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SYNTHESIS OF 3-BENZYLOXY-17-MALEYLOXY-16,17-SECOESTRA--1,3,5(10)-TRIENE-16-NITRILE

ABSTRACT: Under the conditions of Beckmann fragmentation reaction, 3-benzyloxy-17β-hydroxyestra-1,3,5,(10)-triene-16-one oxime (**2**) gave the D-seco derivative **3.** Sodium borohydride reduction of this compound afforded 3-benzyloxy-17-hydroxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4**). The esterification of seco-cyanoalcohol **4** was achieved by action of maleic acid anhydride in dry pyridine, yielding 3-benzyloxy-17-maleyloxy-16, 17-secoestra-1,3,5(10)-triene-16-nitrile (**5**).

KEY WORDS: Steroids, 16,17-seco-estrone derivatives, synthesis, Beckmann fragmentation reaction, esterification, hemiesters

INTRODUCTION

In the frame of a broader project directed towards obtaining potential antiestrogens, a series of new 16,17-seco-estrone derivatives has been prepared [Petrović et al., 1990; Pejanović, 1991; Sakač, 1997; Petrović et al., 1998; Jovanović-Šanta, 2000; Jovanović-Šanta et al., 2000]. One of them, 3-benzyloxy-17-hydroxy-16,17-secoestra-1,3,5(10)-trien--16-nitrile (**4**, Scheme 1), exhibited high antihormone action. We assumed that this activity could be increased by functionalizing this compound with a moiety possessing a free carboxyl group. Namely, it is known that steroid hormone derivatives having a side chain with a carboxyl function react with the ε-amino group of testosterone-binding globulin (TEBG), forming amide bonds [Erlanger et al., 1957]. Therefore, it can be expected that dicarboxylic acid monoesters of seco-cyanoalcohol 4 will behave in the same way with estrogen receptors, thus enhancing the desired antihormone activity.

EXPERIMENTAL

Melting points were determined in open capillary tubes on a Büchi SMP apparatus and are uncorrected. NMR-spectra were taken on a Bruker AC 250E spectrometer and are reported in parts per million downfield from a tetramethylsylane internal standard; symbols s, bs, d, dd, q and m denote singlet, broad singlet, doublet, double doublet, quartet and multiplet, respectively.

3-Benzyloxy-17 -hydroxyestra-1,3,5(10)-triene-16-one oxime (2)

Compound **1** (1 g, 2.57 mmol) was dissolved under heating in a mixture of methanol (20 cm^3), methylene chloride (8 cm^3) and 1% aqueous solution of KOH (30 cm^3). To the cooled solution, $NaBH_4$ (0.95 g, 25.11 mmol) was added portionwise. The reaction mixture was stirred for 20 min at room temperature and then refluxed for 40 min. After cooling, acetic acid was added to pH 5 and the white precipitate collected and washed thoroughly with water (0.98 g, 98.00% yield, mp 193—195°C). Recrystallization from methanol afforded analytically pure **2**: 0.83 g (83.00%), mp 195—196°C.

^{1}H-NMR ($CDCl_3$): 0.80 (s, 3H, CH_3, C_{18}); 3.68 (bs, 1H, C_{17}); 4.20 (s, 1H, C_{17}-OH); 5.13 (s, 2H, O-C**H**$_2$-C_6H_5); 6.72—7.34 (group of signals, 8H, aromatic protons); 8.87 (bs, 1H, C=N-OH).

^{13}C-NMR ($CDCl_3$): 11.22 (CH_3, C_{18}); 69.93 (O-**C**H_2-C_6H_5); 156.81 (C_3); 165.59 (C=NOH).

3-Benzyloxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (3)

-Hydroxy oxime **2** (1 g, 2.56 mmol, finely ground and dried for 3 hrs at 90°C) and *p*-toluenesulfonyl chloride (1.53 g, 8 mmol) were dissolved in absolute pyridine (15 cm^3). The reaction mixture was kept at room temperature for 3 hrs and than poured into an excess of cold diluted HCl. The separated precipitate of the crude 3-benzyloxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3**) was collected, washed with water and dried (0.954 g, 95.40%). Column chromatography on silica gel (70 g, toluene-ethyl acetate, /95:5/) afforded 0.72 g (71.66%) of pure compound **3**, mp 137—138°C.

^{1}H-NMR ($CDCl_3$): 1.18 (s, 3H, CH_3, C_{18}); 2.95 (d, 2H, C_{15}); 5.08 (s, 2H, O-C**H**$_2$-C_6H_5); 6.78—7.35 (group of signals, 8H, aromatic protons); 9.40 (s, 1H, CHO).

^{13}C-NMR ($CDCl_3$): 13.11 (CH_3, C_{18}); 69.97 (O-**C**H_2-C_6H_5); 118.63 (C N); 156.94 (C_3); 204.76 (C=O).

*3-Benzyloxy-17-hydroxy-16,17-secoestra-1,3,5(10)-triene-16- nitrile (**4**)*

3-Benzyloxy-17-oxo-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**3**, 1g, 2.68 mmol) was dissolved under heating in methanol (35 cm^3). To the cooled solution $NaBH_4$ (0.81 g, 22.2 mmol) was added portionwise. After stirring for 30 min at room temperature and refluxing for 20 min, the reaction mixture was diluted with water (100 cm^3). The white precipitate was filtered off, washed with water and dried, yielding 0.98 g (98.00%) of crude secocyanoalcohol **4**. The product was purified on a silica gel column (100 g, toluene-ethyl acetate

/2:1/), whereby 0.96 g (96.00%) of analytically pure 3-benzyloxy-17-hydroxy--16,17-secoestra-1,3,5(10)-triene-16-nitrile (**4**), mp 135—136°C, was obtained.

^{1}H-NMR($CDCl_3$): 0.95 (s, 3H, CH_3, C_{18}); 2.16 (d, 1H, OH); 2.54 (dd, 1H, H_a-C_{15}, J_{gem} = 16.08 Hz, $J_{I5a,\ 14}$ = 6.80 Hz); 2.68 (dd, 1H, H_b-C_{15}, $J_{15b,\ 14}$ = 7.15); 3.44 (q, 2H, C_{17}); 5.03 (s, 2H, O-C**H**$_2$-C_6H_5); 6.78—7.35 (group of signals, 8H, aromatic protons).

^{13}C-NMR ($CDCl_3$): 15.47 (CH_3, C_{18}); 69.87 (O-**C**H_2-C_6H_5); 70.90 (C_{17}); 119.94 (C N); 156.81 (C_3).

*3-Benzyloxy-17-maleyloxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**5**)*

Seco-cyanoalcohol **4** (1.16 g, 3.10 mmol) and maleic acid anhydride (0.91 g, 9.3 mmol) were dissolved in absolute pyridine (10 cm^3). The reaction mixture was intensively stirred at room temperature for 6 hrs, than poured into a mixture of ice and water and acidified with diluted HCl (1:1) to pH 5. From the formed suspension compound **5** was extracted with diethyl ether (3 x 30 cm^3), the extract was dried over anhydrous sodium sulphate and evaporated to dryness. The crude product was purified by column chromatography on silica gel (70 g, methylene chloride-methanol /1:1/) giving 0.58 g (39.73%) of analytically pure 3-benzyloxy-17-maleyloxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**5**), in the form of a pale yellow oil.

^{1}H-NMR ($CDC1_3$): 1.05 (s, 3H, CH_3, C_{18}); 2.95 (d, 2H, C_{15}); 3.98 (d, 1H, H_{17a}); 4.24 (d, 1H, H_{17b}, J_{gem}=11.61 Hz); 5.07 (s, 2H, O-C**H**$_2$-C_6H_5); 6.40 (d, 2H, HC=CH); 6.79—7.50 (group of signals, 8H, aromatic protons); 9.38 (s, 1H, COOH).

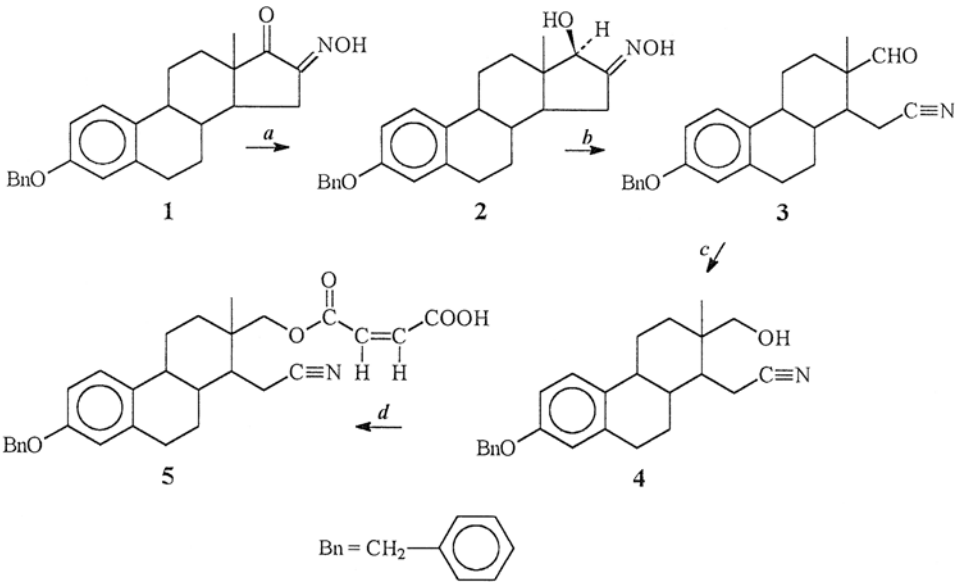
^{13}C-NMR ($CDCl_3$): 15.29 (C_{15}); 15.73 (CH_3, C_{18}); 69.54 (O-**C**H_2-C_6H_5); 72.32 (C_{17}); 119.15 (C N); 156.56 (C_3); 165.83 (COOH); 166.96 (COOR).

RESULTS AND DISCUSSION

As already mentioned, the aim of this paper was the synthesis of a new D-seco-estrone derivative possessing a free carboxyl function in the side chain at C-17, namely 3-benzyloxy-17-maleyloxy-16,17-secoestra-1,3,5(10)-triene-16--nitrile (**5**, Scheme 1).

Starting from oximinoketone **1**, oximinoalcohol **2** was obtained in a high yield by reducing of **1** with sodium borohydride in a mixture of methilene chloride, methanol and water.

The Beckmann cleavage of oximinoalcohol **2** was carried out under the action of *p*-toluenesulfonyl chloride in dry pyridine, yielding seco-cyanoaldehyde **3** in 95.4% yield. Further, by sodium borohydride reduction this compound was converted, in a high yield, into the corresponding derivative **4**. The esterification of seco-cyanoalcohol **4** was achieved by action of maleic acid anhydride in dry pyridine, yielding its maleic hemiester, 3-benzyloxy-17-maleyloxy-16,17-secoestra-1,3,5(10)-triene-16-nitrile (**5**) in the form of a pale yellow oil.



a $NaBH_4/CH_2Cl_2$, MeOH, 1% aq. KOH
b TsCl/Py
c $NaBH_4$/MeOH
d maleic acid anhydride/Py

Scheme 1

According to the described procedure, syntheses of other hemiesters of compound **4** are in progress, in order to study the influence of the side chain length at C-17 on the biological activity.

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СИНТЕЗА 3-БЕНЗИЛОКСИ-17-МАЛЕИЛОКСИ-16,17-СЕКОЕСТРА--1,3,5(10)-ТРИЕН-16-НИТРИЛА

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Резиме

У раду је описана вишефазна синтеза једног новог Д-секо-естронског деривата са карбоксилном функцијом у бочном низу, 3-бензилокси-17-малеилокси--16,17-секоестра-1,3,5(10)-триен-16-нитрила (**5**). Ово једињење је добијено естерификацијом секо-цијаноалкохола **4** са анхидридом малеинске киселине у апсолутном пиридину, док је једињење **4** добијено по познатом поступку из оксиминокетона **1**.

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OXIDOREDUCTASE IN RATS INTOXICATED WITH CADMIUM

ABSTRACT: There are a lot of literature data concerning the toxicity of cadmium on liver and kidney. The present work is concerning with the investigation of the effect of two plant extracts: *Alloe* and *Allium sativum* and an alcoholic Propolis extract on the behavior of the antioxidant systems. There were studied especially the activity of three enzymes: catalase, methaemoglobine reductase and superoxid dismutase consecutive an installed oxidative stress after cadmium administration in single doze.

The changes which appear in the protection enzyme's activity are different in the red blood cells and in liver. The natural extracts had a different influence on the enzymes activity. The alcoholic propolis extract was more efficient on catalase and superoxid dismutase activities in comparison with the *Allium sativum* extract. The last one had an important role in the activity of superoxid dismutase.

KEY WORDS: rat, oxidative stress, catalase, superoxid dismutase, methaemoglobin reductase

INTRODUCTION

It is well known the toxicity of cadmium on kidney and liver (Cenuşe et al., 1998). By the inhibition of the glutathion peroxidase activity, the lipid peroxidation was induced by the toxicity of cadmium (Chow, 1979; Cenuşe et al., 1998).

The antioxidant systems formed by glutathione peroxidase, glutathione reductase, catalase, glucozo 6 phosphat dehidrogenase had a major role in removing the lipid peroxides formed under cadmium influence (Cenuşe et al., 1998).

The present work is concerning with the investigation of the effect of some plant extracts on the behavior of the antioxidant systems consecutive an installed oxidative stress after cadmium treatment in single dose.

MATERIALS AND METHODS

The experiment was carried on 30 Wistar, one year old male rats having a body weight of 280—300 g divided in 5 batches. The first batch (L1) was the control and was daily administrated with 0.5 ml of physiologic serum per os. A single dose of 20 mg of cadmium was administered to the second batch (L2) and from the second day only physiologic serum. The third batch (L3) was administered with 20 mg of cadmium in single dose and from the second day of the experiment was treated daily with 0.5 ml of an *Alloe* aqueous extract for three weeks. The fourth batch (L4) was administered with 20 mg cadmium in single dose and from the second day of the experiment was treated daily with 0.5 ml of a Propolis alcoholic extract till the end of the experiment. The fifth batch (L5) was administered with 20 mg cadmium in single dose and from the second day till the end of the experiment was treated with an aqueous extract of *Allium sativum*. At the end of the experiment the rats were anaesthetized with chloroform and the blood was prelevated by heart puncture. There were also prelevated liver and kidney. There were determined the blood and liver catalase activity by Sinha method (Iordachescu, 1988), methaemoglobin reductase activity (Manta et al., 1976) and the blood and liver superoxide dismutase activity by the method with Nitroblue tetrazolium (NTB) (Iordachescu, 1988) by colorimetric methods (Chişu, 2000).

Data processing was done using the multiple interval test (Duncan), and testing was done for the level of significance of = 0.05.

RESULTS AND DISCUSSION

As a consequence of the administration of the natural extracts there were revealed important changes in the activity of the protection enzymes (Table 1, Fig. 1).

Tab. 1. — The activity values of catalase (Cat) (UI), superoxid dismutase (SOD) (UI) and methaemoglobin reductase (MetHb-red) (UI) in rats intoxicated with $CdCl_2$

Batch	Catalase blood	Catalase liver	SOD blood	SOD liver	Mhb-red % MHb
L1	70.5±2.44a	74.6±10.2a	4.55±0.12a	5.92±0.16c	17.5±2.56a
L2	53.5±3.6d	54.6±9.2b	1.86±0.15c	7.42±0.48b	13.97±1.02ab
L3	59.3±1.70c	67.5±4.25a	1.76±0.11c	3.37±0.17e	14.8±3.5ab
L4	64.6±3.70b	68.0±3.4a	3.30±0.12b	5.35±0.31d	11.97±0.67b
L5	58.5±2.75c	43.3±10.4c	4.55±0.37a	8.82±0.49a	13.7±0.45ab

* Values with the same letter do not differ significantly at = 0.05

There was observed a decreasing of both the blood and the liver catalase consecutive the administration of a single dose of 20 ppm Cadmium at the second batch (intoxicated and untreated with natural extracts). The same observations were made to the batches (L3—L5) which were treated with the natural extracts.

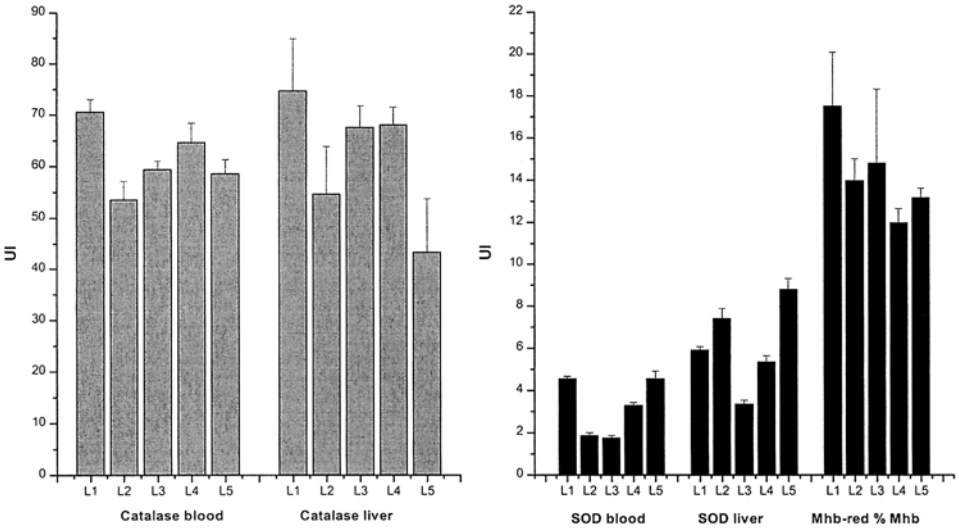

Fig. 1. — The activity values of enzimes in rats intoxicated with $CdCl_2$

After the treatment of the natural extracts there was observed an increasing of the blood catalase activity at the L3, L4 and L5 batches. The same observation could be made at the L3 and L4 in relation with the liver catalase activity but not at the 5th batch (L5) treated with *Allium sativum* aqueous extract, where there were registered lower values of the enzyme activity. This response of the blood catalase was a consequence at the induced oxidative stress (SO), observation which was confirmed by former literature data (G h e r g a r i u , 1997).

Between the administrated natural extracts, the most efficient one, on the blood catalase activity was the alcoholic propolis extract; the aloe and the *Allium sativum* extract had similar effect on the blood catalase activity.

The liver catalase had a different behavior at the treatment with different natural extracts. So, the aloe and the propolis extract have both the same effect (an increasing with 10% in comparison with the control) but at the treatment with the *Allium sativum* extract was registered a semnificative decreasing in both the blood and the liver catalase activity (—17% as the values registered at the control).

The superoxide dismutase activity (SOD) registered a decreasing at the treated batches in comparison with the control but were semnificative different between the treated batches. So, at the aloe treated batch (L3) the blood SOD activity registered a decreasing with 6% as the intoxicated and untreated batch (L2) but an increasing of the enzyme activity with 70—144% was registered at the propolis (L4) and *Allium sativum* (L5) treated batches. The liver SOD activity decreased at the aloe and propolis treated batches and increased at the *Allium sativum* treated one but registered similar values with the control.

As a consequence of the installation of the oxidative stress, the methaemoglobin reductase (metHb-red) activity decrease at the batches treated with

aloe and propolis, but not at the batch treated with *Allium sativum* aqueous extract, where there was registered a decreasing of the methaemoglobin reductase activity.

CONCLUSION

The changes which appear in the protection enzyme's activity are different in blood and in liver. After the oxidative stress was installed both the red blood cells enzymes and the liver enzymes registered a decreasing of their activity.

The natural extracts had a different influence on the enzymes activity. So, the alcoholic propolis extract was more efficient on the catalase and superoxid dismutase activities in comparison with the *Allium sativum* extract. The last one had an important role in the activity of superoxid dismutase.

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ОКСИДОРЕДУКТАЗА КОД ПАЦОВА ТРЕТИРАНИХ КАДМИЈУМОМ

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Резиме

Познато је да кадмијум утиче токсично на бубреге и јетру, као и да оксидоредуктазе могу утицати на токсичност кадмијума у организму. У раду су испитани ефекти неких биљних екстраката на понашање антиоксидативних система после стреса кадмијумом. Промене које су се појавиле као последица заштитне улоге ензима су различите у црвеним крвним зрнцима и јетри мада им је заједничко смањење инхибиторног дејства кадмијума. Природни екстракти имају различит утицај на ензимску активност. Тако је алкохолни прополис екстракт више утицао на каталазну и супероксиддисмутазну активност у поређењу са екстрактом из лука — *Allium sativum*.

