit dem früh und spät Datum des Frühlingswachstum. Fichten wachsen auf der internationaler Provenienzversuchs IPTNS-IUFRO 1964/68 in Krynica (Polen). Die genetische Variation der sieben Enzyme wird untersucht, die bei elf Loci kodiert wurde. Fichten, die früh and spät Knospen treiben, eine ähnliche genetische Variation der folgenden Parameter hatten: die mittlere und die effektive Zahl der Allele je Locus sowie die beobachteten und erwarteten Heterozygosity. In jedem Jahr der Beobachtung, eine signifikante Korrelation zwischen dem Datum des Frühlingswachstum und Allelfrequenz im Locus B von Leucin-Aminopeptidase (*Lap-B*) und im Locus A von *Glutamatde dehydrogenase* (*Gdh-A*) wurde gezeigt. Die geographische Variation der *Gdh-A, Mdh-B* und *Shdh-A* Loci wurde beobachten.

1. Introduction

The Norway spruce [*Picea abies* (L.) Karst.] is one of the most important forest tree species in Europe due to significant economic and protection role and high participation in the stands of this area. It is also sensitive to environmental conditions such as heat, drought, and frosts [Jaworski 2011]. The most dangerous condition for the Norway spruce is late spring frosts which occur at the time of the development of buds and during young shoot elongation. Most of the economic losses caused by such environmental conditions were observed during the production of seedlings in nurseries and at high altitude crops. There has also been a decline of mature trees [Duffeld 1956]. In comparison to the late flushing spruces, the early budding provenances are more vulnerable to frost damages because during these conditions they are still at the stage of bud burst or at early stages of photosynthetic system development. The late flushing provenances, which are still in winter dormancy, cannot be damaged by spring frosts.

The first observations of Norway spruce flushing were made by Engler [1905] in the experiment that was established in Switzerland. Since then, the analysis of the spring phenology of *Picea abies* was the subject of many studies conducted in Europe and North America [Svoboda 1953; Lacaze 1969; Schmidt-Vogt 1977]. However, most of the information on the variability of the time of spruce bud burst were derived from the provenance studies coordinated by the International Union of Forest Research Organizations (IUFRO) [Tyszkiewicz 1968; Sabor 1984; Giertych 1976, 77; Krutzsch 1975]. Based on the results of these works, it was concluded that the populations with early flushing, and therefore susceptible to spring frosts, were from Scandinavian, Siberian, and Alpine provenances of Austria and Italy, while the spruce resistant to late frost was of the north-eastern and Romanian provenances. It was also found that the time of bud burst, which is positively correlated with the resistance to late frosts,

is characterized by a very high heritability ranging from 0.56 to 0.95 [Schmidt-Vogt 1977] and the pronounced clinal variability from south to north [Danusevičius and Gavrilavicius 2001; Sabor 1989; Søgaard et al. 2008].

There are few studies describing the molecular aspect of the time of vegetation start being positively correlated with the resistance to late frost in Norway spruce. It has only been indicated that the time of spruce flushing is controlled by multiple genes with small additive effects [Eriksson et al. 1978]. The analysis of quantitative trait loci (QTLs) in Douglas-fir identified three loci responsible for spring bud burst [Jermstad et al. 2001] that are mapped on the same location as QTLs for spring cold hardiness. This observation confirms the relationship between the analyzed traits and also suggests the possibility of an effective resistance selection of trees based on the time of bud flushing. Frost resistance was also analyzed in *Populus* and *Betula* species indicating that it is controlled respectively by six [Frewen et al. 2000] and four loci [Tsarouhas 2003].

The analysis of *Populus* ssp., *Picea abies*, and *Pinus sylvestris* mutant collection shows that the time of vegetation start, correlating with late frost resistance, is controlled by genes responsible for phytohormone biosynthesis (*PHYB1, PHYB2*) [Howe et al. 1995, 1998; Clapham et al. 2001]. Phenotypic variation in the time of the bud burst is most likely affected by abscisic acid-insensitive genes (*ABI1B, ABI3*) [Frewen et al. 2000] along with a gene family involved in transcription activation (*CBF/DREB1*) [Thomasow 2001]. In recent years, the analysis has also been focused on the role of phytohormones which are the components of signaling pathways, namely, abscisic acid, auxins, and gibberellins in the willows [Olsen et al. 1995], poplar [Frewen et al. 2000; Eriksson and Moritz 2002] and birch [Li et al. 2003].

