Electrophoretically detected genetic variability of yellow sweet clover *Melilotus officinalis* (L.) Lam populations

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Summary

Six natural populations of sweet clover (*Melilotus officinalis*) were examined for allelic frequencies at 7 electrophoretically detected polymorphic loci in five enzyme systems: LAP, AP, ME, EST and PX. According to Nei's statistics, the extent of genetic variability in all populations shows that intra-population genetic variability is expressed by higher values of GST coefficient (mean value for all loci 0.135) which exceeds values of inter-population differences expressed by DST=0.059 coefficient. Gene flow between populations is rather low (Nm=1.60). Hierarchical clustering (UPGMA) of Hedrick's genetic distances (when examined simultaneously in all the allozymes), demonstrated three groups of populations suggesting certain tendency to geographic connections.

Key words: *Melilotus officinalis, genetic diversity, enzyme electrophoresis*

INTRODUCTION

Sweet clover (*Melilotus officinalis*) represents a biennial plant, encountered on wastelands or balks, railway embankments and other vastly insolated sites. It is a species characteristic for *Molinio-Meloliteum* and *Erisimo-Meliloteum* complexes. It grows in Eurasia, beginning at West Europe and ending at China. It represents a valuable fodder plant, even if it is less tasty than white clover (*Melilotus alba*) due to a higher content of coumarin in young leaves, flower buds and pods [1]. Therefore, consumed by cattle in excess it may be even harmful.

Due to its variable therapeutic properties, sweet clover was known already in antiquity, it was described by Hippocrates and Plinius [2], and at present it is harvested both from natural populations and herbal plantations and it is the main component of therapeutic mixtures. It is a valuable melliferous plant, the honey from which used to be added to other honeys to improve their taste and smell.

The sweet clover manifests extensive adaptive abilities. Brought from Europe to North America, in difficult climatic conditions of West Canada it proved to be a highly fertile cultivated species [3].

Because of its high adaptability to various soil and climatic conditions, sweet clover seems to be an interesting plant for genetic investigations.

MATERIAL AND METHODS

Plant material

The seeds were collected from geographically distant populations originating from:

1- Tuczno (N=24), West Pomerania province,
2- Tumlin (N=39), Świętokrzyskie province,
3- Łączna (N=33), Świętokrzyskie province,
4- Skarżysko-Kamienna (N=36), Świętokrzyskie province,
5- Poznań, ul. Lutycka (N=30), Wielkopolskie province,
6- Poznań-Dębiec (N=30), Wielkopolskie province.

Electrophoretic procedures

The fresh leaves of individual seedlings cultivated in similar greenhouse conditions were analyzed by electrophoresis. Leaves were ground, each with a drop of double-distilled water, the extract was soaked into 3 x 5 mm paper wicks (Beckmann No. 3119329) which were placed into 11% starch gel (Sigma) based on lithium-borate buffer system pH 8.3. The electrophoresis was conducted at 200 V and 40 mA at 5°C. Five enzyme systems with distinct and reproducible patterns were analyzed: LAP, EC 3.4.1.1 (leucine aminopeptidase); AP, EC 3.1.3.2 (acid phosphatase); ME, EC 1.1.1.40 (malic enzyme), EST, EC 3.1.1. (esterase) and PX, EC 1.11.1.7 (peroxidase).

The staining procedure closely followed that described by Show and Prasad [6]. The bands belonging to the same zone, having a consistent composition typical for homo- or heteromorphic allozymes, were considered as belonging to the same locus. Alleles in each locus were numbered according to their electrophoretic migrations toward anodic or cathodic parts of the gel (fig.1).