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GEOCHEMISTRY OF IRON IN SOILS OF VOJVODINA, ŠUMADIJA AND NORTHERN POMORAVLJE

ABSTRACT: Iron is one of the most common elements in the Earth's crust and it is fourth on the list of abundance after oxygen, silicon and aluminum. It plays an important role in the biosphere. In plants it is necessary for the formation of chlorophyll, while in animals it acts in transferring oxygen from air or water to animal tissue.

During a large-scale sample collection, a regular orthogonal 10 x 10 km grid has been used to avoid bias in site location. From the set of about 3000 samples from Vojvodina, Šumadija and Northern Pomoravlje, a selection of samples (from the arable layer 0—25 cm) has been taken to represent the most important soil types. Total iron content has been determined by the atomic absorption spectrophotometric method (AAS). The soil samples have also been assayed for metal on a phase-specific basis following procedures according to the EC protocol. Spatial distribution of iron content over the investigated area has been presented in a pedogeochemical map.

The presented results have shown a wide range of iron contents, from 0.73 to 10.86% Fe. Statistical analysis of the results obtained from 103 samples has shown an average value of 4.06% Fe, with the standard deviation of 1.682 and the coefficient of variation of 41.49%. Iron contents lower than 2.10% have been found in 4.32% of the samples, medium and average values (2.10—4.97% Fe) have been found in 55% of the samples, values higher then the average have been found in 26.3% of the samples and the contents higher than 7.86% have been found in 13.96% of the samples.

Arenosols and rigosols developed on aeolian sands have shown the lowest levels of total iron, from 0.73 to 1.82% Fe. On the other side, ranker developed on serpentinite has shown maximum contents, between 8.53 and 10.86% Fe. Soils developed either on loess or tertiary clay parent rocks (halomorphic soils, some marshy humogleys and vertisols) have shown a wider range of results (1.33—4.65% Fe) with a shift of results towards average values.

The majority of the investigated soils that have fallen within the group between 3.00 and 4.96% Fe were represented by pseudogley, eugley, luvisol, fluvisol, eutric cambisol, ranker and rendzina. Semigley and chernozem have shown a wider range of distribution of results, from 2.18 to 7.72% Fe. Generally, the analyzed soils have shown lower average results compared with the available literature data.

Chemical speciation has shown that an average iron content of 84.24%, with the range from 70 to 92%, was primarily associated with residual forms bound to the silicate lithogenic fraction. An average of 12.69%, with the range from 6 to 26%, has been found as Fe-Mn-oxide/hydroxide fraction. Organic-matter-bound iron (1—9%) and exchangeable and carbonate-bound iron (0.09—1.92%) have been present to a lesser extent.

KEY WORDS: iron, soil, minerals, chemical speciation, pedogeochemical map

INTRODUCTION

Iron is one of the most common elements in the Earth's crust and it is fourth on the list of abundance after oxygen, silicon and aluminum. It is found as the main constituent in all classes of minerals. In igneous rocks, it is found associated with magnesium in silicates: olivine, pyroxene, amphibole and mica. Minor amounts of pyrite, pyrrhotite, magnetite and ilmenite are also found in these rocks. In exogenic processes of surface weathering, in soils and sedimentary rocks, iron is oxidized to goethite, hematite, siderite, or other secondary iron silicates such as chamosite, glauconite, greenalite or stilpnomelane.

In surface environments iron is very quickly oxidized and changed from the ferrous to the ferric state in minerals. The susceptibility of iron to surface oxidation is one of the major characteristics of its geochemical cycle. Ferric oxide may oxidize organic mater and at the same time become reduced to the ferrous state, but again it could be reoxidized by oxygen from the atmosphere. So iron acts as a catalyst of carbon cycling in nature and their cycles are closely bound to the oxygen cycle. Iron plays an important role in the biosphere. In plants it is necessary for the formation of chlorophyll, while in animals it acts in transferring oxygen from air or water to animal tissue.

Tab. 1. — Average iron content (%) of various rocks and environments

Earth's crust	5.00	Limestone	0.86
Ultrabasic rocks	9.85	Clay and shales	4.72
Basic rocks	8.56	Soils	3.80 (0.3—55%)
Intermediate rocks	5.85	Biosphere	0.010
Granite	2.70	River water	0.005
Sandstone	2.80	Sea water	0.0015

From: Beus (1975), Bowen (1979), Vinogradov (1962)

An average content of iron in the Earth's crust is 5%. Igneous ultrabasic rocks may contain up to 9.85% of iron, while many acidic rocks contain no more then 1%, but on average 2.70% of total iron. Iron is a typical siderophile element, which is concentrated in early magmatic crystallizates such as magnetite, ilmenite or chromite. Similar ionic radii of Fe and Mg allow continuous substitution of elements in feromagnesian silicate minerals, olivine, pyroxenes and amphiboles. In acid rocks significant amounts of iron enter into the structure of biotite.

In exogenic environments, during rock weathering and sedimentation, large quantities of ferric oxide or hydrated ferric oxide or hematite and goethite are formed, which are trapped in subtropical and tropical ferruginous soil and laterites. In temperate climates iron is involved in the formation of clay minerals and some soils may contain from 0.3—55% Fe or an average of 3.80%. Also, clays and shales may contain an average of 4.72% Fe. Under acidic or reducing conditions of some organic humose soils, iron may transform into ferrous-bicarbonate solution. Some quantities of iron may move to surface

streams as colloidal ferric oxide. Ferrous carbonate will precipitate when carbon dioxide is removed, whereas ferric oxide may precipitate from solution as a result of a change in the oxidation potential. Therefore, small deposits of iron carbonate or iron oxide are formed in swamps and lakes from weathering solutions.

Iron content of live tissues is as low as 0.010%, indicating its low biophile activity, which is very similar to titanium and other oligoelements. Concentrations of 10 to 200 mg/l are toxic to plants, while amounts of 200 mg/day are toxic for men.

MATERIAL AND METHODS

During a large-scale sample collection for a project financed by the Ministry of Science and Technology, a regular orthogonal 10 x 10 km grid was used to avoid bias in site location. From the set of about 3000 samples a collection of samples from Vojvodina, Šumadija and Northern Pomoravlje was taken to represent the most important soil types. Selection was restricted to the samples from the arable layer with 0—25 cm depth.

After collection, samples were air-dried and sieved to pass through a 2 mm sieve. A 50 g subsample of soil was obtained by conning and quartering and then ground, to less then 150 mesh, in an all-agate planetary ball--mill. Sample digestion was carried out by hydrofluoric-perchloric acid mixture ($HF+HClO_4$). Iron content was determined by the atomic absorption spectrophotometric method (AAS), on PERKIN-ELMER 373 instrument.

The soil samples were sub-sampled and assayed for metal on a phase--specific basis following procedures for soils, outlined by Tessier, Campbell and Bisson (1979), which was carried out according to the EC protocol. The extraction procedures have partitioned soil-associated metal into four fractions:

1. exchangeable and carbonate-bound metal ions
2. metal ions bound to Fe-oxides/hydroxides
3. organically-bound metal ions
4. residual metal ions bound to silicate lithogenic fraction

Histograms of the frequency distribution and summary consisting of the mean, mode, percentiles, quartiles and range in a log-normal distribution of iron were made by means of a GENSTAT program package. The classes used to represent the data on the map were chosen from the box and whiskers analysis, according to Kuerzl (1986). Thus, the raster map showing the distribution of iron has 5 classes and it was computer-generated using the UNIRAS subroutine. Each square on the map represents the results from one 10 by 10 km grid scale unit.

RESULTS AND DISCUSSION

The results of AAS analysis of total iron in the soils (A horizon) are presented in Table 2 and Figure 1. Spatial distribution of iron content over area and within soils investigated is presented in Figure 2.

Tab. 2. — Statistical analysis of iron content (%) in various soil types

Soil type	N	Min	Q_1 25%	Md 50%	Q_3 75%	Max	Average	St. dev.	CV %
Arenosol	4	0.73	1.06	1.24	1.42	1.82	1.32	0.586	44.39
Vertisol	4	1.47	1.75	3.07	3.46	4.65	3.20	1.218	42.15
Halo-morphic	5	1.81	1.93	2.95	3.15	3.39	2.72	0.685	25.18
Humogley	7	1.33	2.27	2.98	3.45	4.20	2.93	0.893	30.48
Pseudogley	5	2.32	2.48	3.01	3.12	3.57	2.97	0.468	15.76
Eugley	5	2.59	2.67	3.16	3.71	4.20	3.05	0.908	29.77
Luvisol	5	2.96	3.06	3.56	3.68	3.90	3.42	0.397	11.61
Fluvisol	8	2.90	3.00	3.20	4.33	4.85	3.58	0.802	22.40
Eutric cambisol	14	2.85	3.36	3.81	4.14	5.01	3.82	0.552	14.45
Ranker	5	2.36	1.56	3.26	4.28	6.15	3.34	1.874	56.11
Semigley	20	2.50	3.53	4.98	5.98	7.82	4.99	1.630	32.67
Chernozem	18	2.18	4.05	5.45	6.26	7.10	4.87	1.853	38.05
Ranker (serp.)	3	8.53	8.92	9.64	10.21	10.86	9.75	1.934	19.84
All soils	103	0.73	3.00	3.68	4.97	10.86	4.06	1.682	41.49

The analysis of the results presented in diagrams in Figures 1a and 1b and Table 2 showed a wide range of iron contents, from 0.73 to 10.86% Fe. The statistical analysis of the results obtained from 103 samples showed an average value of 4.06% Fe, median of 3.38 and mode of 3.20, with the standard deviation of 1.682 and the coefficient of variation of 41.49%, indicating a slight shift of the results for the investigated area towards lower iron contents.

The classes presented in the map were calculated from the log-normal box and whiskers analysis by using lower (3.00%) and upper (4.97% Fe) quartile, as proposed by K u e r z l (1986). The lower and upper percentile-whiskers were obtained in the same manner, thus the map provided in Figure 2 has 5 classes. The iron content lower than 2.10% was shown by 4.32% of the samples, medium and average values (2.10—4.97% Fe) were shown by 55% of the samples, values higher than the average were shown by 26.3% of the samples and the content higher then 7.86% was shown by 13.96% of the samples.

Arenosols and rigosols developed on Deliblato and Subotica aeolian sands showed the lowest levels of total iron, from 0.73 to 1.82%. On the other side, ranker developed on serpentinite, an initial undeveloped soil with strong influence of the parent rock, showed maximum contents, between 8.53 and 10.86% Fe.

Soils developed on loess or tertiary clay parent rocks (halomorphic soils, some marshy humogleys and vertisols) showed a wider range of results (1.33—4.65% Fe) with a shift of results towards average values.

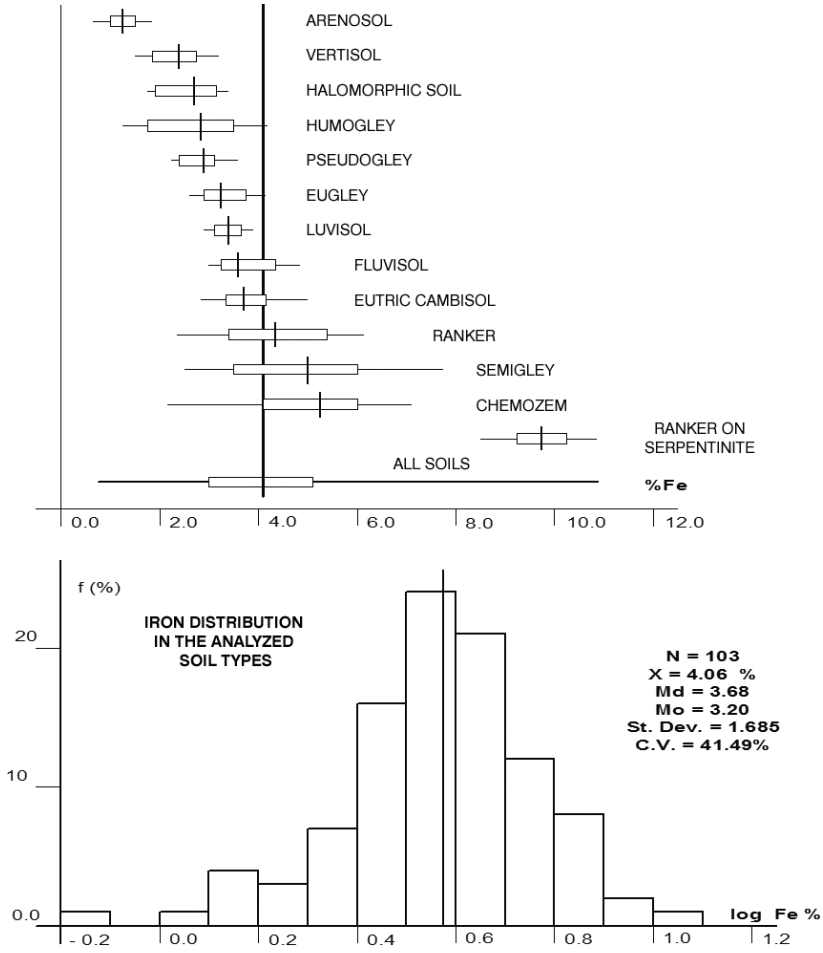

Fig. 1. — Statistical review of iron content in the analyzed soil types

The majority of the investigated soils that came within the group between 3.00 and 4.96% Fe were represented by pseudogley, eugley, luvisol, fluvisol, eutric cambisol, ranker and rendzina. Semigley and chernozem showed a wider range of distribution of results, from 2.18 to 7.72% Fe, the results scattering towards lower values. Generally, our soils showed lower average results as compared with the available literature data.

The pedogeochemical map given in Figure 2 presents the spatial distribution of total iron. It can be seen in this map that the lowlands of northern Vojvodina, which make the largest part of the Bačka region, showed minimum to average values, while the hilly areas around the Fruška Gora Mountain, some parts of Šumadija and Pomoravlje showed high to maximum iron contents. Wide areas of Banat and large parts of western and central Šumadija showed average iron contents, but mottled sporadically with small patches of lower or higher contents.

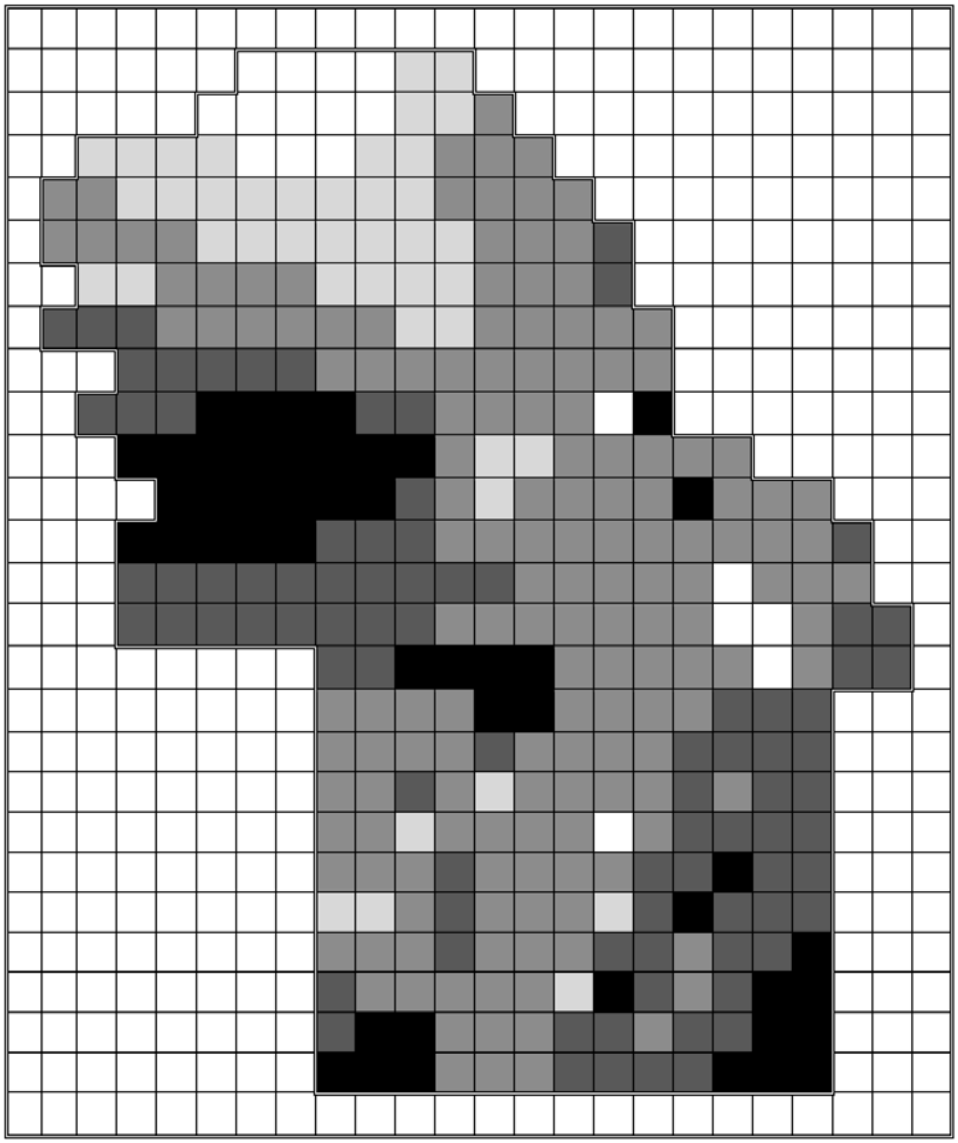

Fig. 2 — Pedogeochemical map of iron distribution in the analyzed soils (square grid 10 x 10 km)

Class limit (Fe %)	0.73—2.10	2.10—3.00	3.00—4.96	4.96—7.86	7.86—10.86	
Raster value						Total
All soils (km2)	1800	4200	15600	8700	4700	35000
Vojvodina (%)	7.90	16.75	43.72	20.00	11.63	100
Šumadija and Pomoravlje (%)	0.74	4.44	45.92	32.60	16.30	100

Minimal values were noticed in Bačka and Banat, in areas of Subotica and Deliblato, respectively, where arenosols had developed on silicate-carbonaceous aeolian sands. Low iron contents were also observed over loess plateaus and terraces around aeolian sands where some chernozem and semigley are developed, as well as on wider part of loess terraces in middle Banat, with halomorphic soils, humogley and semigley developed on alluvial and loessial derivatives.

Areas around the Fruška Gora Mountain and the region of Srem showed increased iron contents, in consequence to high chlorite and biotite contents in the clay fraction of rendzina, rankers on vulcanite and serpentinite, chernozem, semigley, as well as eugley-marshy soils and fluvisols along the Sava River.

South of the Sava and Danube Rivers, in the regions of Šumadija and Pomoravlje, average iron contents could be found in parts where pseudogley, eutric cambisol and vertisol had developed on neogene loam and clay sediments. Fluvisols and semiglays of Pomoravlje showed increased iron contents.

Maximum average iron contents were found in rankers developed on serpentinite, as well as in some eutric cambisols and rendzine, which had developed on the hills and mountains of Šumadija, on schists, cretaceous flish, neogene clay sediments, andesite and their tephra.

These distribution patterns are an expression of the parent rocks on which the soils had developed. In Vojvodina, loess and neogene sediments and their derivatives such as alluvial sediments and aeolian sands have influenced an even spread of iron in the soils developed on such parent materials. In Šumadija and Pomoravlje on the other hand, a number of soils had developed on parent rocks differing widely in age, weathering history and petrographic composition. Alluvial sediments can be found along the river valleys, loess, neogene and Mesozoic sediments and Paleozoic schists can be found in hilly areas, while andesite, dacite, granite and serpentinized peridotite are present in mountain regions.

In order to establish a relationship between the mineralogy and pedology of the soils on one side and base cations and iron on the other, a multivariate cluster analysis was carried out. Correlation between variables was performed according to Pearson and clustering was done by the weighted pair-group average (WPGA) method.

The resulting dendrogram is presented in Figure 3. Minimal coefficient of correlation for 103 degrees of freedom and the significance level of using these parameters, the 20 variables were clustered into 3 groups.