The aim of this study was to determine the genetic polymorphism and find genetic markers associated with the time of spruce flushing (indirectly correlated with resistance to late frosts). The working hypothesis was that environmental conditions of mother trees formed differences in the frequency of alleles, which contributed to the changes in the genetic variability of early and late phenological forms of Norway spruce.

2. Methods

2.1 The plant material

The analysis included the progeny of 24 provenances of Norway spruce (7 early and 17 late flushing provenances and each was represented by 10 randomly selected trees) growing in the IPTNS-IUFRO 1964/68 experiment test in Krynica, Poland. The characterization of seed source of analyzed provenances was presented in Table 1.

Table 1: Characteristics of analyzed Norway spruce population of IPTNS-IUFRO 1964/68 experiment test in Krynica [Bałut and Sabor 2002]

Tabelle 1: Eigenschaften von ausgewählten Herkünften von Fichten des Provenienzversuchs IPTNS-IUFRO 1964/68 auf der Versuchsfläche in Krynica

No IUFRO	Provenance	Geographical region	Indices of spring flushing			Geografical coordinate (°)	
			1975	1976	1977	latitude	longitude
0111	Bricalovic	75–Belarus	2.645	4.642	2.893	53.3	28.7
0146	Puszcza Białowieska	70–Białowieża Primeval Forest; Poland	2.742	5.043	3.014	52.6	23.6
0326	Knyszyn	69–Augustów Lakeland, Podlasie; Poland	5.368	5.354	2.76	53.3	22.9
0340	Cimpeni, XV Valea Mare 24	58–Bihor Mts, Transylvania; Romania	5.757	5.271	3.192	46.3	23.0
0351	Ukmerges	71–Vilnius Lakeland, Belarus Lakeland; Lithuania, Belarus	5.28	5.085	2.85	55.3	24.7
0417	Stanz-Kindtal-Allerheiligen	32–Styria (N–E) 1; Austria	6.478	6.118	3.652	47.5	15.5
0439	Dorna Cindreni, II Rosia, 50A	59–East Carpathians; Romania	5.542	5.186	2.416	47.4	25.4
0441	Deutschlandsberg	33–Styria (S–E); Austria	6.65	6.194	3.71	46.8	15.2
0447	Borki Knieja	68–Masurian Lakeland; Poland	5.282	4.881	2.302	54.1	22.1
0451	Seewiesen, Seereith	32–Styria (N–E) 1; Austria	6.617	5.882	3.669	47.6	15.3
0487	Cucureasa, 65	59–East Carpathians; Romania	5.484	5.08	3.054	47.3	25.0
0700	Cosna, Cucureasa 4A	59–East Carpathians; Romania	2.602	4.554	2.261	47.3	25.2
0735	Knittelfeld	32–Styria (N–E) 1; Austria	4.071	5.931	3.712	47.2	14.8
0749	Cucureasa, 65	59–East Carpathians; Romania	2.748	4.621	2.464	47.3	25.0

0761	Liemberg-wald/Zell Am See, 59	28–Tyrol–Salzburg; Austria	3.891	5.993	3.466	47.3	12.8
0765	Stuebing/ Gamskogel	32–Styria (N–E) 1; Austria	3.844	5.946	3.045	47.3	15.6
0834	Molvotitsk	76–East Russia (Valdai Hills); Russia	5.325	4.588	2.668	57.5	32.5
0841	Ignalino	71–Vilnius Lakeland, Belarus Lakeland; Lithuania, Belarus	5.495	4.525	2.567	55.3	26.2
0856	Roslavi	78–Russia 2 (Central Russian Upland, Smolensk–Moscow Heights)	5.442	4.714	2.641	54	33.0
0917	Mikaszowka	69–Augustów Lakeland, Podlasie; Poland	5.656	5.2	3.07	53.9	23.4
0922	Cucureasa, 65	59–East Carpathians; Romania	5.062	4.634	2.882	47.3	25.0
0925	Tarnawa	60–East Beskids (Tarnawa); Poland	5.55	5.086	3.154	49.1	22.8
0986	Foelz, Mayerberg	32–Styria (N–E) 1; Austria	6.582	6.04	3.893	47.5	15.2
1147	Mogilevskoje Oblast	75–Belarus	4.933	4.822	2.476	53.7	30.0