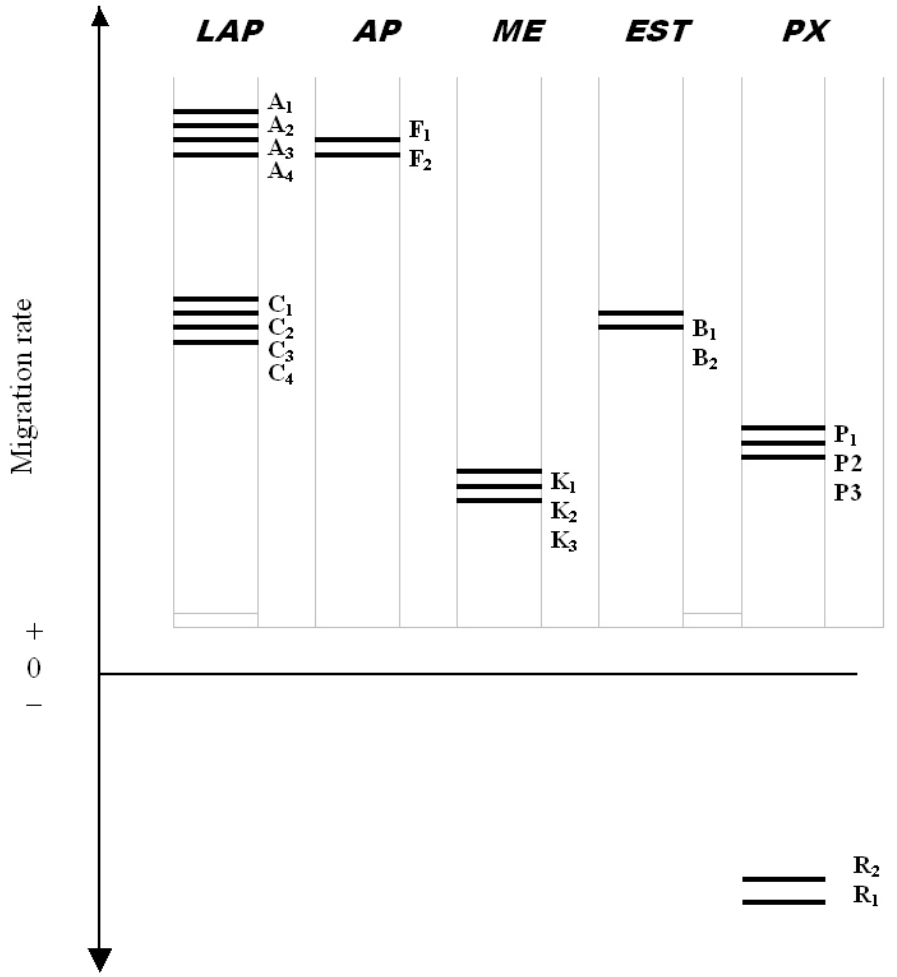

Figure 1. Schematic allozyme migration of the following enzyme systems: leucine aminopeptidase (LAP), acid phosphatase (AP), malic enzyme (ME), esterasae (EST) and peroxidase (PX)

Data analysis

Calculations of genetic parameters, such as observed heterozygosity (Ho), expected heterozygosity (He), total genetic diversity (HT), fixation indices (F), polymorphism indices of genotypes (Pg), relative measures of genetic differentiation between populations (GST, DST) and genetic similarities among populations based on genotype frequencies [5] were performed using the GEN computer software.

RESULTS AND DISCUSSION

Leucine aminopeptidase (LAP)

The electrophoretically detected band patterns were grouped in two zones, each with four allozymes. The enzyme showed a monomeric behaviour since heterozygotic genotypes showed two-banded phenotypes. Genetic distances between populations (Fig.2A) [5] showed a separate character of the monomorphic population 1 and a similar character of the polymorphic populations.