The first group showed the highest clustering of carbonates and soil reaction (pH). In this group of minerals, calcite and dolomite (Ca and Mg bases) were closely correlated with soil pH followed by the associations of mixed-layer-silicates (MSS) of (14—14) type or chlorite — smectite and (10—14) type or ilite-smectite as well as humus content in soil. The group MSS had been formed during the weathering of primary minerals in the presence of humic acids, as a transition from primary chlorites and mica to clay fraction illite and chlorite. The latter minerals, contain significant amounts of Mg and Fe, respectively.

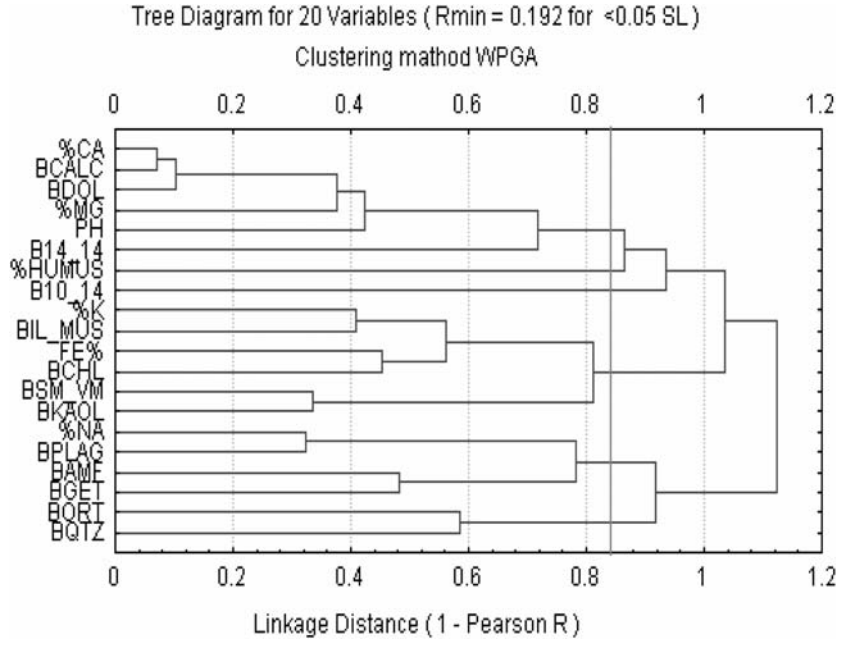

Fig. 3 — Dendrogram of cluster analysis of mineralogy and chemistry of the analyzed soils

The second group comprised hydrated alumosilicates and K% and Fe% contents. There were two subgroups, the first one including clay minerals kaolinite, smectite and vermiculite, the second one including micas, illite and chlorite, with K% bound to the first two minerals and Fe% bound to the last mineral. The mineralogical analysis of the soils showed the presence of small amounts of mica — biotite, illite of the phengite type, vermiculite, smectite of the ferro-beidelite type and chlorite from the ripidolite-chamosite mineral series.

The third group generally comprised primary minerals and it could be divided into two subgroups. The first subgroup showed significant correlations of Na% contents and plagioclase, which is normal since most feldspars present in the soils were of the acid plagioclase group (albite-ologoclase). This group included also goethite and alkali amphibole. The second subgroup was represented by quartz and orthoclase. Quartz was the most common mineral in the analyzed soils. While it is a stable mineral in the prevailing temperate climate, the minor amounts of orthoclase that accompanied quartz are less stable and in close association with parent rocks. The group of primary minerals showed a slight negative correlation, without statistical significance, with the other two groups, i.e., clays and carbonates.

In order to ascertain the bioavailability of iron, an additional sequential selective extraction of 20 selected samples of the most common soils was carried out according to the EC protocol. Chemical speciation of iron in the soils and the distribution of exchangeable, oxide, organic and residual forms are presented in Table 3 and Figure 2.

Tab. 3. — Statistical analysis of chemical speciation of Fe (%) in 20 soil samples

	Min	Q_1 25%	Md 50%	Q_3 75%	Max	Average	St. dev.	C.V. %
Exchangeable	0.09	0.18	0.47	0.86	1.92	0.61	0.54	89.84
Oxide	6.02	7.75	9.74	16.46	25.67	12.69	6.38	50.31
Organic	1.42	1.75	2.05	2.48	8.04	2.50	1.49	59.53
Residue	69.68	80.18	86.49	90.04	92.21	84.24	7.14	8.48

From Table 3 and Figure 2 it is evident that an average iron content of 84.24%, with the range from 70 to 92%, was primarily associated with residual forms bound to the silicate lithogenic fraction. An average of 12.69%, with the range from 6 to 26%, was found as Fe-Mn-oxide/hydroxide fraction. Organic-matter-bound iron (1—9%) and exchangeable and carbonate-bound iron (0.09—1.92%) were present to a lesser extent.

Maximal concentrations of residual iron were found in the soils with high content of chlorite and minor concentrations of biotite, indicating that these silicate minerals have suffered the least transformations during argilogenesis. Due to the relatively semi-arid climate with about 600 mm of annual rainfall and high summer temperatures, transformations of minerals in chernozems were relatively minimal in relation to the other investigated soils.

However, as the rainfall increased, so increased its influence on the mineral weathering and there occurred a gradual decrease in residual iron with slight increases in other „free" forms of iron, such as that in goethite and occasionally in hematite. The largest changes were found, as expected, in luvisols and dystric cambisols, in which „free oxides" reached 20—25% of the total iron content. All of the investigated soils were grouped between these two values. It is interesting to mention that the hydromorphic soils shown an increased content of „free oxides" in relation to the automorphic soils.

The organic-bound iron was found in the concentrations between 1.4 and 8% and it occurred unanimously in all soils. Minimal concentrations (1.4—2.2%) were found in the automorphic soils, chernozem and eutric cambisol, which had developed on well-drained substrates in aerated environments. However, in the fluvisol and various gleysols, which had low soil aeration and amplified concentration of organic matter because they had been saturated for a short or long period of time with either surface or underground waters, the contents of organic-bound iron were increased (2—3%). Maximal concentrations of organic-bound iron were found in rankers developed on serpentinite and acid forest soils, which could be attributed to the decay of litter rich in organic matter and the ratio of humic to fulvic acids in these soils.

The contents of exchangeable and soluble forms of iron (amorphous ferihydrite or Fe carbonate) were quite low, with the exception of pseudogley, semigley and fluvisol that had developed on alluvial planes and loess terraces, in which the total iron content was slightly higher, ranging between 1 and 2%.

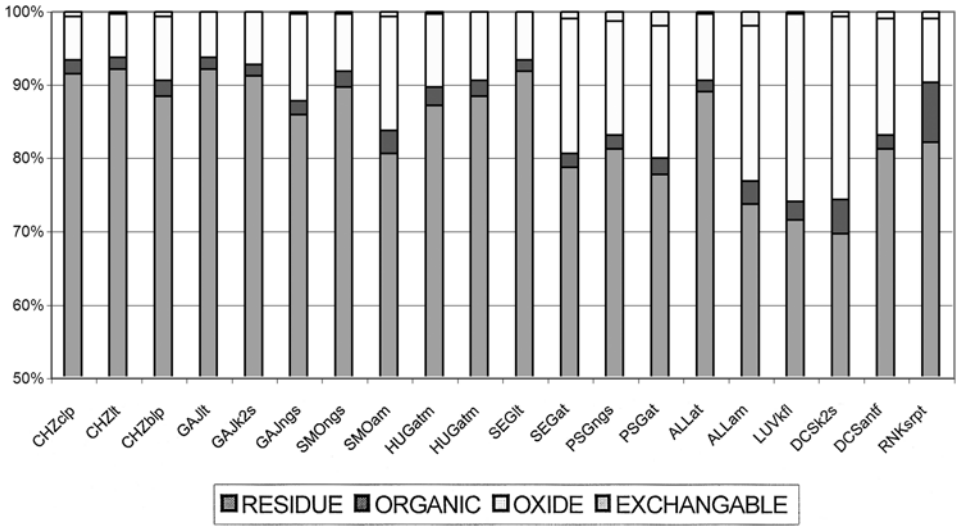

Fig. 4 — Chemical speciation of iron in the investigated soils

CONCLUSIONS

The comprehensive investigation of iron in the soils from Vojvodina, Šumadija and Northern Pomoravlje, the main agricultural regions of the country, showed a wide range of concentrations of the investigated element. Based on the obtained results, the following conclusions were drawn.

The lowest contents of iron were found in the northern parts of the regions of Bačka and Banat, in Subotica and Deliblato aeolian sands, where arenosols had developed on silicate-carbonate sand dunes. Low average contents were also registered for the loess plateaus and terraces around the aeolian sands, where chernozem and semigley had developed, as well as for a part of the wide loess terrace in central Banat, where humogley, semigley and saline — halomorphic — soils had developed.

South of the Sava and Danube Rivers, the areas of Šumadija and Pomoravlje with pseudogley, eutric cambisol and vertisol developed on Neogene loamy and clayey sediments showed average contents of iron. In the region of Pomoravlje, increased iron contents were found in fluvisols and semigleys developed on the alluvial plain of the Morava River.

Enhanced iron contents were found in a wider area around the Fruška Gora Mountain and in the area of lower Srem. They were due to high concentrations of chlorite and mica (biotite) in the clayey fractions of rendzinas, due to rankers developed on volcanic rocks as well as due to semigley, fluvisol and eugley in marshy areas along the Sava River.

Maximum average contents of iron were associated with chernozem, eutric cambisol and semigley on the slopes of the Fruška Gora Mountain, with ranker developed on serpentinite, and with eutric cambisols and rendzinas,

which had developed at the highest points of the hilly Šumadija region, on schists, cretaceous flysch, neogene sediments and andesite-effusive rocks and their tuffs.

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ГЕОХЕМИЈА ГВОЖЂА У ЗЕМЉИШТИМА ВОЈВОДИНЕ, ШУМАДИЈЕ И СЕВЕРНОГ ПОМОРАВЉА

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Резиме

Гвожђе је један од најчешћих елемената у Земљиној кори, четврти у низу по заступљености иза кисеоника, силицијума и алуминијума, где се налази као главни градивни елемент у свим класама минерала. Оно игра веома важну улогу у биосфери. У биљкама је неопходно за стварање хлорофила, а код животиња служи у трансферу кисеоника из ваздуха или воде до виталних органа.

У току вишегодишњег пројекта извршено је детаљно узорковање терена Војводине, Шумадије и Поморавља, по квадратној мрежи са основом 10 x 10 km. како би се избегла грешка узорковања. Сакупљено је 3000 узорака а од тог броја узорака извршена је селекција узорака из орничког слоја са дубине од 25—30 cm. Садржај гвожђа одређен је методом атомске апсорпционе спектрофотометрије (ААС).

Анализа представљених резултата показује интервал појављивања гвожђа од 0.73 до 10.86% са скоро правилном лог-нормалном дистрибуцијом резултата.

Просечни садржај гвожђа износи 4.06%, са стандардном девијацијом 1.682 и коефицијентом варијације 41,49%. Географска дистрибуција садржаја гвожђа приказана је на педогеохемијској карти у тексту. Наша земљишта показују генерално ниже просечне садржаје гвожђа у поређењу са литературним подацима.

Најнижи садржаји гвожђа констатовани су на просторима Бачке и Баната, у Суботичкој и Делиблатској пешчари, где су развијени ареносоли на силикатно-карбонатним песковима. Нижи до просечни садржаји такође прате лесне платое и терасе око пешчара, где су развијени чернозем и семиглеј, као и део шире ласне терасе у средњем Банату, где су развијени хумоглеј, семиглеј и слана — халоморфна — земљишта.

Јужно од Саве и Дунава, простор Шумадије и Поморавља показује просечне садржаје гвожђа, где су развијени псеудоглеј, еутрични камбисол и вертисол на неогеним иловастим и глиновитим седиментима. У Поморављу са флувисолима и семиглејем на алувијалној равни Мораве појављују се повишени садржаји гвожђа.

Шири простор око Фрушке горе и подручје Срема показују повећане садржаје гвожђа, што је последица више концентрације хлорита и лискуна (биотита) у глинама рендзина, ранкера на вулканитима и серпентинитима, чернозема, семиглеја, као и еуглејно-мочварних земљишта и флувисола око реке Саве.

Највише просечне садржаје гвожђа показују чернозем, гајњача и семиглеј на обронцима Фрушке горе, затим ранкери развијени на серпентинитима, као и неки еутрични камбисоли и рендзине, који су развијени на шкриљцима на највишим котама Шумадије.

У циљу утврђивања дистрибуције хемијских облика гвожђа у земљиштима урађена је хемијска специјација по ЕЕЦ протоколу. Добијени резултати показују значајне варијације у појединим облицима као и у самим земљиштима. Генерално посматрано, гвожђе је у резидуалном делу везано за силикате (69—92%). Значајније количине гвожђа јављају се у облику „слободних оксида", најчешће ферихидрита, гетита и ређе хематита (6—25%), затим као органски везано гвожђе (1.4—8%) и најмање као приступачно-изменљиво и за карбонате везано гвожђе (0.1—1.9%).

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SEED PROTEIN VARIABILITY IN SAFFLOWER

ABSTRACT: Total seed proteins in two safflower species (*Carthamus tinctorius* L. and *C. lanatus* L.) have been separated by the SDS-PAGE method. Their molecular masses ranged from 120 to 20 kDa. All *C. tinctorius* genotypes under study exhibited identical electrophoretic patterns which differed from the pattern exhibited by the wild species *C. lanatus* in the number and position of protein bands. Differences in protein profiles occurred in regions around 60 kDa, from 43 to 36 kDa and around 30 kDa. Statistically significant differences in seed protein content were found among safflower genotypes from different countries as well as among genotypes from the same country but from different sites. The highest seed protein content was found in a genotype originating from the USA.

KEY WORDS: electrophoresis, safflower, seed proteins

INTRODUCTION

Safflower is an annual plant from the family *Compositae*. It is referred to in literature as cultivated (*Carthamus tinctorius* L.) and wild species (*C. lanatus* L.). Africa and Asia are mentioned as places of origin, with the Mediterranean as the main region of distribution. Safflower is an important aromatic and medicinal plant. Due to high oil content in seed, it is also cultivated as an oil crop. Cartamine ($C_{21}H_{22}O_{11}$), a coloring substance found in the flowers of this plant, is used as colorant in food-processing industry (Hegi, 1954; Rapoti and Romvari, 1972).

Kiss et al. (2001) found significant differences in the contents of total fatty oil, oleic and linoleic acids in safflower seeds collected in different countries and those collected from different sites in the same country.

The importance of evaluation and preservation of wild and less cultivated plant species was recognized during the 20th century. Broad source of useful genes is essential for successful breeding process. The first step is to characterize genetic resources and to evaluate polymorphism in germplasm. In the next phase, useful genes from wild relatives are introduced into cultivated material by conventional breeding methods or methods of genetic engineering. Within the scope of safflower breeding programs, isozyme variability (Zhang, 2001) and PCR-based RAPD analysis (Yazd-Isamadi et al., 2001) were carried out to detect genetic diversity in the genus *Carthamus*. The results showed that two classes of markers could be useful for the classification of germplasm and identification of safflower landraces.

The aim of this paper was to evaluate seed protein variability in different *Carthamus tinctorius* genotypes and in *C. lanatus*.

MATERIAL AND METHODS

The study was carried out on safflower cultivated genotypes (*Carthamus tinctorius* L.) and its wild relative (*C. lanatus* L.). The experimental material, obtained from the gene bank of the Institute of Agrobotany, Tapioszele, Hungary, included 15 cultivated genotypes from different countries and one wild safflower (Table 1):

Tab. 1. — Analyzed safflower genotypes

Sample	Species	Seeds origin	Sample	Species	Seeds origin
1	*C. tinctorius*	Hungary, Nyiregyhaza	9	*C. tinctorius*	Germany
2	*C. tinctorius*	Hungary, Nyiregyhaza	10	*C. tinctorius*	Hungary, Nyiregyhaza
3	*C. tinctorius*	Romania, Nagyvarad	11	*C. tinctorius*	India
4	*C. tinctorius*	England	12	*C. tinctorius*	Hungary, Cegled
5	*C. tinctorius*	USA	13	*C. tinctorius*	Germany
6	*C. tinctorius*	Germany	14	*C. tinctorius*	Hungary, Mateszalka
7	*C. tinctorius*	Slovakia	15	*C. tinctorius*	Germany
8	*C. tinctorius*	Poland	16	*C. lanatus*	Germany

SDS-PAGE was carried out with the buffer system described by Laemmli (1970). Embryoless mature seeds (20 mg/0.5 ml) were suspended in a sample buffer of TRIS-HCl (pH 6.8), 2% SDS, 5% mercaptoethanol and 20% glycerol. After shaking for 2 h at room temperature, the suspension was boiled for 2 min. After centrifugation (5000 g) for 5 min at room temperature, 50 µl of protein solution were applied to a gel that consisted of 11.84% acrylamide and 0.16% bisacrylamide (1.35% C). Electrophoresis was carried out in vertical slab gels (180x160x1.5 mm). Two gels were run at 40 mA for 30 min and

80 mA for 3 h. Proteins were stained with 0.02% Coomassie Brilliant Blue R 250 in 40% methanol, 10% glacial acetic acid.

Seed protein content was calculated by multiplying total nitrogen content with factor 6.25. Total nitrogen content was determined by the micro-Kjeldahl method. The results were statistically processed for LSD.

RESULTS AND DISCUSSION

Total seed proteins were separated by the SDS-PAGE method. Their molecular mass ranged from 120 to 20 kDa. Four regions could be distinguished in gel slabs, each possessing characteristic arrangement and number of bands. All cultivated genotypes of *C. tinctorius* had identical electrophoretic patterns, which differed from that found in the wild species *C. lanatus* (Figure 1).