The selection of trees was based on the results of research of Bałut and Sabor [2001, 2002], who in the spring of 1975, 1976, and 1977 observed bud burst, assuming a 9-point scale proposed by Krutzsch [1973]. The authors determined the relative speed of bud burst in particular time of observation and incremental cycle in 1975, 1976, and 1977. They established the indices of spring flushing. Consequently, the geographical location of spruce vegetation with different time of vegetation period start was determined. The detailed description of the spring flushing of Norway spruce was presented by Balut and Sabor [2001, 2002].

2.2 Analysis of isozymes

The genetic structure of Norway spruce was determined using seven isozymes encoded in 11 loci (Table 2). Protein separation was carried out using 12% starch gel electrophoresis [Conkle et al. 1982]. Individual buffers for visualization and electrop-

horesis were prepared according to Könnert and Maurer [2004]. The evaluation of obtained zymograms was performed based on the patterns developed by Könnert and Maurer [1995].

Table 2: The analyzed isoenzymatic systems

Tabelle 2: Untersuchte Enzymsysteme

Name of enzyme	Abbreviation	Number E.C.	Number of loci
Glutamate-oxaloacetate dehydrogenase	GOT	2.6.1.1	3
Glutamate dehydrogenase	GDH	1.4.1.2	1
Isocitrate dehydrogenase	IDH	1.1.1.42	1
Malate dehydrogenase	MDH	1.1.1.37	3
Shikimic dehydrogenase	SHDH	1.1.1.25	1
Leucine aminopeptidase	LAP	3.4.11.1	1
Phosphogluconic isomerase	PGI	3.5.1.9	1

2.3 Data analysis

The average number of alleles per locus (N_a) and the observed heterozygosity (H_o) were determined. The distribution of allelic variants of early and late flushing spruce was evaluated using the effective number of alleles at one locus (N_e) [Bergman and Gregorius 1979]. The expected heterozygosity (H_e) was assessed in accordance with the model given by Nei and Roychoundry [1974]. Based on the estimated genetic distance, analysis of molecular variance (AMOVA) was conducted. The parameters of genetic variation were calculated using GeneAlex ver. 6.41 [Peakall and Smouse 2006]. The significance of differences among the obtained parameters of genetic variability for early and late flushing provenances were estimated by Student's t-test using Statistica ver 9.0 [StatSoft Inc. 2010].

The relationship between the tree phenotype (expressed as average flushing rates estimated in 1975, 1976, and 1977) and the frequency of alleles of particular isoenzyme systems was analyzed using the nonparametric Spearman correlation method. The calculations were performed using Statistica ver. 9.0 [StatSoft Inc. 2010].

3. Results

3.1 Genetic diversity of phenological forms

Among the examined enzymes, the locus C of glutamate-oxaloacetate transaminase (*Got-C*), the locus B of phosphoglucomutase (*Pgi-B*), and the locus B of leucine aminopeptidase (*Lab-B*) were characterized as highly polymorphic. The loci *Got-A Got-B*, *Idh-B*, and *Mdh-A* were monomorphic (the frequency of the most common allele was greater than 0.99), while the loci *Gdh-A, Mdh-B, Mdh-C*, and *Shdh-A* were semi-monomorphic (the frequency of the most common allele ranged from 0.95 to 0.99).

The differences in allele frequency between the different populations were observed. For example, only north-eastern populations were characterized by genetic variation in the locus *Gdh-A*. A similarity in locus B of malate dehydrogenase was observed. Genetic variation in this locus was observed only in Alpine populations. In the other provenances from north-eastern and Carpathian regions, the locus Mdh-B was monomorphic.

The dominant allele (A_3) in the locus *Shdh-A* occurred in all of the north-eastern populations with the exception of 0917—Mikaszówka population from Augustów Lakeland (69 Krutzsch region). In the Alpine and Carpathian populations, the frequency of the allele A_1 in the locus *Shdh-A* was higher.