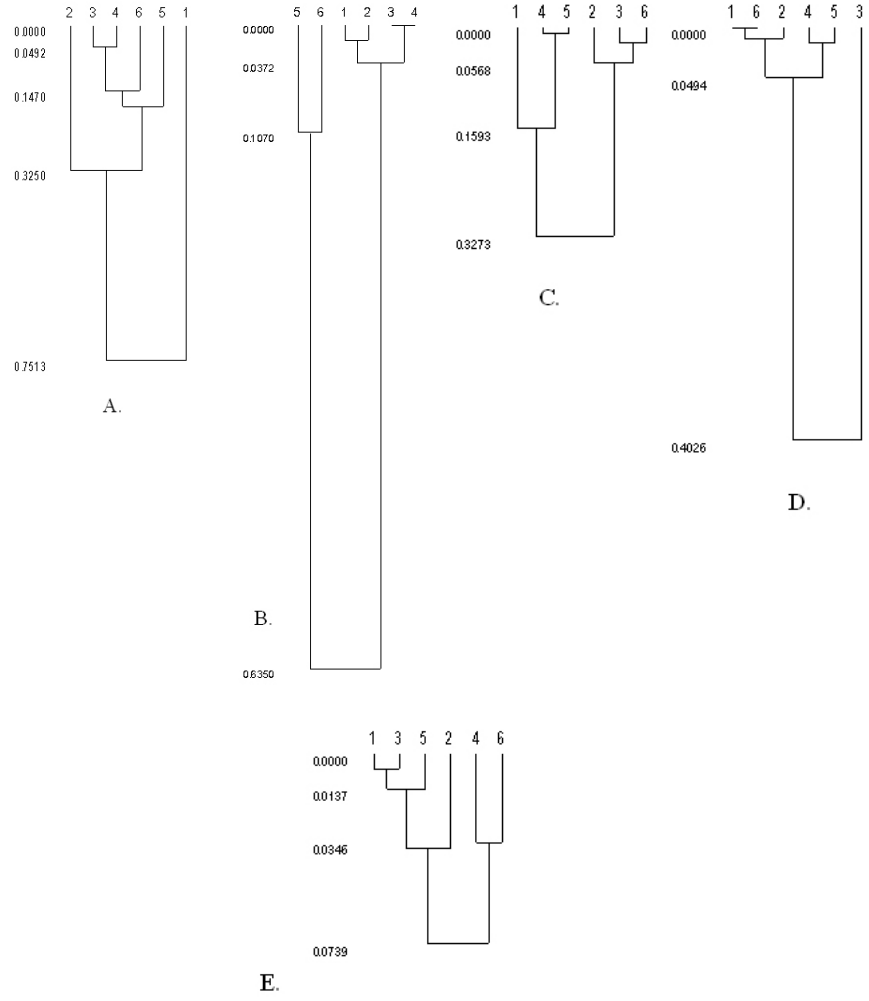

Figure 2. Dendrograms (UPGMA) constructed on the basis of genetic distances for separate loci: leucine amino-peptidase (LAP) – A; acid phosphatase (AP) – B; malic enzyme (ME) – C; esterase (EST) – D: peroxidase (PX) – E

Acid phosphatase (AP)

This enzyme system involved a single locus with two alleles. Populations compared in respect to genotype frequencies (fig. 2B) showed two genetically similar groups: populations 5 and 6 from Poznań (growing in urban conditions) composed separate group, whereas all remaining populations showed a high degree of similarity.

Malic enzyme (ME)

Three alleles were found in one locus. Single-banded electrophoretic phenotypes were considered to represent homozygotes at the locus, whereas heterozygotes were double-banded, indicating a monomeric structure of the enzyme. Dendrogram constructed on the basis of genotypes frequency (fig. 2C) revealed two groups of populations separated by a distance of more than 400 km.

Esterase (EST)

In the case of EST, the relative frequencies of phenotypes, composed of two allozymes, separated the populations into two main groups. The most distinct was the population 3 from Łączna, whereas all other populations showed a high genetic similarity (fig. 2D).

Peroxidase (PX)

The electrophoretically detected two loci (fig. 1) revealed existence of three anodally migrating allozymes in locus P and three cathodally migrating alleles in locus R. Genetic distances between populations calculated for both loci demonstrated (fig. 2E) two groups of populations: one clusters the two (4 and 6) distanced of about 400 km, the second composed of populations 1 and 5 as well as quite distant populations 3 and 2.

A summary of population statistics is given in table 1. The most heterozygotic was the population 5, in view of acid phosphatase and in respect of peroxidase locus R, the most heterozygotic were populations 1, 2 and 3. From all populations, the most polymorphic was population 4 (Pg=0.825) and population 2 (Pg=0.82), both for LAP – locus A.

Table 1

Genetic parameters for the six *Melilotus officinalis* populations: He – expected heterozygosity, Ho – observed heterozygosity, F – Wright's fixation index, Pg – polymorphism coefficient

locus	populations	He	Ho	F	Pg
LAP A	2	0.7009	0.4615	0.3415	0.8205
LAP A	3	0.6488	0.7273	-0.1210	0.7952
LAP A	4	0.6748	0.5000	0.2590	0.8256
LAP A	5	0.6306	0.3333	0.4714	0.7711
LAP A	6	0.5583	0.1000	0.8209	0.6089
LAP C	2	0.5759	0.3590	0.3767	0.7337
LAP C	3	0.2185	0.1818	0.1681	0.3600
LAP C	4	0.3438	0.1389	0.5960	0.4522
LAP C	5	0.2867	0.2667	0.0698	0.4378
LAP C	6	0.0644	0.0667	-0.0345	0.1244
AP F	1	0.3950	0.1250	0.6835	0.4965
AP F	2	0.4527	0.0769	0.8301	0.5207
AP F	3	0.2975	0.0606	0.7963	0.3526
AP F	4	0.2778	0.0556	0.8000	0.3287
AP F	5	0.4994	0.9000	-0.8020	0.1844
AP F	6	0.4550	0.6333	-0.3919	0.4867
ME K	1	0.4861	0.0000	1.0000	0.4861
ME K	2	0.6091	0.2051	0.6632	0.7272
ME K	3	0.5785	0.3333	0.4238	0.7420
ME K	4	0.4614	0.1111	0.7592	0.5448
ME K	5	0.4450	0.2000	0.5506	0.5689
ME K	6	0.5950	0.3000	0.4958	0.7356
EST B	2	0.0973	0.1026	-0.0541	0.1841
EST B	3	0.4224	0.6061	-0.4348	0.4775
EST B	4	0.2589	0.0833	0.6781	0.3318
EST B	5	0.3750	0.1000	0.7333	0.4600
PX P	1	0.2266	0.2500	-0.1034	0.4063
PX P	2	0.3024	0.3077	-0.0174	0.4602
PX P	3	0.1942	0.1515	0.2199	0.3177
PX P	4	0.1794	0.1944	-0.0839	0.3287
PX P	5	0.1561	0.1000	0.3594	0.2422
PX P	6	0.2311	0.2667	-0.1538	0.3911
PX R	1	0.4922	0.8750	-0.7778	0.2188
PX R	2	0.4882	0.8462	-0.7333	0.2604
PX R	3	0.4835	0.8182	-0.6923	0.2975
PX R	4	0.3885	0.4722	-0.2155	0.5262
PX R	5	0.4911	0.7333	-0.4932	0.4178
PX R	6	0.4550	0.6333	-0.3919	0.4867