In the region around 60 kDa, the cultivated genotypes had two bands and the wild species three. Most striking differences in both the number and arrangement of bands occurred in the region from 43 to 36 kDa (Figure 2). The cultivated genotypes had eight bands and the wild species nine, all of which

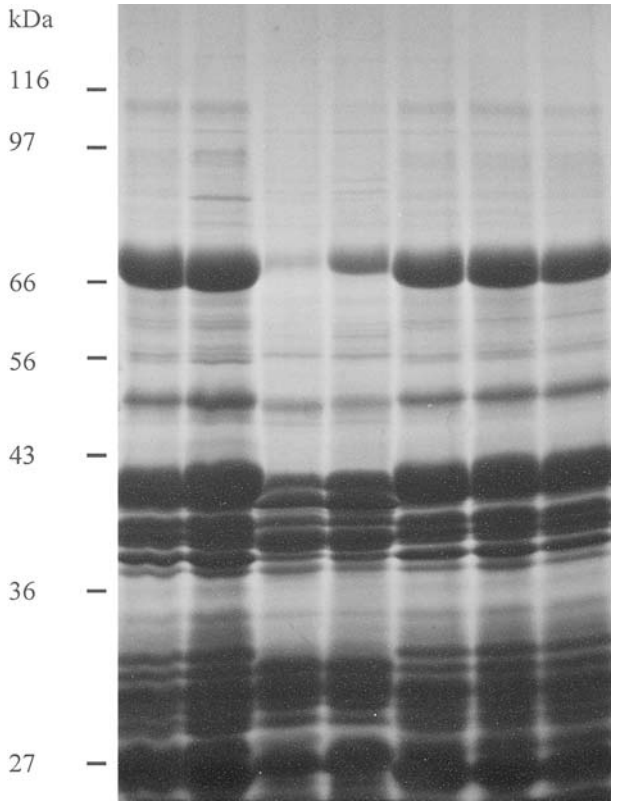

Fig. 1. — Total seed protein electrophoregram of safflower genotypes (from left to right: *C. tinctorius* genotypes 1, 12; *C. lanatus; C. lanatus; C. tinctorius* genotypes 3, 4, and 5)

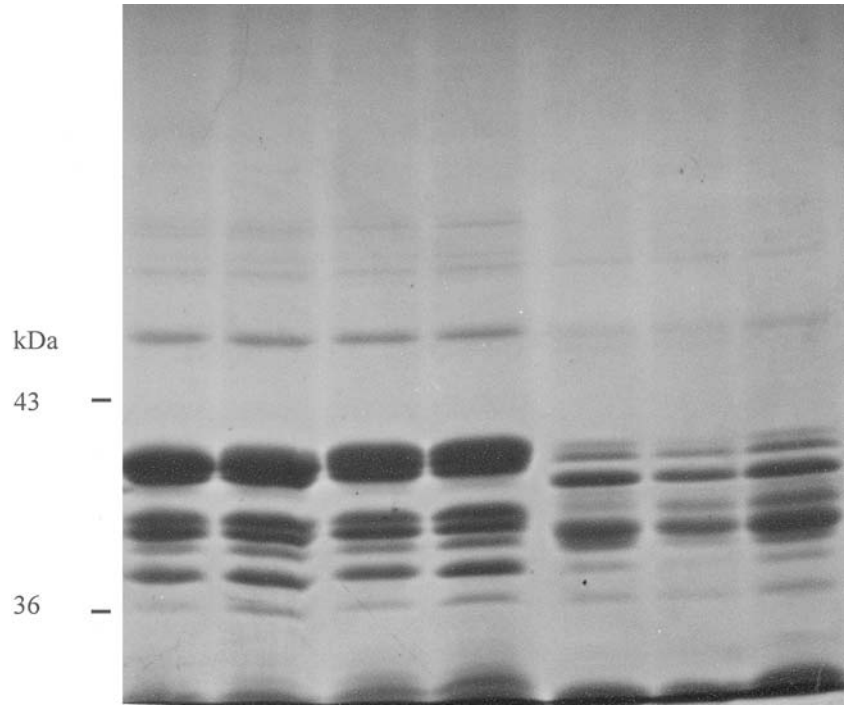

Fig. 2. — Total seed protein electrophoregram of safflower genotypes, major differences between two species were observed in the range of 43—36 kDa

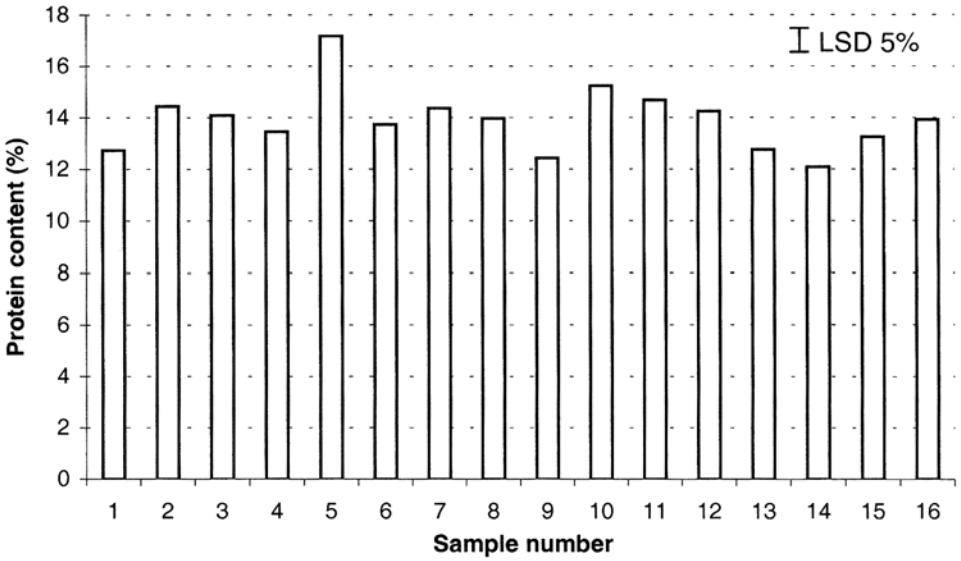

Fig. 3. — Total seed protein content of safflower genotypes

differed in intensity and mobility. Differences in the number and arrangement of bands also existed in the region of 30 kDa. The cultivated genotypes had 6 bands, the wild species four.

Significant differences in seed protein content were found among safflower genotypes from different countries as well as among genotypes from different sites in the same country (Figure 3). The highest protein content was found in the sample from the USA. In an earlier study, Kiss et al. (2001) had found the highest oil content in the same sample. Our result was thus unexpected because a negative correlation typically exists between protein and lipid contents in seed. Low carbohydrate contents in safflower seed samples form the USA seem to be the only explanation for this. Regarding the seed protein content in the wild relative, it was in agreement with the average content of the cultivated genotypes.

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ВАРИЈАБИЛНОСТ ПРОТЕИНА СЕМЕНА ШАФРАНА

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Резиме

Укупни протеини семена две испитиване врсте шафрана (*Carthamus tinctorius* L. и *C. lanatus* L.) су раздвојени методом SDS-PAGE и њихова молекулска маса се кретала од 120 до 20 kDa. Сви гајени генотипови врсте *C. tinctorius* имали су идентичне електрофоретске путање које су се разликовале по броју и положају протеинских трака од дивље врсте *C. lanatus*. Разлике у протеинским профилима јављале су се у регионима: око 60 kDa, од 43—36 kDa и 30 kDa. Утврђена је статистички сигнификантна разлика у садржају протеина у семену шафрана пореклом из различитих земаља, као и између семена пореклом из исте земље са различитих локалитета. Највећи садржај протеина утврђен је у семену пореклом из САД.

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GENETIC VARIABILITY AND DIVERSITY OF CORN BREEDING MATERIAL ORIGINATING FROM DOMESTIC AND FOREIGN POPULATIONS DETERMINED ON THE BASIS OF BIOCHEMICAL-GENETICAL MARKERS

ABSTRACT: Isozymes can serve as genetic markers and their number should be large enough in order to make the coverage of genomes as complete as possible and in order to use these methods for gene marking for required agronomic traits. These markers are the products of 21 mapped genes, which is relatively reliable number for their application in mapping for certain agronomic traits. Genetic variability and diversity are significant for populations and for selfpolinated lines as basic material in breeding and creation of new corn hybrids. For that reason, several groups of corn populations of different origin were analyzed. Two groups of Yugoslav populations, Italian, Portuguese and French collections were assessed on the basis of detected alleles of 21 loci and standard genetic distances between genotypes. Yugoslav corn collections had shown high heterozygosity, on the basis of isozymes as gene markers. Genetic diversity of Italian populations was pronounced on the basis of some loci, and the Portuguese populations had more polymorphic and more heterozygous loci than French populations. Inter-genetic variability between populations and their geographical location are very important in breeding crops for creation of heterosis.

KEY WORDS: Genetic variability, diversity, genetical markers, isozymes, corn, populations.

INTRODUCTION

Biochemical, physiological and genetical studies should be connected at the level of gene, i.e. molecular level. Research and application of new molecular methods should, above all, find its way in breeding and seed science. Application of molecular markers is multilateral and is used for:

— identification of genes for desirable agronomic traits
— identification of genes for disease resistance
— identification of genes for qualitative seed traits

Role of molecular markers in breeding is based on „linkage" of gene markers and genes for required quantitative and qualitative traits. This kind of research is widely used in the world. They encompassed laboratory analysis of markers and integration of these methods with classical breeding methods. These methods can be applied in seed science, in control of specific seed quality, i.e. genetic purity. For their application the wide spectra of methods by which screening tests are made possible should be introduced.

Spectra of genetic changes of cultivated species and their wild relatives are kept and maintained in seed banks and field gen banks world widely (Simpson, Withers, 1986). Markers in gene-banks have potential application in identification of collection samples and are different among tested samples, clones, pure lines, inbreeding of population groups which differ in genetic variability and demand different treatments.

The aim of this investigation was to determine genetic variability and diversity of breeding corn material of different origin on the basis of isozymes as gene markers. Used isozymes were products of certain polymorphous loci on the basis of which some genetic characteristics of great number of populations of certain groups were determined as well as their significance for creation of heterosis hybrids.

According to many authors (Salanoubat, Pernes, 1986, Veldboom, Lee, 1996), result of adaptation process to biotic and abiotic is highly heterogeneous population. Modifications of pure lines are according to Hallauer (1990) continued with new sources of germplasm. Methods for parent identification, and for heterozygous pairs crossing, are the molecular markers, according to the same author. Isolation and identification of DNA sequences and genes, are meant to be efficient tools for development of lines and identification of best crossing. Genetic classification of lines originating from different population groups can be done on the basis of molecular markers far more efficient than on the basis of field testing of genotypes of unknown heterosis effect (Mumm, Dudley 1994). This type of estimation is made on the basis of isoenzymic markers for hybrid identification, for efficient selection, and discovering the genetic traits of elite hybrids (Smith, 1989.)

Usage of molecular markers is being introduced into a basis of genetic researches by which all components of breeding are connected and have a key role in genetical, biochemical, physiological and molecular basis of heterosis (Smith and Chin, 1993).

The aim of this investigation was to determine genetic variability and diversity of breeding corn material of different origin on the basis of isozymes were products of certain polymorphous loci on the basis of which some genetic characteristics of great number of populations of certain groups were determined as well as their significance for creation of heterosis hybrids.

MATERIAL AND METHODS

Several groups of corn populations of different origin were analyzed. Yugoslav populations encompassed two groups: 17-hard dents and 18-soft flints.

These populations were obtained by hybridization of populations belonging to other groups.

Collections from Italy came from different regions of this country, 50 populations from the collection of Portuguese and 20 populations from French collection. These populations originated from different geographical and ecological regions and they differed in vegetation period.

Genetic characters of the populations were assessed on the basis of allozymic genotypes belonging to 20 loci. The tested materials were analyzed for the frequency of detected alleles, polymorphism and heterozygosity of the loci and standard genetic distances after N e i (1978). Genetic diversity between populations of certain groups was determined by cluster analysis according to Euclidean distances.

Application of genetic markers, their hromozomic location and the methods of reading were done according to S t u b e r et al. (1988) on the basis of polymorphism of enzymic systems and 20 loci.

RESULTS AND DISCUSSION

Domestic populations from two groups: 17-hard dents and 18-soft flints were analyzed on the basis of 21 isoenzymic loci on which 66 alleles were found (Tab. 1). Populations of these groups were obtained by hybridization of populations belonging to some other groups and that is way they are considered to be the „youngest". On the basis of certain loci (Mdh1,2, Adh1, Got2) differences, between populations and groups to which they belong, were found.

Tab. 1 — Alleles detected in examined Yugoslav collections

No.	Loci	Allele-designated	Total
1.	Acp 1	2, 3, 4, 6	4
2.	Adh 1	4, 6, N	3
3.	Cat 3	7, 9, 12, N	4
4.	Enp 1	6, 7, 8	3
5.	Est 8	3, 4, 4.5, 5, 6	5
6.	Glu 1	1, 2, 3, 6, 7, N	6
7.	Got 1	4, 6	2
8.	Got 2	2, 4, 6, N	4
9.	Got 3	4	1
10.	Idh 1	4, 6, N	3
11.	Idh 2	4, 6	2
12.	Mdh 1	1, 6, 9, 10.5, N	5
13.	Mdh 2	3, 3.5, 6, N	4
14.	Mdh 3	16, 18	2
15.	Mdh 4	8, 12	2
16.	Mdh 5	12, 15	2

17.	Pgm 1	9, 16, 17, N	4
18.	Pgm 2	2, 3, 4	3
19.	Pgd 1	2, 3.8	2
20.	Pgd 2	2.8, 5	2
21.	Phi 1	3, 4, 5	3
			Total: 66

Genetic diversity of the populations inside groups was analyzed on the basis of standard genetic distance (D). For these groups the cluster analysis was given (Fig. 1 and 2). The populations C133; C432, C214 and C433 were similar in group 17. In this group division of populations into two groups, the first one from C133 to C76 which was more homogenous, and the second from C612 to C382, was noticed. In group 18 the similar populations were C139, C576, C533, C647 and C326. In this group two populations were distinguished from the rest of the analyzed populations in this group (C124 and C127) which were genetically rather distant. In order to broaden the genetic basis for corn breeding the populations with greater variability and diversity, although these two factors can, but don't necessarily have to be in correlation, can be of some significance.

The populations from Italy gathered at the Istituto Sperimentale per la Cerealicoltura, Bergamo, were analyzed. Thirty (30) populations from different regions of Italy were studied. Genetic properties of the populations were determined on the basis of analyzed eleven enzymic systems controlled by twenty (20) loci. The alleles in these populations were detected, their frequencies and heterozygosity of the studied loci was determined, and finally the standard genetic distance among all analyzed populations i.e. genetic diversity between all pairs of populations were determined (Tab. 2).

Tab. 2 — Alleles, polymorphism of Loci, Heterozygousity and Standard Genetic Distances (D) in Italian Populations

Number of population	Number of alleles	Average alleles per locus	Mean proportion of polymor. loci (%)	Heterozyg ous. (He)	Mean of D
1	33	1.6	55	0.173	0.072
2	32	1.6	45	0.168	0.076
3	32	1.6	45	0.129	0.067
4	34	1.7	60	0.224	0.080
5	31	1.5	50	0.175	0.076
6	32	1.6	50	0.169	0.091
7	33	1.6	45	0.181	0.058
8	34	1.7	55	0.185	0.067
9	37	1.8	60	0.212	0.058
10	34	1.7	60	0.203	0.063
11	33	1.6	55	0.171	0.068
12	31	1.5	50	0.202	0.073
13	33	1.6	55	0.223	0.046

14	32	1.6	50	0.209	0.070
15	30	1.5	45	0.179	0.069
16	38	1.9	70	0.242	0.072
17	41	2.0	75	0.235	0.069
18	35	1.7	55	0.220	0.055
19	34	1.7	45	0.212	0.059
20	37	1.8	60	0.212	0.075
21	36	1.8	60	0.220	0.048
22	35	1.7	55	0.207	0.054
23	36	1.8	60	0.232	0.057
24	31	1.5	50	0.218	0.056
25	35	1.7	50	0.217	0.044
26	33	1.6	50	0.292	0.058
27	35	1.7	55	0.182	0.054
28	32	1.6	50	0.190	0.053
29	31	1.5	50	0.222	0.070
30	32	1.6	45	0.200	0.063

The comparison of the frequencies of alleles in Italian and Yugoslav maize populations showed that frequencies for most alleles were similar, but there were alleles which frequencies were different in these collections. On the basis of some alleles which were rare or more frequent in the populations some differences were found among Italian and Yugoslav maize collections (Acp1, Adh1, Mdh5).

The mean polymorphism of the analyzed loci was 45 to 75% (Tab. 2). On the basis of the obtained frequencies of the detected alleles, the genetic distances (D) were calculated for all analyzed populations. Standard genetic distance ranged from 0.044 to 0.091.

Open pollinated corn collections were examined, 50 from Portuguese and 20 from French collections. The populations originated from different geographical and ecological regions. Their genetic characters were assessed on the basis of isozymes as gen markers controlled by 20 loci. A range of allelic frequencies was detected (Tab. 3). The detected alleles were common to almost all populations and their frequencies were usually high. Several new but rare alleles were found in both collections. These populations differed in frequency of the alleles on loci Acp1, Glu1, Mdh2, Pgd2. The total number of alleles per population was 31 to 43 for Portuguese collection, i.e. an average of two alleles per locus (Tab. 4).

Tab. 3 — Average frequencies for the detected alleles in Portuguese and French collections

Locus-allele	Frequency	Locus-allele	Frequency
Portugal		France	
Acp 1—2	0.45	Acp 1—2	0.44
3	0.03	3	0.01
4	0.49	4	0.56
6	0.03	6	0.01

Adh 1—4	0.65	Adh 1—4	0.65
6	0.35	6	0.35
N	0.01	N	0.01
Cat 3—7	0.06	Cat 3—7	0.11
9	0.62	9	0.64
12	0.28	12	0.24
N	0.04	N	0.01
Est 8—4	0.50	Est 8—4	0.49
4.5	0.49	4.5	0.49
5	0.01	5	0.02
6	0.01	6	0.01
		N	0.01
Glu 1—1	0.01	Glu 1—1	0.01
2	0.20	2	0.18
3	0.06	3	0.08
6	0.05	6	0.02
7	0.61	7	0.44
9	0.01	8	0.01
10	0.01	10	0.01
N	0.08	N	0.26
Got 1—4	0.97	Got 1—4	0.98
6	0.03	6	0.02
		N	0.01
Got 2—2	0.11	Got 2—2	0.13
4	0.89	4	0.87
		N	0.01
Got 3—4	1.00	Got 3—4	1.00
Idh 1—4	0.99	Idh 1—4	0.98
6	0.01	6	0.01
		N	0.01
Idh 2—4	0.64	Idh 2—4	0.44
6	0.36	6	0.56
Mdh 1—1	0.03	Mdh 1—1	0.05
6	0.91	6	0.94
10.5	0.06	10.5	0.01
Mdh 2—3	0.21	Mdh 2—3	0.16
3.5	0.03	3.5	0.02
4.5	0.01	4.5	0.01
6	0.76	6	0.82
Mdh 3—16	0.99	Mdh 3—16	0.99
18	0.01	18	0.01
Mdh 4—12	1.00	Mdh 4—12	1.00
Mdh 5—12	0.80	Mdh 5—12	0.92
15	0.20	15	0.08
Pgm 1—9	1.00	Pgm 1—9	0.99
		16	0.01
Pgm 2—2	0.02	Pgm 2—2	0.09
3	0.07	3	0.05
4	0.91	4	0.86
Pgd 1—2	0.23	Pgd 1—2	0.46
2.8	0.03	3.8	0.54
3.8	0.74		

Pgd 2—2	0.01	Pgd 2—2	0.01
2.8	0.01	2.8	0.01
5	1.00	5	0.98
		10	0.01
		N	0.01
Phi 1—3	0.01	Phi 1—3	0.01
4	0.97	4	0.96
5	0.03	5	0.03

The average heterozygosity of loci in Portuguese populations varied between 0.129 and 0.269, and in French populations between 0.098 and 0.223. The results of the analyses indicate that the Portuguese populations had more polymorphic and more heterozygous loci than French populations (Tab. 4).

Tab. 4 — Polymorphism of loci and heterozygosity in Portuguese and French populations

Population	Mean proportion of loci polymorphism (%)	Hetero-zygosity	Population	Mean proportion of loci polymorphism (%)	Hetero-zygosity
Portugal					
1.	60	0.186	26	60	0.238
2.	50	0.173	27	65	0.246
3.	40	0.129	28	65	0.262
4.	65	0.207	29	65	0.245
5.	65	0.230	30	70	0.239
6.	70	0.225	31	60	0.216
7.	60	0.214	32	70	0.210
8.	70	0.227	33	60	0.195
9.	80	0.241	34	60	0.228
10.	70	0.267	35	55	0.217
11.	60	0.185	36	65	0.229
12.	55	0.231	37	70	0.203
13.	55	0.201	38	60	0.219
14.	60	0.218	39	80	0.234
15.	60	0.209	40	60	0.214
16.	65	0.221	41	55	0.236
17.	60	0.197	42	55	0.225
18.	70	0.222	43	55	0.238
19.	65	0.244	44	60	0.205
20.	65	0.199	45	65	0.228
21.	65	0.214	46	50	0.197
22.	65	0.261	47	65	0.241
23.	70	0.247	48	55	0.195
24.	60	0.223	49	60	0.176
25.	70	0.269	50	75	0.245

France					
1.	45	0.137	11	55	0.201
2.	60	0.200	12	30	0.128
3.	50	0.150	13	55	0.190
4.	60	0.214	14	65	0.180
5.	60	0.211	15	50	0.158
6.	55	0.223	16	50	0.208
7.	60	0.184	17	60	0.134
8.	55	0.195	18	75	0.145
9.	55	0.208	19	35	0.098
10.	45	0.171	20	60	0.208

The significance of inter-genetic variability between populations and link between their structure and geographical location are very important in breeding programs and usage of germplasma collections by breeders (Salanoubat, Pernes, 1986).

Genetic diversity of corn breeding material and its combining ability can be determined on the basis of several markers such as: morphological, isoenzymic, components of storage proteins, DNA fragments (Smith and Smith, 1989). Their different possibilities and need for applying different methods were also determined. Advantage of RFLP markers over testing in the field and isozymes come from their greater efficiency and more complete genome coverage. They are better in determining the complete variability identification and diversity of genotypes.

CONCLUSION

— Isozymes as genetic markers are products of genes and as such serve for assessment of starting breeding material i.e. populations which serve as „gene-banks" in the breeding hybrid process.

— Genetic and molecular markers can serve to point out the degree of variability and diversity of breeding material. Creation of hybrids depends on choice of breeding material.

— Yugoslav corn collections had shown on the basis of polymorphism of analyzed loci fairly high heterozygosity. Genetically very similar, but also very distant populations were found.

— Identification of Italian populations was based mainly on alleles similar to Yugoslav, with the difference concerning the variability level inside them, and genetic diversity of Italian populations was more pronounced on the basis of certain loci (Phi1, Adh1).

— The Portuguese populations were generally found to contain the same common alleles, but some new, rare alleles were also found.