There were also differences in locus *Got-C*, which had no relationship with the geographical distribution of species or seed collection. The populations of Tyrol and south-eastern Styria were characterized by a higher frequency of allele C_4, whereas for the provenance of north-eastern Styria, allele C_2 was more specific (Table 3).

Table 3. Allele frequencies in spruce provenances

Tabelle 3. Häufigkeiten von Allel in Fichten Provenienzen

Locus	North – ekstern populations										Carpathian populations								Alpine populations					
	0111	0146	0326	0351	0447	0834	0841	0856	0917	1147	0340	0439	0487	0700	0749	0922	0925	0417	0441	0451	0735	0761	0765	0986
Gdh-A2	0.85	0.90	1.00	0.90	0.65	0.85	0.85	1.00	1.00	0.85	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00
Gdh-A3	0.15	0.10	0.00	0.10	0.35	0.15	0.15	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	0.00	0.00	0.00	0.00	0.00	0.00
Got-A3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Got-B2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Got-C2	0.55	0.70	0.50	0..6	0.35	0.25	0.40	0.30	0.50	0.20	0.45	0.30	0.30	0.35	0.30	0.40	0.50	0.50	0.20	0.65	0.45	0.05	0.40	0.65
Got-C4	0.40	0.30	0.50	0.40	0.65	0.75	0.60	0.70	0.50	0.80	0.55	0.70	0.70	0.65	0.70	0.60	0.50	0.50	0.80	0.35	0.55	0.85	0.60	0.35
Got-C5	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.00
Idh-B1	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Idh-B2	0.95	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Lap-B2	0.05	0.05	0.00	0.05	0.00	0.05	0.00	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.05	0.20	0.15	0.05	0.05	0.10	0.00
Lap-B3	0.00	0.00	0.00	0.05	0.05	0.00	0.05	0.00	0.10	0.10	0.00	0.15	0.05	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.05	0.00	0.00	0.25
Lap-B4	0.95	0.95	1.00	0.90	0.95	0.95	0.95	0.95	0.85	0.90	1.00	0.85	0.95	1.00	1.00	0.95	0.90	0.90	0.80	0.85	0.90	0.95	0.90	0.75
Mdh-A2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Mdh-B2	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	0.95	1.00	1.00	1.00
Mdh-B4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.05	0.00	0.00	0.00
Mdh-C2	0.00	0.05	0.05	0.00	0.00	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00	0.00	0.15	0.25	0.15	0.00	0.00	0.10	0.20	0.05	0.00	0.00
Mdh-C4	1.00	0.95	0.95	1.00	1.00	0.95	0.95	0.95	0.95	1.00	1.00	1.00	1.00	1.00	0.85	0.75	0.85	1.00	1.00	0.90	0.80	0.95	1.00	1.00
Pgi-A2	0.35	0.35	0.25	0.35	0.00	0.55	0.40	0.30	0.30	0.40	0.35	0.35	0.00	0.00	0.20	0.45	0.40	0.40	0.00	0.00	0.00	0.40	0.25	0.25
Pgi-A3	0.65	0.65	0.75	0.65	1.00	0.45	0.60	0.70	0.70	0.55	0.75	0.65	1.00	1.00	0.80	0.55	0.60	0.60	1.00	1.00	1.00	0.60	0.75	0.75
Pgi-A4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Shdh-A1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.05	0.00	0.10	0.10	0.00	0.00	0.05	0.15	0.00	0.10	0.00	0.00	0.00	0.05	0.00	0.15
Shdh-A3	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	0.95	1.00	0.90	0.90	1.00	1.00	0.95	0.85	1.00	0.90	1.00	1.00	1.00	0.95	1.00	0.85

Both early and late flushing spruce populations had similar parameters of genetic variation estimated by isoenzyme markers. This result indicates Student's t-test, which proved no statistically significant differences in values of genetic variation of spruces with early and late vegetation period start.