The dendrogram based on data obtained for all enzyme systems (fig. 3) displayed genetic distances between populations and showed that the most different was the population 1 from Tuczno. Two populations from Poznań made one subgroup and three populations from Świętokrzyskie province also clustered together. The populations formed three main clusters, suggesting some connections with their geographic distribution.

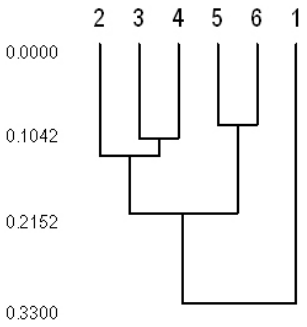

Figure 3. Dendrogram (UPGMA) constructed on the basis of genetic distances according to Hedrick [5] for all enzyme systems

According to statistics of Nei [4], the extent of genetic variability in all populations shows that intra-population genetic variability is expressed by higher values of GST coefficient (mean value for all loci amounted 0.135), which exceeds values of inter-population differences expressed by DST=0.059 coefficient. This is in accordance with other investigations in natural populations, where intra-population variability is usually higher than that among populations. Gene flow between populations is rather low (*Nm*=1.60).

Results of investigations included in this report have demonstrated that sweet clover *(M. officinalis*) shows an extensive enzymatic polymorphism. A conviction prevails that genetic polymorphism is of an adaptive significance since a higher genetic variability based on gene-environment interaction may increase some chances for invading a new habitats by the species in question.

REFERENCES

1. Mowszowicz J. Przewodnik do oznaczania krajowych roślin trujących i szkodliwych. Warszawa 1982.
2. Nowiński M. Dzieje upraw i roślin leczniczych. Warszawa 1983.
3. Goplen BP. Polara, a low cumarin cultivar of sweetclover. Can J Plant Sci 1971; 51:249-51.
4. Nei M. Genetic distance between populations. Amer Natur 1972; 106:283-92.
5. Hedrick PW. Genetic similarity and distances: comments and comparisons. Evolution 1974; 29(2):362-6.
6. Show CR, Prasad R. Starch gel electrophoresis of enzymes, a compilation of recipes. Bioch Gen 1970; 4:297-320.

GENETYCZNA ZMIENNOŚĆ POPULACJI NOSTRZYKA ŻÓŁTEGO (*MELILOTUS OFFICINALIS*) WYKRYTA METODĄ ELEKTROFOREZY ENZYMÓW

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S t r e s z c z e n i e

Sześć naturalnych populacji nostrzyka zółtego (*Melilotus officinalis*) przebadano pod względem częstości występowania 7 elektroforetycznie wyodrębnionych polimorficzmych loci w pięciu układach enzymatycznych: LAP, AP, ME, EST i PX. Zastosowanie statystyki Neia [4] dla wyrażenia wewnątrzpopulacyjnego (GST=0,139) i międzypopulacyjnego (DST=0,059) zróżnicowania genetycznego wykazało większe różnice międzypopulacyjne. Przepływ genów miedzy populacjami okazał się raczej niski (Nm=1,60). Porównanie populacji metodą najbliższego sąsiedztwa (UPGMA) pozwoliło wykreślić dendryt, na którym połączenia między populacjami (grupując 2 populacje z Wielkopolski i trzy populacje z rejonu Gór Świętokrzyskich) mogą sugerować podobieństwa geograficzne.

***Słowa kluczowe**: Melilotus officinalis, różnorodność genetyczna, elektroforeza enzymów*