— Compared to the French populations, the Portuguese populations were more variable, but also more closely related.

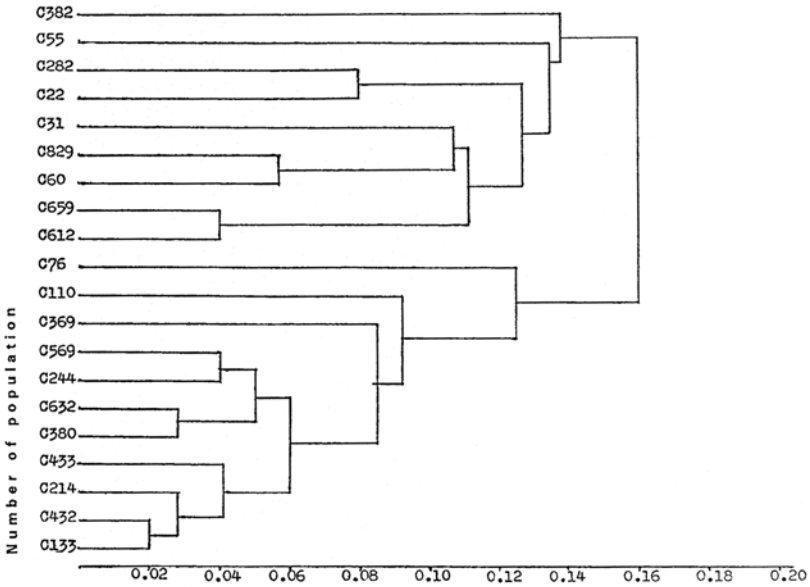

Fig. 1 - CLUSTER ANALYSIS OF POPULATION GROUP 17

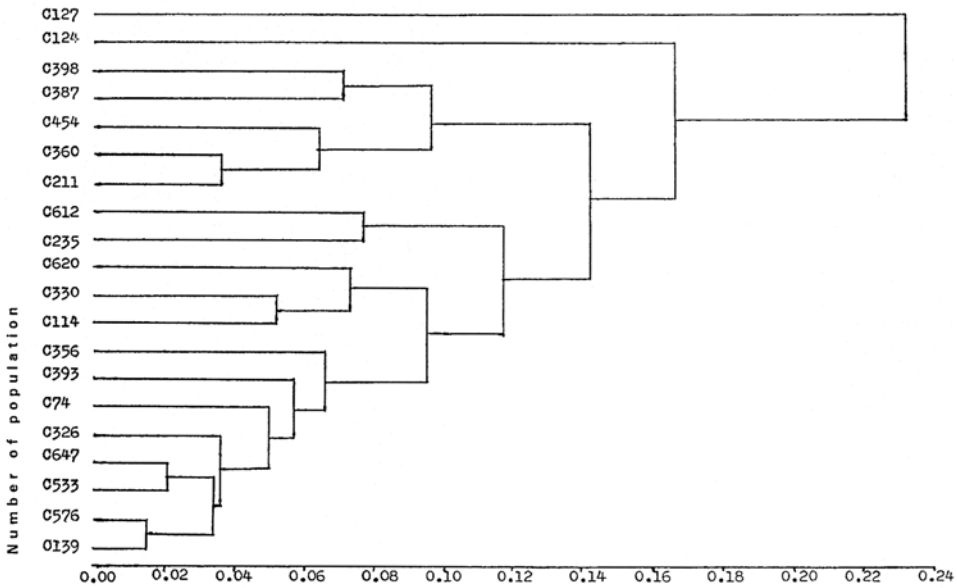

Fig. 2 - CLUSTER ANALYSIS OF POPULATION GROUP 18

A relatively low variability and an increased diversity in the French populations are possible due to an increased number of homozygous loci; even those loci which are usually polymorphic in open pollinated populations were homozygous in the French material.

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ГЕНЕТСКА ВАРИЈАБИЛНОСТ И ДИВЕРГЕНТНОСТ СЕЛЕКЦИОНОГ МАТЕРИЈАЛА КУКУРУЗА КОЈИ ПОТИЧЕ ОД ДОМАЋИХ И СТРАНИХ ПОПУЛАЦИЈА, ДЕТЕРМИНИСАНА НА БАЗИ БИОХЕМИЈСКО-ГЕНЕТСКИХ МАРКЕРА

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Резиме

Изоензими се користе као генетски маркери, а њихов број треба да буде довољно велик да би покривеност генома била што већа и као такви су маркери гена за тражена агрономска својства. Употребљени маркери су производи 21 мапираног гена, што представља релативно поуздан број за тумачење генетске основе одређених особина генотипа. Генетска варијабилност и дивергентност су значајне за популације и самооплодне линије, као основни материјал у селекцији и стварању нових хибрида кукуруза. Из тог разлога анализирано је неколико група популација кукуруза различитог порекла. Две групе југословенских популација, италијанска, португалска и француска колекција биле су оцењене на основу алелне варијабилности за 21 локус и стандардне генетске удаљености унутар сваке популације. Југословенске колекције кукуруза су показале високу хетерогеност на бази изоензима као ген. маркера. Генетска дивергентност италијанске колекције је наглашена на бази неких локуса, а португалска је имала више полиморфних и хетерозиготних локуса од француске колекције, што значи већи потенцијал генетске варијабилности. Интер-генетска варијабилност између популација и њихове географске локације веома су значајан услов у селекцији биљних врста и њихов хетерозис.

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IDENTIFICATION OF GENETIC CHARACTERISTICS OF MAIZE (*Zea mays* L.) USING GENETIC MARKERS

ABSTRACT: Different genetic markers are used for estimation of breeding material, its characteristics and potential for ultimate aim — heterosis of hybrids. They also point out to the qualitative seed traits at the level of linkage with genes responsible for desirable agronomic traits. This program encompasses testing methodologies for the new seed technology. Genetic analysis of breeding material during certain phases is comprised of isozymic gene expression and degrees of their variability, but it is continued (in order to be evaluated) until determination of presence or absence of some genes, existing or introduced for certain traits. Using combination of different molecular methods such as PCR, RAPD, and AFLP based on polymorphism of DNA fragments, the definite aim — identification of newly created products of improvement is achieved. Testing of traits of breeding material, its genetic variability and diversity is the first stage in analysis of the maize genome. It is also the condition for determination of presence of certain genes, used for obtaining the ultimate aim — attest of identity of the genotype.

KEY WORDS: Maize (*Zea mays L.*), biochemical-molecular markers, isozymes, PCR, RAPD, SSR, AFLP, RFLP, heterosis.

INTRODUCTION

Genetic identification of corn is necessary in the process of improvement as well as in the estimation of its quality. The source breeding material (Gene-pool), and generations included in the improvement program (self-pollination, and development of homozygous lines), and finding of parent components and their crossing must be included in the above mentioned. The result of these processes of maize improvement is the final product with certain agronomic traits, which genetic characteristics should be identified.

Biochemical investigations are included in the improvement program and analysis of qualitative seed traits. The biochemical-molecular markers are isozymes, expression of genes, and DNA sequences leading to complete genome coverage giving the genetic basis of quantitative and qualitative traits of breeding products. The introduction of the necessary methods on which deter-

mination of genetic diversity and relationship between European maize inbreds are given in the results of Hahn et al. (1995), where lines were of different origin, tested by ranging of methods starting from isozymes, via molecular methods with polymerase chain reaction (PCR) of DNA as a basis. Identification of QTL_S using molecular markers has a key approach for interpretation of genetic basis of these traits, and their improvement (Stuber et al., 1992). The chromosome location of 76 isozymes and RFLP markers linked with QTL_S for grain yield was shown. The development of maize inbred lines and their origin can be interpreted using molecular markers and these results can be used for fundamental and applied studying of maize (Lee, 1994). Description of inbred lines is given on the basis molecular polymorphism of the nuclear genome of maize, including several methods, ranging from cytology, via components of storage and functional proteins-enzymes, to basic molecular methods (PCR) and those leaning on the PCR.

Some of the physiologically different quantitative traits concerning maize development and its yield are mapped using molecular markers. So, the putative associations of developmental genes generally coincide with the location of homeotic genes (Khavkin and Coe, 1997). Drought-stressed maize plants are tested using genetic map with different RFLP loci, on the level of leaf abscisic acid (ABA) concentration in maize (Tuberosa et al., 1998).

Biotechnology of corn includes biochemical-genetic-molecular markers at the level of protein, RNA, and DNA respectively. They are used for gene mapping for desired traits, and the ultimate goal is genetic identification of genotype being tested and developed using method of breeding.

Maize is an excellent representative of open-pollinated plant species with present polymorphism of enzyme systems, allelic variability of isoenzymic loci, which is the crucial parametar in identification of material in the final stage of plant improvement process. Applicative side of genetics comprises new technologies in plant breeding with genetic engineering included. The food production is primary goal besides improvement of maize plant production, development and usage of genetic potential. New biotechnology methods make possible to develop the plant genotypes with new traits at the level of recombinant DNA.

Usage of molecular markers is being introduced into a basis of genetic researches by which all components of breeding are connected and have a key role in genetic, biochemical, physiological, and molecular basis of heterosis (Smith and Beavis, 1996).

The aim of this review is to give the pathway of using biochemical-molecular markers in genetic identification of breeding products.

MATERIAL AND METHODS

Maize, as an open-pollinated plant species plays special role at biotechnological and molecular level in determining the linkage genes for all physiological-genetic important agronomic traits. Biochemical-molecular markers based

on protein polymorphism, fragments of RNA and DNA have the advantage in determining this relationship.

PCR — based DNA markers are:

Random Amplified Polymorphic DNA ($RAPD_S$)
Simple Sequence Repeat (SSR) or Microsatellite
Amplified Fragment Length Polymorphism (AFLP)

Various methods are used to characterize plant varieties, lines and their hybrids, they include pedigree, agro-morphological and cytogenetic data, calculations of genetic distance, which is the condition for heterosis. Recent molecular technologies provide a tool for „fingerprinting" germplasm. These data may be required to provide sufficient information for the classification of populations or groups of lines in order to study genetic structure and genetic diversity of genotypes.

Fingerprinting of genotypes is particularly applied especially in the case of crops, such as maize in which hybrid breeding is a lucrative business. It could provide ways of estimating genetic distances between lines, and so genetic diversity existing within and between breeding populations and lines. In the case of maize, fingerprinting profiles may determine the heterosigosty between lines in the process of improvement and their crossing, which is at the same time the information concerning their genetic background.

RESULTS AND DISCUSSION

Genetic markers, isoenzymes, are components of protein, which are gene expression, and sequences of RNA and DNA can be used as a molecular markers. The complete coverage of genome is achieved by combination of these methods making real identification of the observed genotype possible. Components of storage and functional proteins are direct gene products freed from environmental factors, and as markers participate in genetic estimation of breeding and seed material (Z l o k o l i c a et al., 1996). Biochemical and molecular markers are involved in pedigree data as a valuable source of information about genetic relationships among maize inbreds, which are at the base of certain isoenzymes, such as the genetic variability of lines, originating from domestic populations, and their hybrids (Fig. 1, Tab. 1). Allelic variability of genes was used for identification of genetic distance and cluster analysis. Corn inbred lines (78) originating from domestic populations are very different. Genetic distances range from very small to very big, and according to them the observed lines are grouped i.e. separated. Mapped protein loci were unevenly and insufficiently distributed in all plant species, so the genome coverage was not complete. They should be accompanied by techniques at DNA level, such as PCR, RAPD, SSR, RFLP, and by some others which are complementary. Their combinations are given full taxonomic, genetic, and physiologic information of genotypes.

Tab. 1. — Enzyme systems used to characterize maize hybrids

Enzyme system	Locus	Chromosomal location
Aconitase	Aco 1	4
Acidphosphatase	Acp 1	9L
Alcohol dehydrogenase	Adh 1	1L
Arginine aminopeptidase	Amp 1	1
Diaphorase-glucosidase	Dia 1	2
Diaphorase-glucosidase	Dia 1	4
Glutamate oxalacvetate transaminase	Got 1	3L
Glutamate oxaloac trans.	Got 1	5L
Hexokinase	Hex 2	6L
Isocitrate dehydrogenase	Idh 1	8L
Isocitrate dehydrogenase	Idh 2	6L
Malatedehydrogenase	Mdh 1	8L
Malatedehydrogenase	Mdh 2	6L
Malatedehydrogenase	Mdh 3	3L
Malatedehydrogenase	Mdh 5	5S
Glucosidase	Glu 1	10L
Phosphoglucomutase	Pgm 1	1L
Phosphoglucomutase	Pgm 2	5S
6-posphogluconate dehydrogenase	Pgd 1	6L
6-phosphogluconate dehydrogenase	Pgd 2	3L
Phosphohexose isomerase	Phi 1	1L

The polymerase chain reaction (PCR) is one of the basic methods of molecular markers such as RAPD, SSR, AFLP, RFLP. PCR includes extraction, sequencing, and separation of amplified DNA fragments, and is related to usage of randomly selected, or specific oligo-nucleotides (primers), used for detection of variability of DNA fragments and their amplification. Maize seed was analyzed on the basis of 3 random primers A, B, C and one specific 35S primer (D), of which different amplification of DNA fragments between genotype groups, but not between analyzed genotypes for individual primers was achieved (Fig. 2). The identification of species can be initiated by PCR method which is the basis for new molecular markers such as SSR (Simple sequence report), AFLP (Amplified fragment length polymorphism), and indirectly for RFLP (Restriction fragment length polymorphism) used for marking most of the desirable genes for monogene and polygene traits such as quality, quantity, resistance etc.

Genetic distances between genotypes are highly correlated with the distances based on known pedigrees, and the distances based on isozymes and RFLP are also highly correlated with coefficient of parentage and with the distances between inbred lines. Genetic similarity among hybrids is related by pedigree (S m i t h and S m i t h , 1992).

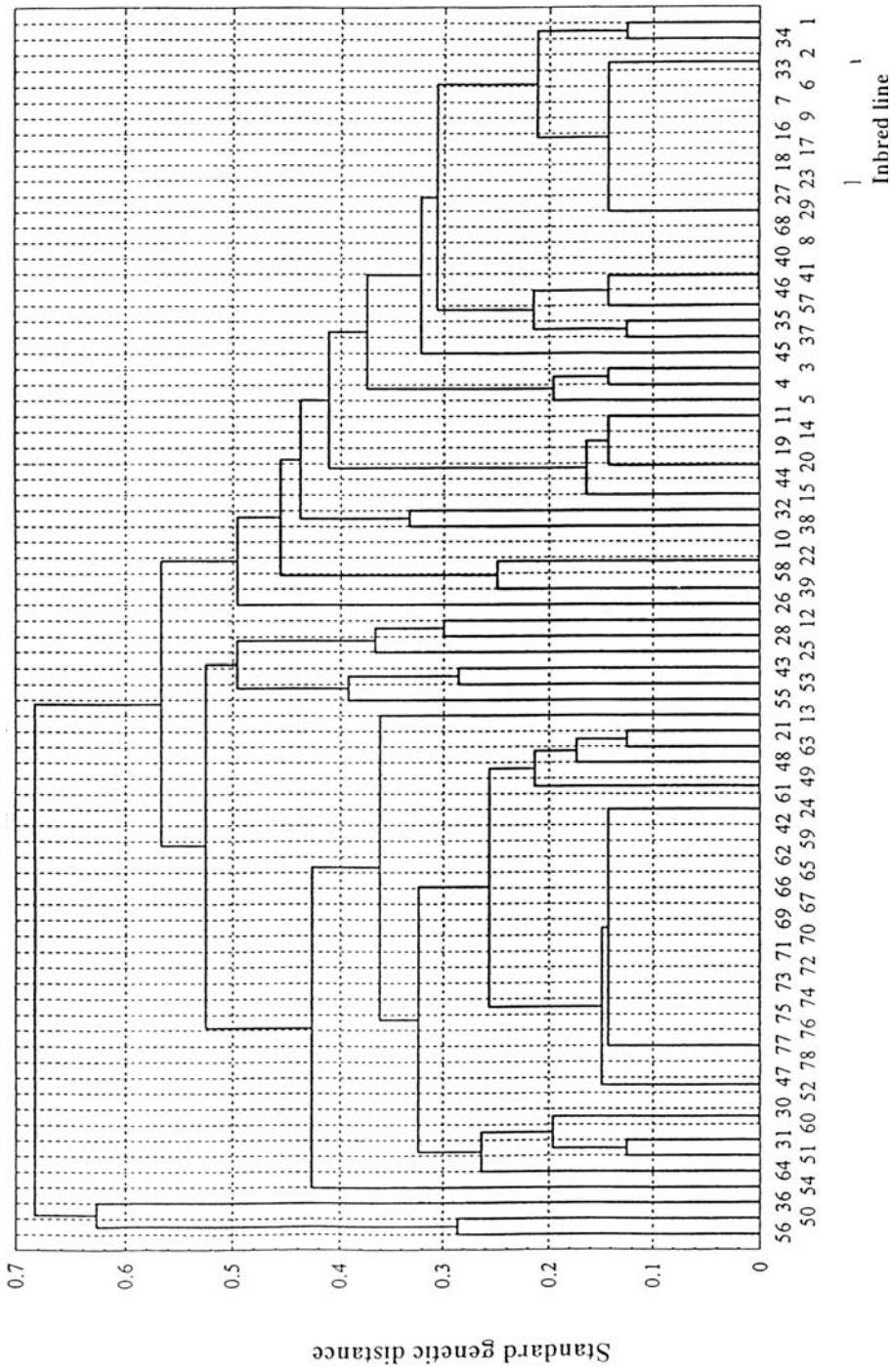

Fig. 1. — Cluster analysis of corn inbred lines (78) originating from domestic populations

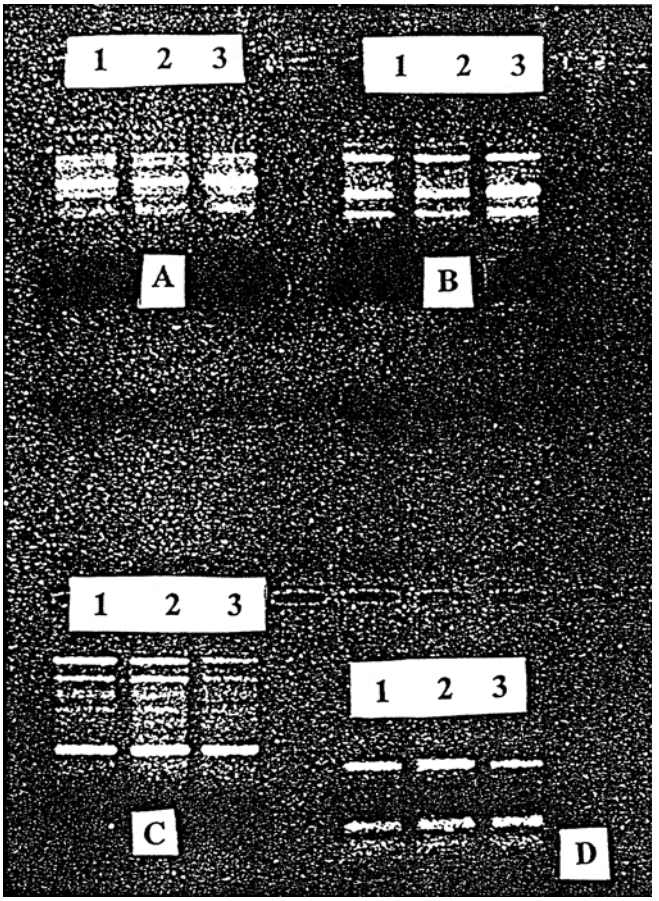

Fig. 2. — RAPD polymorphisms derived from whole corn seed using three random primers 1 (A), 3 (B) and 15 (C) as well as one specific primer for 35S promoter (D). From left to wright: 1—178 genotype, 2—182 genotype, 3—186 genotype

Simple sequence repeats (SSR_S), microsatellites are highly polymorphic markers and due to that are highly informative in plants. Their methodology approach is based on PCR, and they are codominant inherited. They are consisted of tandem repeated DNA sequences with the core sequence of 2—5 bp in eucaryotes genome, where they are uniformly distributed within genome (Akkaya et al. 1995, Rongwen et al. 1995). SSRs can show the difference between close relatives and they useful in determination of breeding material (Mudge et al., 1997). These markers have some advances such as large polymorphism, they are codominant, and fast accorging to PCR reaction, and equally distributed along the genome.