The highest value of polymorphic loci (V%) was obtained for the late flushing provenances. The average and effective number of alleles per locus calculated for the early forms were 1.220 and 1.186, respectively. For late flushing spruces, the values of these parameters were, respectively, 1.385 and 1.188. A strong similarity of the observed and expected heterozygosity was demonstrated for both early and late flushing spruces, which shows a comparable proportion of homozygotes to heterozygotes (Table 4).

Table 4: The parameters of isoenzyme and genetic polymorphism of early and late flushing provenances of Norway spruce

Tabelle 4: Die Parameter der genetischen Polymorphismus der Fichten, die früh und spät Knospen treiben

Forms	Isoenzyme polymorphism				
	V%	N_a	N_e	H_o	H_e
Early	37.66	1.22	1.186	0.113	0.114
Late	36.9	1.385	1.188	0.118	0.11
Average	37.28	1.3025	1.187	0.1155	0.112

V% - percentage of polymorphic loci, N_a – the mean number of alleles per locus, N_e – the effective number of alleles per locus, H_o - observed heterozygosity, H_e - expected heterozygosity

The analysis of molecular variance (AMOVA) showed that 95% of the observed variation was located within the early or late spruce provenances, respectively. The rest of the variation was located between populations (Table 5). The differences between the analyzed early and late flushing populations were statistically insignificant.

Table 5: Analysis of molecular variance

Tabelle 5: Die Analyse molekularer Varianz

Source of variance	Degrees of freedom	Sum of squares	Mean square	Wariation	%
Among populations	23	43.883	1.908	0.067	5%
Within populations	216	267.200	1.237	1.237	95%
Total	239	311.083	-	1.304	100%

3.2 The relationship between flushing and genetic variability of spruce

Using the nonparametric Spearman correlation method, a significant correlation between the indices of spring flushing estimated in 1975, 1976, and 1977 and the frequency of alleles in locus B of leucine aminopeptidase (*Lap-B*) was found. In this locus, allele 4 occurred with higher frequency in the late forms than in early flushing provenances. A high correlation was also observed for both allelic variants at the locus A of glutamate dehydrogenase (*Gdh-A*). Allele 2 had a higher frequency in the early forms of spruces, whereas allele 3 was more characteristic for late flushing provenances. The relationship between the flushing factor estimated in the growing cycle of 1977 and the allele frequency of the locus B malate dehydrogenase (*Mdh-B*) has been demonstrated. In this locus, allele 4 had a higher frequency in the early form of spruce, whereas allele 2 was more characteristic for late flushing provenances (Table 6).

Table 6: The relationship between the flushing factor estimated in the growing cycle in the years 1975, 1976 and 1977 and the frequency of isoenzyme alleles

Tabelle 6: Die Beziehung zwischen der Wachstumsperiode in den Jahren 1975, 1976 und 1977 und die Frequenz des Isoenzyms Allele

Loci/allel	Indices of spring flushing			Loci/allel	Indices of spring flushing		
	1975	1976	1977		1975	1976	1977
Gdh-A	0.291	0.446*	0.448*	Lap-B	-0.436*	-0.647***	-0.427*
allel 2				allel 4			
Gdh-A	-0.311	-0.431*	-0449*	Mdh-A	monomorphis locus		
allel 3				allel 2			
Got-A	monomorphis locus			Mdh-B	-0.131	-0.392	-0.435*
allel 3				allel 2			
Got-B	monomorphis locus			Mdh-B	0.131	0.392	0.436*
allel 2				allel 4			
Got-C	0.132	0.158	-0.366	Mdh-C	-0.089	-0.106	0.093
allel 2				allel 2			
Got-C	-0.129	-0.156	0.078	Mdh-C	0.088	0.106	-0.093
allel 4				allel 4			
Got-C	-0.366	0.078	0.137	Pgi-A	0.161	0.009	0.009
allel 5				allel 2			
Idh-B	0.316	0.166	0.015	Pgi-A	-0.169	0.019	0.019
allel 1				allel 3			
Idh- B	-0.316	-0.166	-0.015	Pgi-A	-0.135	-0.226	-0.226
allel 2				allel 4			
Lap-B	0.075	0.337	0.408*	Shdh-A	0.276	0.216	0.216
allel 2				allel 1			
Lap-B	0.263	0.336	0.063	Shdh-A	-0.276	-0.216	-0.216
allel 3				allel 2			
Significant at *0.05 level, ** 0.01 level, *** 0.001 level							