Combination of different molecular markers (isozymes, RFLP, SSR) is the best way to mark a trait of genotype, and they can be used for QTLs detection in segregating maize populations, derived from exotic germplasm (Kozumplik et al. 1996).

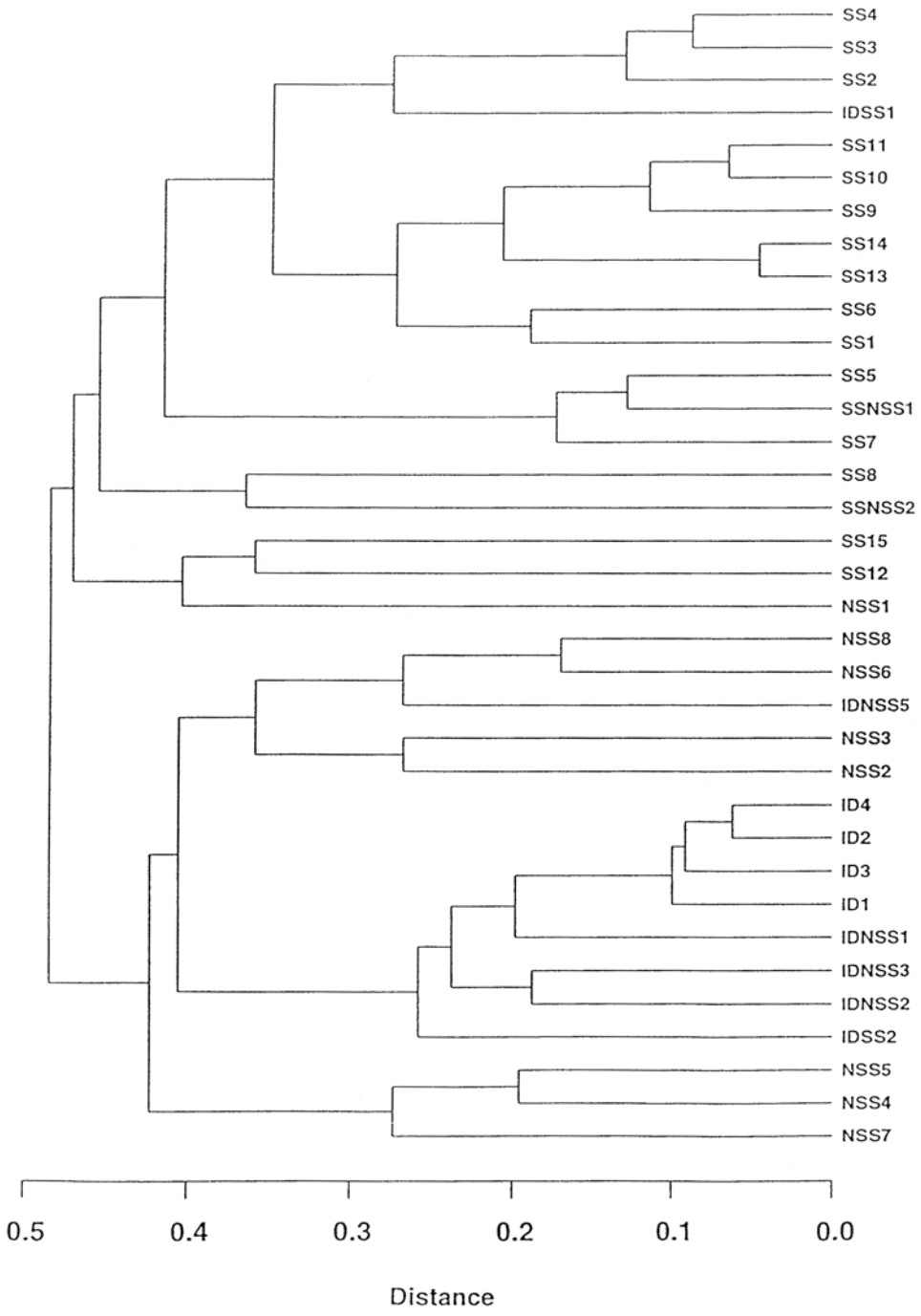

Fig. 3. — Cluster analysis of maize inbred lines in studying heterosis on the basis of AFLP

AFLP markers play significant role in the program of plant improvement and approval of the results. AFLP is based on selective amplification of restriction fragments from total genome DNA. Due to its technique of performance, AFLP technology is very efficient, the most reproductive, and it is the fastest way of maximum mapping of genes responsible for a series of desirable traits. According to many authors it is the ideal method for determination of identity of species. On the basis of electrophoresis — separation of DNA fragments, the differences between AFLP profiles are great, noticeable as presence or absence of fragments. These differences are inherited and thus marking the potential value of material being analyzed. On the basis of these markers obtained polymorphism is very high, being the most important trait in genotype identification, and in determination of diversity between second group of inbred lines, which is the basic condition for hybrid heterosis (fig. 3). Polymorphism of DNA fragments has the advantage in marking of polygene traits controlled by 20—100 loci. They are used for identification of individuals, populations, inbred lines, and hybrids, making the description of the species at the molecular level and its protection possible.

Restriction fragment length polymorphism (RFLP) analysis requires high level of DNA quality and quantity. These markers are based on variation of genome DNA sequence. Unique sequences of DNA are cloned from the nuclear genome to detect homologous sequences in plant DNA. Alleles are identified by differences in the size of the restriction fragments to which the probes hybridize.

Comparison of RAPD and RFLP marker-system for mapping F2 generation in maize was used (B e a u m o n t et al., 1996). Higher possibility (80%) of RFLP marker in relation to RAPD (between 37% and 59%) was achieved. However it was pointed out to great advantage of using combination of these markers for construction of genetic linkage map.

PCR technique is in the basis of a great number of methods used as markers in detection of plant variability. It is used for identification of germplasm in plant breeding. This marker technology must be synchronized between laboratories (J o n e s et al., 1997). Standardization must be directed toward reproducibility of the results. The reproducibility of three popular molecular marker techniques (RAPD, AFLP, and SSR) was tested in this way in several European laboratories. Different results obtained in the compared laboratories, for example RAPD markers proved difficult to reproduce, $AFLP_S$ proved easy to reproduce, and SSR alleles were amplified by all laboratories, with differences determined.

The results of heterosis are followed by biochemical data related to isozyme variability, but the large number of restriction fragment length polymorphisms, has allowed the development of linkage maps, with high degree of resolution useful in locating and manipulating of quantitative trait loci (QTLs), according to T s a f t a r i s (1995).

CONCLUSION

— Markers can be introduced for testing agronomic traits at the morphologic, biochemical and molecular level.

— Biochemical — molecular markers must be polymorphic in order to be used for linkage of qualitative and quantitative genes, and they must cover the genome, or the segments of interest.

— Genetic maps in plants are obtained using different DNA markers such as: RAPD, SSR, and RFLP.

— AFLP markers have the best possibilities. They are very efficient and reproducible, and they provide genetic maps 10—100 times denser.

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ИДЕНТИФИКАЦИЈА ГЕНЕТСКИХ ОСОБИНА КУКУРУЗА (*Zea mays L.*) КОРИШЋЕЊЕМ ГЕНЕТСКИХ МАРКЕРА

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Резиме

Различити генетски маркери се користе за тумачење селекционог материјала, његових особина и основе за крајњи циљ — хетерозис хибрида. Они су такође показатељи квалитета семена на нивоу везаних гена за одређена, тражена агрономска својства. Овај програм укључује нове методологије за тестирање селекционог и семенског материјала. Генетске анализе биљака у фазама оплемењивања обухватају изоензимску експресију гена, и степен њихове варијабилности, која се наставља до детерминације присуства или одсуства гена, одговорних за одређена својства. Примењује се комбинација различитих генетско-молекуларних метода, као што су изоензими, PCR, RAPD, AFLP, RFLP, базирани на полиморфизму протеина и секвенци ДНК, чији је крајњи циљ идентификација новостворених генотипова и њихово унапређење. Утврђивање особина селекционог материјала, његове генетске варијабилности и дивергентности је прва фаза у спознаји генома кукуруза. То је такође услов за детерминацију присуства одређених гена, потребну за постизање завршне фазе, потврде о идентитету генотипа.

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BACTERIAL AND MYCOTIC FLORA OF THE ORAL CAVITY IN PATIENTS USING A POSTRESECTION PROSTHESIS

ABSTRACT: The surgeries in the middle third of the face that distort the anatomic and topographic integrity of the oral cavity, the nose and the sinuses, besides functional damage to these regions, also induce changes in their microfloras of diverse hypothetical combinations. This was the reason to start an investigation on the qualitative and quantitative structure of the microfloras of these regions and their changes. The investigation included 35 patients using a postresection prosthesis. The material for bacteriologic and microbiologic analyses was obtained by single swab sampling from six different localizations, cultured on the blood, TKV, ECV, TYC.S, SABOURAND and ENDO agar over 24—48 hours, in aerobic and anaerobic conditions. Species belonging to the pyogenic cocci family, enterobacteria, oral streptococci and fungal microflora were detected in both the oral and the postresection cavity. Frequency distribution and Spearman's range correlation coefficient ($R = 0.961$; $SS = 17$; 14.280) ($p < 0.01$) reveal the microfloras of the two cavities were almost identical regarding the species/families of the isolated microorganisms.

KEY WORDS: oral cavity, microflora, prosthetic rehabilitation, postresection prosthesis

INTRODUCTION

Traumatic or much more often postresection loss of the oral cavity vault result in an unnatural integration of the oral cavity and the nasal, sinus and upper pharyngeal cavities (Dimitrijević, Stefanović, 1992). Generally, the patients with such acquired deformity are submitted to prosthetic rehabilitation with a postresection prosthesis. This rehabilitation is aimed at reseparating the mentioned cavities, i.e. at restoring the integrity of the oral cavity (Rodney, Nicholas, 1984). However, the separation is functional, resulting from the nature and possibilities of prosthetic reconstruction (Aramany, 1987). Peroral food intake and speaking ability are restored, preventing air circulation through the oral cavity during breathing.

The microfloras of these regions are apt to changes due primarily to the fact that the previous condition, in terms of anatomy, has not been restored. Besides, the microfloras of the mentioned cavities may themselves be altered due to the applied antibiotic, irradiation or immunosupressive therapy. In addition to the already mentioned microflora mixing, there are other factors that probably contribute to the alteration in the types of microorganisms present in these regions, such as: fixing and removal of the prosthesis, good or poor hygiene of both the prosthesis and the oral cavity as well as of the created postresection cavity (Dimitrijević, 1976), air turbulation disorders during breathing, the saliva break into the postresection cavity, changed physical and chemical properties (humidity, temperature, ion concentration).

Even today, almost all patients with either a face or jaw damage are considered (not only by unprofessionals) potentially dangerous for their environment as a possible source of infection. It has been established that these patients suffer from fear of being marked and banished from the society (Dimitrijević, 1984). That fear is grounded and it can be well documented. Research in this field might have been postponed for so long in order to avoid raising a „delicate" issue among the professionals, i.e., doctors and their assisting staff.

OBJECTIVE OF THE RESEARCH

The surgeries in the middle third of the face that disturb the anatomic and topographic integrity of the oral cavity and the nose and sinuses, besides functional damages to these regions, induce changes in their microfloras which may appear in a number of hypothetical combinations.

The research was therefore aimed at determining the types and quantities of microorganisms found in these regions and the changes in their microfloras.

MATERIAL AND METHODS

During the first or second week following surgery, the operated patients were first rehabilitated by an immediate prosthesis, which has been succeeded by a temporary one and finally by a permanent one.

The same procedure of prosthetic rehabilitation was performed in both the patients operated for either malignant or benign tumors and the patients suffering physical injuries.

The research included 35 patients in whom the swab samples of the oral cavity were taken from certain parts of postresection cavities in direct contact with the suprastructure of the postresection prosthesis. Swab sampling was also performed from certain parts of supra and infrastructure surfaces of acrylic postresection prosthesis. None of the patients exhibited a manifest infection. In each patient the sampling was performed with single swabs from six localizations:

swab sample a — from the oral cavity;

swab sample b — from the postresection cavity — the region to the nasal cavity;

swab sample c — from the postresection cavity the region to the sinus cavity;

swab sample d_1 — from a part of the suprastructure surface of the prosthesis to the nasal cavity;

swab sample d_2 — from a part of the suprastructure surface of the prosthesis to the sinus cavity;

swab sample d_3 — from the infrastructure surface of the prosthesis to the oral cavity.

The obtained material for bacteriologic and microbiologic identification (Bergey, 1991), (Honell, 1990), was then cultivated in the following culture media for cultivation under anaerobic conditions at 37°C during 48—72 hours in Gas Pak (CO2+H2) Bio Merieux: blood agar, kanamycin-vancomycin tripcase (KVT), erythromycin crystal violet agar (CV), TYC-S agar (TYC-SB agar combination).

The following microflora was isolated on the below-listed culture media, Tripcasa-blood agar, Sabourand's agar, Endo agar and TYC-S agar.

— Tripcasa-blood agar for cultivating pyogenic cocci and bacteria of greater nutritional needs:

— *Neisseriae*
— *Staphyl. epidermidis*
— *Staphyl. aureus*
— *Staphyl. haemolyticus*
— *Streptococ. pneumoniae* (bstr g2A)
— *Streptococ pneumoniae*
— *Bacteroides*
— *Leptotrichia*

— Sabourand's agar for cultivating fungal microflora:

— *Candida albicans*
— *Saccharomyces*

— Endo agar for cultivating *Enterobacteriaceae*:

— *Escherichia coli*
— *Proteus* sp.
— *Providencia* sp.
— *Pseudomonas aeruginosa*
— *Acinetobacter*
— *Clebsiella aerobacter*

— TYC-S agar for cultivating oral streptococci:

— *Streptococcus salivarius*
— *Streptococcus mutans*
— *Streptococcus sanguis*

RESULTS

The results of the investigation revealed at least one fact: the approach to and comprehension of the processes taking place after a maxillary resection should be corrected.

The distribution of the frequency of positive microflora findings in the examined areas is shown in Table 1.

Tab. 1. — Distribution of positive microflora findings in the examined areas

Microflora	Localization						Total
	A	D3	B	D1	C	D2	
Staphylococcus epidermidis	11	11	9	10	10	9	60
Staphylococcus aureus	15	16	17	13	16	16	93
Staphylococcus haemolyticus	9	10	6	9	9	10	53
Strep. pyogenes (A)	15	9	11	11	9	11	66
Streptococcus pneumoniae	12	10	14	11	9	13	69
Nesseriae	16	7	13	13	15	9	83
Acinetobacter	4	3	1	3	0	1	12
Bacteroides sp.	0	1	0	1	1	0	3
Leptotrichia	1	0	1	0	0	0	2
Candida albicans	21	15	17	19	15	16	103
Sacharomyces sp.	2	2	2	0	4	2	12
Streptococcus salivarus	34	30	26	32	23	25	170
Streptococcus mutans	34	23	25	32	24	27	165
Streptococcus sanguis	34	18	18	24	19	20	133
Escherichia coli	12	7	9	12	9	7	56
Proteus sp.	3	2	2	3	2	2	14
Providencia sp.	3	3	5	1	2	3	17
Klebsiella/Enterobacter	2	2	2	2	2	2	12
Pseudomonas aeruginosa	0	0	1	1	0	0	2
Total	238	169	179	197	169	173	1125

The distribution of the frequency of positive microflora findings in grouped localizations is shown in Table 2.

Tab. 2. — Distribution of positive microflora findings in grouped localizations

Microflora	Oral cavity	Postresection cavity	Total
Staphylococcus epidermidis	22	38	60
Staphylococcus aureus	31	62	93
Staphylococcus haemolyticus	19	34	53
Strep. pyogenes (A)	24	42	66
Streptococcus pneumoniae	22	47	69
Nesseriae	33	50	83

Acinetobacter	7	5	12
Bacteroides sp.	1	2	3
Leptotrichia	1	1	2
Candida albicans	36	67	103
Sacharomyces sp.	4	8	12
Streptococcus salivarus	64	106	170
Streptococcus mutans	57	108	165
Streptococcus sanguis	52	81	113
Escherichia coli	19	37	56
Proteus sp.	5	9	14
Providencia sp.	6	11	17
Klebsiella/Enterobacter	4	8	12
Pseudomonas aeruginosa	0	2	2
Total	407	718	1125

The frequency distribution of the listed microflora in the oral and postresection cavity of the examined series can be graphically presented as below.

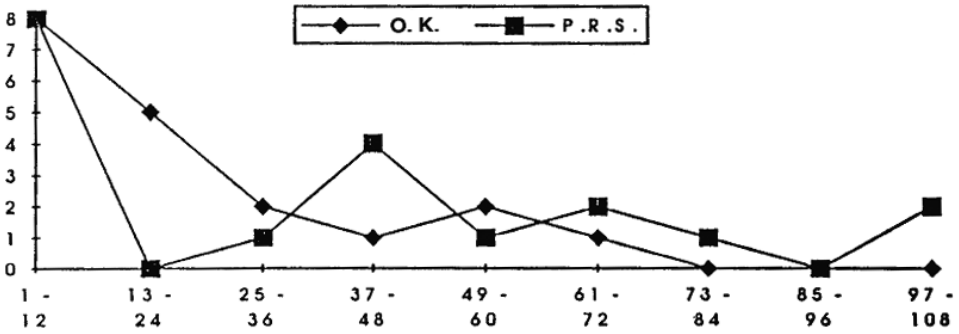

Diagram 1. — The frequency distribution of the listed microflora in the oral and postresection cavity of the examined series

It can be noticed from the diagram that both curves were slightly positively asymmetric since the mean frequency value was higher than the medial one, for the first curve corresponding to the oral cavity 21.4 > 20.5 and for the second curve corresponding to the postresection cavity 37.8 > 37. Standard deviations were exceptionally high — 19.31 for the oral cavity and 33.62 for the resection cavity. Further this results in high variation coefficients — 90.2% for the oral cavity and 88.9% for the resection cavity. The last coefficients signify that an inhomogenous sample is involved. The coefficients $\beta_{1(1)} = 0.621 < 1$, $\beta_{(2)1} = 0.502 < 1$ and $\beta_{1(2)} = 2.554 < 3$, $\beta_{2(2)} = 2.554 < 3$ signify a positive asymmetry and flattening of the curves.

Derived from Table 2. Table 3 presents quantitatively different findings that range from 0.7% to 91.4%.

Tab. 3. — Quantitatively different findings ranging from 0.7% to 91.4%

Microflora	Oral cavity	Postresection cavity	Total
	%	%	%
Staphylococcus epidermidis	31.43	27.14	28.57
Staphylococcus aureus	44.29	44.29	44.29
Staphylococcus haemolyticus	27.14	24.29	25.24
Strep. pyogenes (A)	34.29	30.00	31.43
Streptococcus pneumoniae	31.43	33.57	32.86
Nesseriae	47.14	35.71	39.52
Acinetobacter	10.00	3.57	5.71
Bacteroides sp.	1.43	1.43	1.43
Leptotrichia	1.43	0.71	0.95
Candida albicans	51.43	47.86	49.05
Sacharomyces sp.	5.71	5.71	5.71
Streptococcus salivarus	91.43	75.71	80.95
Streptococcus mutans	81.43	77.14	78.57
Streptococcus sanguis	74.29	57.86	63.33
Escherichia coli	27.14	26.43	26.67
Proteus sp.	7.14	6.43	6.67
Providencia sp.	8.57	7.86	8.10
Klebsiella/Enterobacter	5.71	5.71	5.71
Pseudomonas aeruginosa	0.00	1.43	0.95
Total	30.60	26.99	28.20

There were 28.20% of positive findings obtained on the average; if all examined localizations are taken into account, the oral cavity made 30.60% and the postresection cavity 26.99%. Regarding the microflora species in the examined cavities, they were almost identical; the species isolated from one localization were isolated from the other five localizations. The obtained results therefore lead to a conclusion that the examined series of patients had an almost identical microfloras of both the oral and the postresection cavity.

This qualitative and quantitative correlation was confirmed by Spearman's coefficient of the range correlation between localities (oral cavity vs. postresection cavity). The coefficient had the value of $R = 0.961$. It was a rather high and statistically significant correlation for the number of freedom degrees ($SS = 17$, $T = 14.28$) and the confidence threshold ($p < 0.01$). Such a high correlation signifies that the microflora structure in both the oral and postresection cavity was almost identical in regard to the presence of certain species.