4. Discussion

4.1 The selection of method and research material

In these studies, the isoenzyme markers were used. This technique is useful to determine the genetic variation of spruces [Lewandowski and Burczyk 2002; Masternak et al. 2011] and to identify the relationship between the genetic polymorphism and morphological features of this species [Liesebach 1994; Markussen et al. 2004] or resistance to pollution [Bergmann and Scholz 1985]. The present study included spruce

trees from the inventory provenance test IPTNS-IUFRO 1964/68 in Poland (Krynica) which is a collection of 1096 Norway spruce provenances from the whole range of its occurrence. Research areas are also located in Belgium, Germany, Czech Republic, France, Finland, the UK, Ireland, Canada, Norway, Austria, Sweden, and Hungary. The whole collection has been characterized in terms of spring phenology. It has been frequently showed that the time of bud burst is genetically determined and corresponds with the dates of vegetation start of mother trees [Holzer 1993]. The stability of this trait has been confirmed for spruce [Schmidt-Vogt 1977; Giertych 1989] and other forest trees species [Banach 2002]. Due to its high heritability, reevaluation of individual phenological stages of spruce has been omitted in this study, and the results presented by Balut and Sabor [2002] in 1975–77 have been accepted.

4.2 The genetic structure of phenological forms of Norway spruce

In previous studies, no information on the genetic polymorphism of early and late forms of Norway spruce has been presented. General studies indicate, however, that the estimated genetic variation expressed as the average number of alleles per locus and observed heterozygosity (N_a = 1.39; H_o = 0.12), estimated by the isoenzymatic markers for 24 early and late flushing populations of Norway spruce, was comparable to the polymorphism of trees from the whole range of its distribution as calculated by Langercrantz and Rymann [1990] (N_a = 1.58; H_o = 0.115) and lower than the variability determined by Bergmann and Gregorius [1979] (N_a = 2.14–3.14; H_o = 0.36–0.45).

4.3 Indication of the markers of late frost resistance

The existence of differences in spring phenology of forest trees is the result of the migration history of the species and the environmental pressures resulting in adaptation to habitat conditions. The time of vegetation period start in spruces, such as all physiological features, is strongly influenced by the environmental conditions [Nielsen and Jørgensen 2003], and has the clinal variability from south to north [Danusevičius and Gavrilavicius 2001; Sabor 1989; Søgaard et al. 2008]. Although in our study, there was no difference in the level of genetic variability (N_a, N_e, H_o, H_e) of different phenological forms of Norway spruce, the studies indicated two markers correlating with the time of vegetation start, namely, leucine aminopeptidase (LAP) and glutamate dehydrogenase (GDH). Both enzymes are involved in amino acid metabolism in plants [Kretowicz 1971]. The obtained results indicate that in the studied spruce populations, a gene regulating late induction of vegetation is most likely associated with the described markers. These isozymes, being the products of gene function, may also (at some stage) be involved in the cycle responsible for the development of buds. The start of vegetation growth is a complex phenomenon regulated by nume-

rous metabolic processes including biosynthesis of nucleic acids, structural and enzymatic proteins, polysaccharides or phenols, and the control of growth regulators and membrane properties together with osmotic and energy processes. No publications have yet documented the relation between isozyme systems and spring flushing. However, previous studies indicated a change in the allele frequency in *Lap-B* loci in spruce and pine growing in areas contaminated with zinc salts when compared to the control stands [Hosius 1994; Mejnartowicz and Palowski 1989].

5. Conclusions

The selection of forest trees should consider the increase of wood production, resistance to adverse environmental conditions, and maintenance of the existing genetic diversity. The use of marker-assisted selection technique (MAS) may be a valuable tool in improving the effective selection of forest trees and reducing its duration. The proposed biochemical markers associated with the time of vegetation start, which is correlated with resistance to late frosts, can be applied to further work on the resistance of spruce for this type of damage. To confirm the results of our research, further studies including the analysis using the indicated markers of the forest stands with different time of bud flushing are planned to be conducted.

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