DISCUSSION

The starting premise of the investigation that a prosthetic separation of the involved cavities is physiological but not anatomical has been confirmed

as true. The prosthesis is not crucial for defining the phenomenon of microflora alteration (L a i et al., 1981).

The obtained results show that besides the species normally found in the oral and nasal cavities, the species that are not characteristic for these regions have also been isolated. Namely, the species belonging to the family of enterobacteria (*Echerichia coli, Proteus* sp., *Providencia* sp., *Klebsiella-Enterobacter, Pseudomonas aeruginosa*) have been found to inhabit both the oral and the postresection cavity. Enterobacteria have the digestive tract for their normal habitat (particularly its lower regions), but their occasional presence in the oral cavity need not be alarming either. It is considered transitional — transitional endogenous flora — and need not result in clinical symptoms of infection, as it was the case in the series of patients included in this investigation. However, the possibility that enterobacteria, most often *Escherichia coli*, are identified as infection agents in the face and jaw regions is not excluded. But, as the obtained results show, this family exhibited no special affinity to either the oral or the postresection cavity. The differences in the microfloras of the two cavities were quite irrelevant, so the two cavities were considered as one.

The same situation (F a r m e r and K e l l y , 1990) is found when the presence of certain species from the family of the pyogenic cocci (*Staphylococcus epidermidis, Staphylococcus aureus, Staphylococcus haemolyticus, Streptococcus pyogenes (A), Streptococcus pneumoniae, Neisseriae, Acinetobacter*) is considered: they are found to emerge from their ecological „niches" — nasal cavity and oropharynx — and evenly colonize both the oral and postresection cavity, i.e., the parts of infra- and suprastructure of the posresection prosthesis. Oral streptococci, being an autochthonous oral population, evenly inhabit the mucosa of both the oral and postresection cavity, as well as the infra- and suprastructure of the postresection prosthesis. This can be explained by their well-developed survival mechanisms in the oral cavity, which as a biotype, due to its anatomic and physiologic properties, is extremely difficult for survival in relation to the nasal cavity region.

CONCLUSION

In new anatomic and morphologic circumstances caused by a loss of the natural partition between the oral and the nasal cavity, there occur certain changes in their physiologic microfloras despite the presence of a postresection prosthesis.

Changes in the microfloras of the oral and postresection cavity could be attributed to mixing of the two regions' microfloras, as well as to their colonization with the bacteria from other ecological niches of the organism, such as the respiratory tract and the lower digestive tract regions.

Individual analyses and the analysis of the series showed that the microfloras of the oral cavity and of the postresection cavity were almost identical regarding the presence of the identified species of microorganisms.

Bacteriologic analyses revealed that identical microorganisms inhabited both the infra- and suprastructural areas of a postresection prosthesis, as well

as that the microfloras of these areas were almost identical with those of the complementary epithelialized areas.

The microfloras of both cavities (oral and postresection), as well as of the infra- and suprastructural areas of postresection prostheses, included the normal flora microorganisms of the oral/nasal cavity, the physiologic flora microorganisms of the surrounding regions — oro- and nasopharynx, as well as the physiologic flora of the lower digestive tract regions.

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БАКТЕРИЈСКА И ГЉИВИЧНА ФЛОРА УСНЕ ДУПЉЕ КОД КОРИСНИКА ПОСТРЕСЕКЦИОНИХ ЗУБНИХ ПРОТЕЗА

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Резиме

Хируршки и трауматски, а много чешће постресекциони губици свода усне дупље, доводе до неприродног спајања усне дупље са шупљинама носа, синуса и горњег спрата ждрела. Болесници са овако стеченим губицима по правилу се рехабилитују протетички, постресекционим протезама. Истраживање је укључивало

35 пацијената, корисника постресекционих протеза. Материјал за бактериолошку и миколошку анализу узиман је са појединачних шест различитих локација. Засејаван је на културе крви, TKV, ECV, TYCS, SABOURAND и ENDO агар у временском периоду 24—48 часова у анаеробним и аеробним условима. Дистрибуција фреквенци и Spearman's range correlation coefficient ($R = 0,961$; $SS = 17$; $T = 14, 28$) ($p < 0,02$) говоре нам да је микрофлора изолованих микроорганизама ових двеју шупљина међусобно скоро идентична.

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IDENTIFICATION OF VIRUSES INFECTING PUMPKINS (*Cucurbita pepo* L.) IN SERBIA

ABSTRACT: This study was carried out in order to identify the major viruses infecting pumpkins (*Cucurbita pepo*) grown in Serbia. Leaf samples from virus-infected pumpkin plants were collected in mid-July 2001. Naked-seeded and hulled oil pumpkins, patty pan, zucchini and summer squash from three different locations were included (Table 1). Virus-infected plants showed different symptoms (Table 2 and Figures 1—4). Due to the great variability of the symptoms, the causal viruses could not be fully and precisely determined by visual examination only.

The infected samples were tested by the biotest, as well as by two serological methods, ELISA and EBIA. Polyclonal antibodies raised against cucumber mosaic cucumovirus (CMV), zucchini yellow mosaic potyvirus (ZYMV), watermelon mosaic potyvirus 1 (WMV-1), watermelon mosaic potyvirus 2 (WMV-2) and squash mosaic comovirus (SqMV) were used. In each of the 50 collected samples one or two viruses were detected (Tables 3 and 4). The most prevalent viruses infecting pumpkins were ZYMV (62%) and CMV (58%). WMV-2 was extremely rare.

KEY WORDS: *Cucurbita pepo*, pumpkins, plant viruses, zucchini yellow mosaic potyvirus, cucumber mosaic cucumovirus, watermelon mosaic potyvirus 1, watermelon mosaic potyvirus 2, biotest, ELISA, EBIA

INTRODUCTION

Viruses are the most important pathogenes of cucurbits (cucumber, watermelon, melon and pumpkins) belonging to the *Cucurbitaceae* family. More than 30 infectious viruses causing destructive symptoms and considerable economic losses were reported on these plants (Zitter et al., 1996). Their occurrence, spreading, intensity of infection and destructiveness depend on complex interrelations between the virus, its host plant, the vectors and the environment. It is usually not easy to find appropriate control measures to reduce the extent of destruction.

In order to reduce the harmful effect of a viral disease under field conditions, it is necessary to select and apply appropriate control measures. The first step in this direction is collecting infected plant parts from different locations and from various host genotypes, followed by the development of reliable methods of diagnosing.

Very few researches on cucurbit viruses have been carried out in Yugoslavia (Stakić and Nikolić, 1966; Pejčinovski, 1978; Tošić et al., 1996). Recently, a serious virus infection of pumpkin (*Cucurbita pepo* L.) has been reported by Dukić et al. (2001) for the location of Veliko Selo. The virus was identified as zucchini yellow mosaic potyvirus (ZYMV), known to be one of the most destructive viruses of pumpkins. Further investigation on virus diseases of pumpkins in Serbia thus became necessary. This paper, describing some of the results of our project aimed at studying the virus diseases of pumpkins in Serbia, with special reference to oil pumpkins, is a part of it.

MATERIAL AND METHODS

Collection of infected plant material

Samples of virus-infected pumpkin plants were collected in mid-July 2001 from naked-seeded and hulled oil pumpkins, patty pan, zucchini and summer squash, at three different locations (Table 1). The collected plant material consisted of young leaves and fruits from individual plants showing distinct symptoms of virus infection on the leaves, as well as at the level of the overall appearance of the plant. Each sample represented a single plant. The plants and their corresponding leaf samples were numbered 1—50 for later identification.

Tab. 1 — Samples collected from the virus-infected plants of pumpkin (*C. pepo*) in the field in 2001

Locality	Pumpkin type and cultivar (variety)	Designation of sample
Bački Petrovac	Breeding material of naked-seeded and hulled oil pumpkin	1—8
	Naked-seeded oil pumpkin cv. „Olinka”	9—14
Srbobran	Hulled oil pumpkin cv. „Olivija”	15—16
Torda		42—50
	Naked-seeded oil pumpkin cv. „Olinka”	17—24
	Patty pan cv. „Eva”	25—29
	Zucchini cv. „Zita”	30—36
	Summer squash cv. „Beogradska”	37—41

Pictures of the sampled plants were taken at the time of collection and the symptoms were described in written.

The collected samples were stored at 4°C, until the investigation on the viral nature of the symptoms and the identification of the viruses by the biotest and serological analyses were done.

Biotest

The infectious nature of the disease and the biological characterization of the isolated viruses were performed by mechanical inoculation. Young leaves expressing virus symptoms and surface tissues of the warted fruits were homogenized in cold 0.01 M phosphate buffer of pH 7.0 at the ratio 1:1. The following test plants were used for virus isolation and determination based on the provoked symptoms: *Chenopodium amaranticolor*, *C. quinoa*, *C. foetidum*, *Vigna sinensis*, *Citrullus lanatus* cv. „Crimson sweet", *Cucumis melo* cv. „Ananas", *Cucumis sativus* cv. „Pariski kornišon", *Cucurbita pepo* cv. „Beogradska", *Luffa* sp., *Lagenaria* sp., *Nicotiana tabacum* var. Samsun, *N. glutinosa*, *N. clevelandii* and *N. benthamiana*. The leaves of the test plants were covered with carborundum powder of 400 mesh, followed by rubbing the plant sap into the leaves of the test plants. Two plants of each test plant species were used for mechanical inoculation. The inoculated test plants were kept in glasshouse conditions and checked for symptom development at two-day intervals, up to one month after inoculation.

Serological analyses

All the collected plant samples were tested for virus identification by EBIA (Western blot) according to the method described by O'Donell et al. (1982) and modified by Hewish et al. (1986). The antigens required for EBIA were prepared from the extract of the collected leaves by the method of Laemmli (1970). Polyclonal antibodies (produced by Bioreba AG, Switzerland) raised against cucumber mosaic cucomovirus (CMV), Zucchini yellow mosaic potyvirus (ZYMV), Watermelon mosaic potyvirus 1 (WMV-1), Watermelon mosaic potyvirus 2 (WMV-2) and Squash mosaic comovirus (SqMV) were used at 1:1000 dilution. Goat antirabbit antibodies (produced by Bio-Rad Lab., Richmond, CA, USA) were diluted 1:2500 in skimmed milk. The occurrence of blue-pink color on nitrocellulose membrane was considered as the sign of positive, and its absence as a negative reaction. The molecular weight of the protein subunit of the virus was determined by Prestained SDS-PAGE Standards-Low Range (produced by Bio-Rad Lab., Richmond, CA, USA).

In addition, the samples designated by numbers 2, 6, 10, 14, 16, 20, 27, 31, 32, 36, 38, 41, 45, 46, 48 i 49 were also tested serologically by ELISA test, using polyclonal antisera produced against the following viruses: CMV, ZYMV, WMV-1, WMV-2 and SqMV. For the serological evidence of the viruses the standard direct ELISA (DAS-ELISA), based on the procedure of Clark and Adams (1977), was used with commercial kits of specific antibodies and alkaline phosphatase-labelled conjugate γ-globuline (produced by

Bioreba AG, Switzerland) at 1:1000 dilution in corresponding buffer. Plant extracts for ELISA analysis were ground in the extraction buffer at 1:4 ratio. The reaction was considered positive if the absorption of light at 405 nm was at least twice as high compared with the absorption of the corresponding control.

RESULTS

Symptoms on infected plants under field conditions

Visual inspection of the infected plants revealed various symptoms ranging from mild mosaic, yellowing, spotting and mottling to deformation of leaf lamina. The observed symptoms were classified into 11 symptom categories (Table 2). In many cases simultaneous occurrence of different symptoms was observed on the same plant. Some plants showed virus symptoms only on some of their stems, or on young leaves only. The most frequent symptoms were the deformation of leaf lamina, yellow-green mosaic of different intensity and blistering of leaf lamina.

Tab. 2 — Categories of symptoms on infected plants in the field

Symptoms category	Description of the symptoms
1	mild mosaic
2	yellow-green mosaic
3	yellowing of leaves
4	chlorotic spotting
5	chlorotic mottling
6	netlike mosaic
7	green veinbanding
8	blistering of leaf lamina
9	deformation of leaf lamina
10	plant stunting
11	knobbed fruits

Results of biotest

Based on the reaction of test plant species provoked by the isolated viruses, it could be concluded that the tested plant material was infected by ZYMV, CMV and WMV-2 (Table 3).

Tab. 3 — Reaction of test plants to mechanical inoculation with ZYMV, CMV and WMV-2

Test plant species	Symptoms*					
	ZYMV		CMV		WMV-2	
	local	systemic	local	systemic	local	systemic
Chenopodium amaranticolor	LLc	—	LLn	—	LLn	—
Chenopodium quinoa	LLc	—	LLn	—	LLc	M, D
Chenopodium foetidum	—	—	LLn	M	—	—
Vigna sinensis	—	—	LLc	—	—	—
Citrullus lanatus cv. Crimsonsweet	—	M	LLc	M	—	M
Cucumis melo cv. Ananas	—	M, D	—	M, D	—	M, D
Cucumis sativus cv. Pariski kornišon	—	M	—	M	—	M
Cucurbita pepo cv. Beogradska	—	M, D	—	M, D	r, LLc	M, D
Luffa sp.	—	—	—	M	—	—
Lagenaria sp.	—	M	—	M	—	M
Nicotiana tabacum var. Samsun	—	—	—	M	—	—
Nicotiana glutinosa	—	—	—	M	—	—
Nicotiana benthamiana	—	—	—	—	—	—
Nicotiana clevelandii	—	—	—	M	—	—

* —: no symptoms
LLc: chlorotic local lesions
LLn: necrotic local lesions
M: mosaic
D: deformation of leaves
r: infrequent appearance of lesions

All isolates of each of the investigated viruses had the same host range and caused the same type of symptoms on them. Local symptoms typically appeared about 5—7 days after inoculation, except for local chlorotic spots caused by the isolates of ZYMV on *C. quinoa* and *C. amaranticolor*, which appeared considerably later, 10 days after inoculation.

Some of the test plants exhibited rather characteristic types of reaction that could be useful for identification and differentiation of mechanically transmissible viruses of pumpkins. CMV caused systemic mosaic symptoms on the plants of the genus *Nicotiana* sp., except for *N. benthamiana*, as opposed to ZYMV and WMV-2, which were not infectious for this genus. Of the three viruses identified, only CMV was infectious for *Vigna sinensis*, causing local chlorotic spots, as well as for *C. foetidum* on which local necrotic spots could be observed along with systemic infection. On *C. quinoa*, CMV caused local spots that changed to necrosis rapidly, in a few days. Contrary to the other two viruses, CMV induced systemic infection on *Luffa* sp. expressed as mosaic with ringlike patterns. Of the three viruses studied, only CMV caused mosaic combined with large local chlorotic spots on watermelon. ZYMV and WMV-2 showed the identical host range, but could be differentiated from each other by the reaction on *C. quinoa* and *C. amaranticolor*. On *C. quinoa*, WMV-2 caused local chlorotic spots and easily distinguishable mosaic combi-

ned with slight deformation of the leaf lamina. At the same time WMV-2 caused local chlorotic spots on *C. amaranticolor* which turned to necrosis after a few days, contrary to ZYMV which provoked chlorotic spots that remained chlorotic till the full collapse of the leaf.

The presence of infection caused by a single virus or by different combinations of the three viruses was indicated by the occurrence of characteristic symptoms on the test plants. Infection caused by a single virus was registered in 34 samples (68% of the total sample number), a combined infection by two viruses was determined in 16 samples (32%). ZYMV was determined in 31 samples (62%), out of which 16 samples (32%) were single infections, 14 samples (28%) showed a mixed infection by ZYMV and CMV, and one sample (2%) showed a mixed infection by ZYMV and WMV-2. CMV was detected in 29 samples (58%), out of which 14 samples (28%) showed single, and 15 samples (30%) mixed infection. As far as the combined infections of CMV were concerned, CMV was found in combination with WMV-2 in only 1 sample (2%). WMV-2 was detected in only 6 samples (12%), 4 samples (8%) being single infections. None of the samples were simultaneously infected by all three viruses.

Results of serological analysis

The results of the serological tests were in accordance with the biotest results. Table 4 presents the combined results of the biotest, EBIA and ELISA.

Using the EBIA method and polyclonal antibodies, it was possible to confirm the presence of ZYMV and WMV-2 in the same samples in which they were detected by the biotest. By comparing with the markers of known molecular weight, the molecular weight of protein subunits of these two viruses was estimated at 35 000, which is in accordance with the results of Purcifull et al. (1984). It could be observed in Table 4 that antibodies specific for CMV caused a positive reaction only in samples 18, 30, 33, 34, 41 and 42, in which this virus was isolated from test plants also detected by using the biotest. It was demonstrated by very pale-colored strips on nitrocellulose paper.

All 16 samples tested by the ELISA showed specific reaction with homologous, and absence of positive reaction with heterologous antisera. The presence of viruses in the samples was therefore reliably confirmed, regardless of the infection being single or mixed.

When polyclonal antibodies specific to WMV-1 and SqMV were applied, no positive serological reactions were observed either by the EBIA or the ELISA test.

Relationships between the isolated and identified viruses on one side and the symptom types exhibited in the field on the other are shown in Table 4. ZYMV was isolated from samples with all symptom types, but CMV showed only 9 out of the 11. From infected plant parts showing symptom type 1, 5 and 8, ZYMV or CMV or WMV-2 were isolated alone, but in some cases also complexes of CMV with WMV-2 were determined. Complexes of ZYMV and CMV resulted in symptom types 8 or 5.

Tab. 4 — Viruses identified and their symptom categories on infected plants in the field

Sample	Virus identified*	Symptoms category**
1	CMV[1]	4
2	CMV[1, 3]	2, 9
3	CMV[1]	1, 9
4	CMV[1]	1, 8
5	CMV[1]	2, 8
6	CMV[1, 3]	3, 4, 7, 8
7	WMV-2[1, 2]	1
8	CMV[1]	3, 8
9	WMV-2[1, 2]	1, 8
10	CMV[1, 3], WMV-2[1, 2, 3]	1, 8
11	WMV-2[1, 2]	1
12	CMV[1]	2
13	CMV[1]	1
14	CMV[1, 3]	2, 8, 9
15	ZYMV[1, 2]	4, 8
16	CMV[1, 3]	4, 8
17	ZYMV[1, 2]	1, 2, 3
18	CMV[1, 2], ZYMV[1, 2]	2, 8
19	ZYMV[1, 2]	3, 7
20	ZYMV[1, 2, 3]	3, 9
21	WMV-2[1, 2]	5
22	ZYMV[1, 2]	11
23	ZYMV[1, 2]	1, 9
24	ZYMV[1, 2]	1, 9
25	ZYMV[1, 2]	1, 7
26	ZYMV[1, 2]	1, 3, 7
27	CMV[1, 3], ZYMV[1, 2, 3]	2, 8, 9, 11
28	ZYMV[1, 2]	2, 5, 10
29	CMV[1]	9, 10
30	CMV[1, 2], ZYMV[1, 2]	2, 9
31	WMV-2[1, 2, 3] ZYMV[1, 2, 3]	2, 9
32	ZYMV[1, 2, 3]	2, 9
33	CMV[1, 2], ZYMV[1, 2]	3, 9
34	CMV[1, 2]	5, 9
35	CMV[1], ZYMV[1, 2]	5, 9
36	CMV[1, 3], ZYMV[1, 2, 3]	9, 10
37	CMV[1]	5, 7
38	CMV[1, 3], ZYMV[1, 2, 3]	3, 7
39	ZYMV[1, 2]	5, 7
40	ZYMV[1, 2]	6, 7
41	CMV[1, 2, 3], ZYMV[1, 2, 3]	5, 6, 7

Sample	Virus identified*	Symptoms category**
42	CMV[1, 2], ZYMV[1, 2]	3, 5
43	CMV[1], ZYMV[1, 2]	3, 6, 7
44	CMV[1], ZYMV[1, 2]	3, 7
45	CMV[1, 3], ZYMV[1, 2, 3]	2, 8
46	CMV[1, 3], ZYMV[1, 2, 3]	2, 8, 9
47	CMV[1], ZYMV[1, 2]	3, 7
48	ZYMV[1, 2, 3]	2, 8, 9
49	ZYMV[1, 2, 3]	1, 3
50	ZYMV[1, 2]	2, 8, 9

* Virus identification by 1: biotest, 2: EBIA, 3: ELISA
** Designation for symptom categories from Table 2.

DISCUSSION

Members of the *Cucurbitaceae* family are highly sensitive to virus infection. They are infected by more than 30 viruses, the most important being: Cucumber mosaic cucumovirus (CMV), Watermelon mosaic potyvirus 2 (WMV-2), Zucchini yellow mosaic potyvirus (ZYMV), Watermelon mosaic potyvirus 1 (WMV-1, earlier: Papaya ringspot virus, PRSV) and Squash mosaic comovirus (SqMV) (Zitter et al., 1996).

The investigation reported in this paper confirms the presence of ZYMV, CMV and WMV-2 in our country. These viruses had been described previously in other locations (Đukić et al., 2001). ZYMV and CMV could be considered as widespread. The most frequent virus, ZYMV, was present in 62% of samples. This virus occurred in a large number of samples (30%) in combination with CMV. Compared with the other two viruses, WMV-2 was detected only sporadically.

The symptoms caused by these three viruses in different pumpkin types and cultivars were various. It was not possible to establish a correlation between the type of symptom and the virus, which was an indication that field symptoms cannot be used as reliable indicators, even in the case when infection is caused by a single virus.

Having on mind that the investigations of cucurbit viruses in Serbia have started recently (Đukić et al., 2001), it was necessary in this investigation to study not only the occurrence of viruses in different locations but also some biological characteristics of the isolated viruses. The gathered results should facilitate further diagnosing and monitoring of the viruses of the cucurbits.

Based on the species of host plants and characteristic symptoms it is possible to make a biological characterization of mechanically transmissible viruses of cucurbits. CMV is easiest to prove and differentiate from other viruses, based on its specific reactions on the plants of *Nicotiana* spp. It is also relatively easy to detect WMV-2 in the presence of ZYMV, based on the systemic reaction on *C. quinoa*. ZYMV is not hard to detect in case of single infection, but in the case of mixed infection with WMV-2, detection is possible only ba-

sed on local chlorotic sposts on *C. amaranticolor*. In order to make the detection of ZYMV in mixed infections possible, it is necessary to find a host plant displaying a specific reaction only to ZYMV.

The results of the biotest showed no observable differences among the isolates of the same virus, indicating the lack of variability within individual viruses under the conditions maintained in this study. The isolates of ZYMV as well as of WMV-2 showed very similar but not identical reactions to those published in the literature.

None of our isolates of ZYMV were infectious for *Luffa* sp., although numerous literature data dealing with ZYMV characterization referred to isolates capable of infecting *Luffa acutangula* (Lisa et al., 1981; Lisa and Lecoq, 1984; Provvidenti and Gonsalves, 1984; Prieto et al., 2001) and *Luffa aegyptica* (Lisa et al., 1981). The ZYMV isolate from cucumber described as not infectious to *Luffa acutangula*, differed from our isolates by reaction to *N. benthamiana* (Lesemann et al., 1983).

The isolates of WMV-2 obtained in this study tended to cause the same symptoms as those previously described in literature (Provvidenti and Schroeder, 1970; Purcifull et al., 1984). However, they were not infectious to *N. benthamiana*, which was not in accordance with the results of other authors (Purcifull and Hiebert, 1979; Tobias and Tulipan, 2002), and which made them different from WMV-2 derived from cucumber by Tošić et al. (1996). At the same time, our isolates of WMV-2 caused numerous chlorotic spots on the infected leaves of *C. quinoa*, just as described for most isolates of the same virus, but, contrary to others, our isolates belonged to a small group of isolates capable of systemic infection of this test plant and of causing mosaic and leaf deformation (Lisa and Dellavalle, 1981, Purcifull et al., 1984, Tošić et al., 1996).

The symptoms on test plants induced by our CMV isolates were not much different from those described by other authors (Lastra, 1968; Cohen and Nitzanny, 1963; Tobias and Tulipan, 2002).

The identification of the viruses collected in this study was confirmed by serological methods using appropriate antisera. The ELISA method, used worldwide for routine detection of cucurbits viruses (Menassa et al., 1986; Yuki et al., 2000), appeared to be very sensitive and appropriate for the study of a large number of samples. The EBIA method showed to be suitable for the detection of ZYMV and WMV-2, but for the detection of CMV it is necessary to standardize and increase the sensitivity of this method, in order to make it suitable for the cases when the virus occurs in low concentrations.

Based on our result it could be concluded that serological testing of a large number of samples, especially by the ELISA test, is sufficiently sensitive and appropriate for the detection of the presence of ZYMV, CMV and WMV-2 in cucurbits.

In spite of the fact that viruses cause numerous and very destructive diseases on the cultivated species from the family *Cucurbitaceae*, little attention has been paid to these viruses in our country in the past. In view of the intensified incidence of cucurbit viruses and their growing economic importance in Serbia, it is necessary to continue this study, focusing the attention on ZYMV, one of the most destructive viruses of cucurbits.

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ИДЕНТИФИКАЦИЈА ВИРУСА ИНФЕКТИВНИХ ЗА ОБИЧНУ ТИКВУ (*Cucurbita pepo* L.) У СРБИЈИ

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Резиме

Циљ ових истраживања био је да се идентификују најважнији вируси тикава (*Cucurbita pepo* L.) гајених у Србији. Узорци биљног материјала уљане тикве-голице, уљане тикве са љуском, тиквице за јело, патисона и цукинија, који су били заражени вирусима, сакупљени су у три локалитета средином јула 2001. године (таб. 1). Биљке заражене вирусима показивале су различите симптоме (таб. 2 и сл. 1—4). Тачна детерминација вируса само на основу симптома није могућа због варијабилности самих симптома.

Заражени узорци су тестирани биотестом као и применом две серолошке методе, ELISA и EBIA коришћењем поликлоналних антитела на Cucumber mosaic cucomovirus (CMV), Zucchini yellow mosaic potyvirus (ZYMV), Watermelon mosaic potyvirus 2 (WMV-2), Watermelon mosaic potyvirus 1 (WMV-1) и Squash mosaic comovirus (SqMV).

У 50 испитаних узорака детектован је један или два вируса (таб. 3 и 4). Преовлађујући вируси тикава били су ZYMV (62%) и CMV (58%). WMV-2 је детектован у веома малом броју узорака.

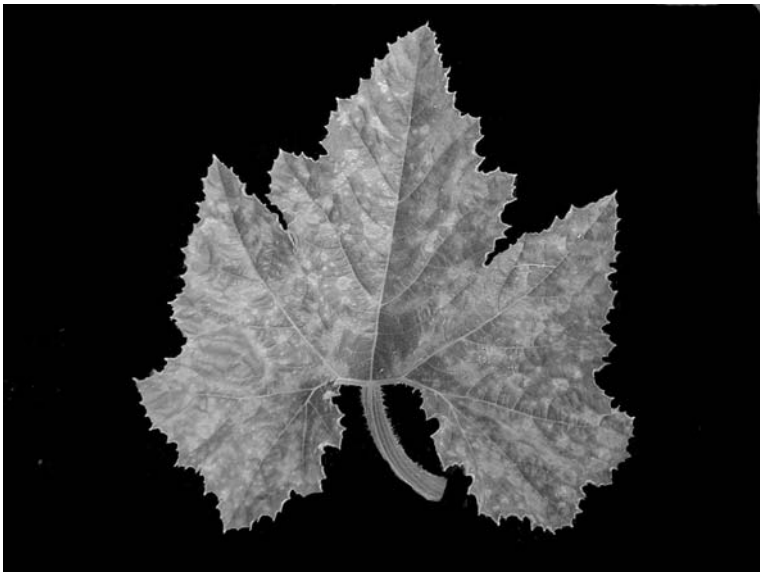

Fig. 1. — Intensive yellow-green mosaic and chlorotic mottling of leaf (sample 28) caused by zucchini yellow mosaic potyvirus

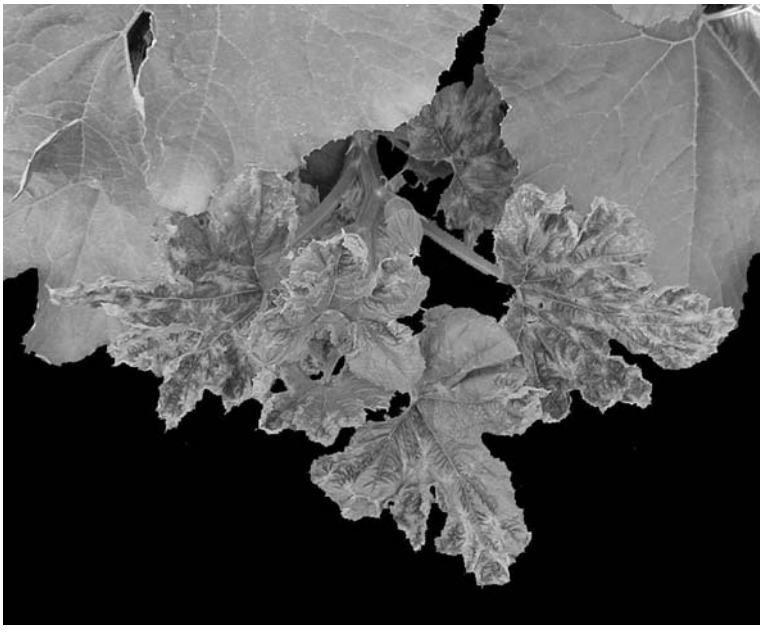

Fig. 2 — Yellow-green mosaic and blistering of leaf lamina (sample 2) caused by cucumber mosaic cucumovirus

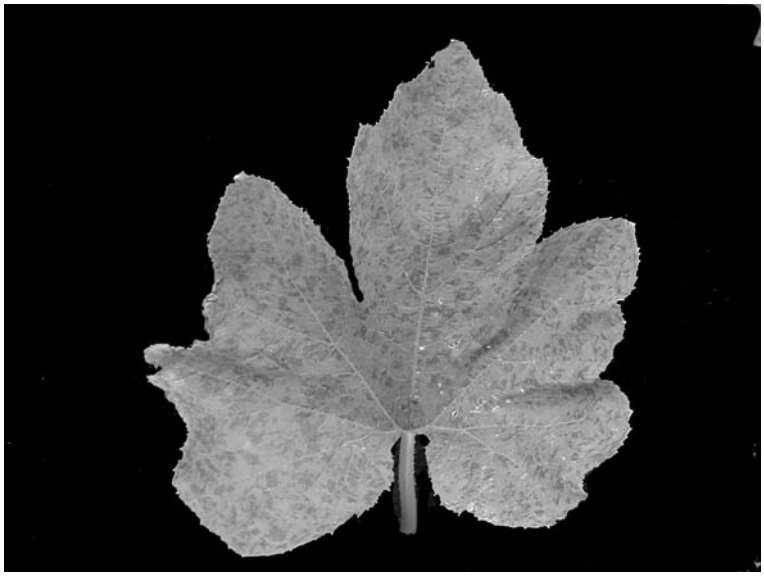

Fig. 3 — Chlorotic mottling of leaves (sample 21) caused by watermelon mosaic potyvirus 2

Fig. 4 — Yellowing and green veinbanding of leaves (sample 38) caused by zucchini yellow mosaic potyvirus

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MORPHOLOGICAL, ANATOMICAL AND PHYSIOLOGICAL CHARACTERISTICS OF *LATHYRUS LATIFOLIUS* L. (*FABACEAE*)

ABSTRACT: The *Lathyrus* species are wild relatives of cultivated peas. Results of morphological, anatomical and physiological analyses of *Lathyrus latifolius* L. are presented in this paper.

Certain measured parameters and observed morphological characteristics are not in agreement with available literature data, whereas no reference to the winged stem width, length of inflorescence pedicle, calyx length and leaflet index can be found. Two forms, f. *rotundifolium* Rchb. and f. *latifolius* Beck. have been determined. Leaflets are amphystomatic, with anisocytic stomata present in almost equal number on both epidermises, dorsoventral, with one-layered palisade and many-layered spongy tissue. Stem in transverse section is rotund to oval with two long, lateral wing-like expansions and cortical bundles. Mineral element analysis shows that the highest N, P and K concentrations were obtained with the flower while Ca and Na with the leaf. Leaf pigment content was higher than stem content while net photosynthesis rate was twice as high as respiration rate.

KEY WORDS: *Lathyrus latifolius*, morphology, anatomy, mineral element concentrations

INTRODUCTION

Wild relatives of cultivated plants are a significant natural resource and an inexhaustible gene pool of important and insufficiently surveyed plant species. Also, they are useful genetic material in breeding, when transferring desirable traits into cultivated plants, developing cultivars with desirable characters (Aarssen et al., 1986; Škorić et al., 1988; Lazić and Jovićević, 1988; Pavićević, 1990; Kojić and Mratinić, 1997; Berenji et al., 1997; Nikolić et al., 1997; Takahashi et al., 1999) or cultivating wild species (Lazić et al., 1995; Lavadinović and Isajev, 1997). Therefore, knowledge of morpho-anatomical, physiological and

biochemical characteristics of autochthonous relatives of cultivated plants and their relationships is important and required. *Lathyrus* species are the closest autochthonous relatives of cultivated peas. In *Flora Europaea* the genus is represented by 54 species (Ball, 1968) while in *Flora of Serbia* 26 species occurring in different habitats are reported (Kojić, 1972).

The purpose of the present study was to survey the morphological, anatomical, and physiological characteristics of the species *Lathyrus latifolius* L. in order to get a better insight into the biological characteristics of the *Lathyrus* species in general.

L. latifolius is a perennial plant with branched rhizome (Kojić, 1972), glabrous or hairy shoot, stem with lateral wing-like expansions, and leaves composed of one leaflet pair with a tendril. Leaflets are linear to ovate or elliptic-rotund with lanceolate to ovate, semihastate stipules. Inflorescences are racemose, with (5) 8—15 flowers that are much longer than leaves. The calyx is campanulate, unequally dentate. The corolla is dark red, rarely whitish. The pod is elongate linear, glabrous, dark brown, with reticulate veins and 8—14 seeds. The seed is semiglobose or oval. The species grows on meadows and forest clearings (Kojić, 1972). *L. latifolius* belongs to east sub-Mediterranean floral element. A number of infraspecies forms have been reported (Gams, 1964; Soó, 1966; Kojić, 1972; Kozuharov, 1976).

MATERIAL AND METHODS

Meadow plant material from the surveyed site (Vrdnik, the Fruška Gora Mountain) was collected at flowering, at the beginning of June. Twenty dried and mounted plants were used for morphological investigations. Plant height, width of winged stem, wing width, length and width of leaflets and stipules, length of inflorescence axis, flower, and calyx, and pod length and width were measured, and the number of leaflet pairs, number of flowers per inflorescence, and number of seeds per pod were counted.

Ten plants were selected for anatomical survey. Characteristics of leaflet epidermal tissue were analyzed on epidermal prints made after Wolf (1954). Light microscopy was employed to determine the size and number of stomata per mm^2 on both adaxial and abaxial epidermis. Leaflet anatomy was analyzed in transverse sections of midleaflet portion by using freezing microtome. Microscopic measurements were performed of the median vein and leaflet 1/4 width, height and width of main vein and its vascular bundle, vessels diameter, thickness of leaflet, mesophyll, palisade and spongy tissues and cuticle, size of palisade and spongy tissue cells, adaxial and abaxial epidermis cells, as well as of the number of layers of spongy tissue. Stem anatomy analysis was done using transverse sections of internodes at mid-stem portion. Stem diameter, cuticle thickness, size of sclerenchyma groups, vascular bundles at rib zone, epidermal cells, parenchyma cells and vessels were measured and the number of vascular bundles counted.

The physiological survey included the analysis of mineral elements concentration in leaf, stem, flower and pod. Nitrogen (N) concentration was deter-

mined using a standard micro-Kjeldahl method (S a r i ć et al., 1990) phosphorus (P) was determined spectrophotometrically, by the ammonium-vanadate-molybdate method (G e r i c k e and K u r m i e s , 1952). Total potassium (K), calcium (Ca), and sodium (Na) were determined from stock solution by the flame photometry method. Concentration of photosynthetic pigments (S a r i ć et al., 1990) was determined after W e t t s t e i n (1957). Net photosynthesis rate and respiration rate were determined polarographically, using a Clark electrode (W a l k e r , 1987).

RESULTS AND DISCUSSION

Morphological characteristics

Plant height, stem wing width, leaflet width and flower length values on the examined plants were lower than data published elsewhere (Table 1), whereas leaflet and stipules length, number of leaflet pairs and the number of flowers per inflorescence were in accordance with the available literature. No data could be found for the width of winged stem, length of inflorescence pedicle, calyx length and leaflet index. Therefore, our results are a contribution to the knowledge of morphological characteristics of the surveyed species.

Two forms were determined, f. *rotundifolium* R c h b . , including individuals with almost rotund leaflets emarginate at the apex, and f. *latifolius* B e c k . , with elliptic to lanceolate leaflets up to 20 mm wide and stem wings approximately 5 mm wide.

Tab. 1 — Morphological characteristics (cm)

	Obtained value	Mean value	Hayek (1927)	Komarov (1948)	Gams (1964)	Ball (1968)	Kojić (1972)	Kožuharov (1972)
Plant height	33—114	79	—	100—200	50—200	60—300	60—300	30—100
Stem width (with wings)	0.4—0.9	0.7	—	—	—	—	—	—
Wing width	0.15—0.4	0.26	0.25—0.6	—	0.25—0.6	—	0.25—0.6	0.5
Number of leaflet pairs	1	1	1	1	1	1	1	1
Leaflet length	3.5—8.1	6.6	4—9	5.5—8 (9.5)	4—9 (10)	(3) 4—15	(3) 4—15	(3) 4—6 (7)
Leaflet width	1.3—3.8	2.5	—	1.2—3 (5)	1.5—5	0.3—5	0.3—5	(0.2) 0.6—3 (3.5)
Leaflet index value	—	2.64	—	—	—	—	—	—
Stipule length	2.1—5.8	3.99	—	2—4	—	(2) 3—6	(2) 3—6	2—4
Stipule width	0.4—1.9	0.9	—	0.8—1.2	—	0.2—1.1	0.2—1.1	(0.7) 1—1.5

Number of flowers/inflorescence	8—13	11	8—14	3—8 (10)	—	5—15	(5) 8—15	—
Inflorescence axis length	5.1—31	15.7	—	—	—	—	—	—
Flower length	0.7—1.8	1.2	—	2—2.5	1.5—3	(1.5) 2—3	(1.5) 2—3	—
Calyx length	0.3—1	0.7	—	—	—	—	—	—
Pod length	—	—	6.2—7.5	5—6	7—8 (11)	5—11	5—11	(4) 5—8 (10)
Pod width	—	—	0.9—1	0.9—1	0.6—0.9 (1.2)	0.6—1	0.6—1	(0.4) 0.5—0.7 (0.8)
Number of seeds/pod	—	—	—	—	8—14	10—15	8—14	10—15

Anatomical characteristics

Leaflet epidermal tissue is formed of rather large cells irregular in shape, with moderately wavy anticlinal walls (Figure 1). Wall waviness of abaxial epidermis is inconspicuous. Stomata are anisocytic (Metcalfe and Chalk, 1957) on both epidermises, situated at the epidermal level. Stomata number and size are almost identical on the adaxial and abaxial epidermis (Table 2).

Fig. 1 — Print of leaflet adaxial epidermis

Tab. 2 — Number and size of leaflet stomata

	Number of stomata/mm^2		Stomata size — ade (μm)		Stomata size — abe (μm)	
	Ade	Abe	Length	Width	Length	Width
Mean value	70	78	26.2	13.7	27.8	14.8
min-max	48—92	59—102	21.4—31.5	8.8—17.6	25.2—32.8	10.1—18.9

Ade — adaxial epidermis; Abe — abaxial epidermis

Leaflets are dorsiventral (Figure 2). The median vein in transverse section is convex abaxially. A collateral vascular bundle is in it. Well developed scle-

renchyma tissue along the phloem portion of vascular bundle. Sclerenchyma fibers incumbent on phloem and xylem. Their lumen grows narrow and walls grow thick towards periphery. Leaflet epidermis formed of a layer of tabulate cells covered with a cuticle somewhat thicker adaxially (Table 3). Palisade tissue structured of a layer of a rather large elongated regular cilyndrical cells. Spongy tissue of 5—7 layers of irregular cells with large intercellulars. Incumbent layer abaxially formed of larger cells. The spongy tissue is twice as thick as the palisade tissue. Leaflet mesophyll with small collateral vascular bundles, enveloped into sheath parenchyma cells.

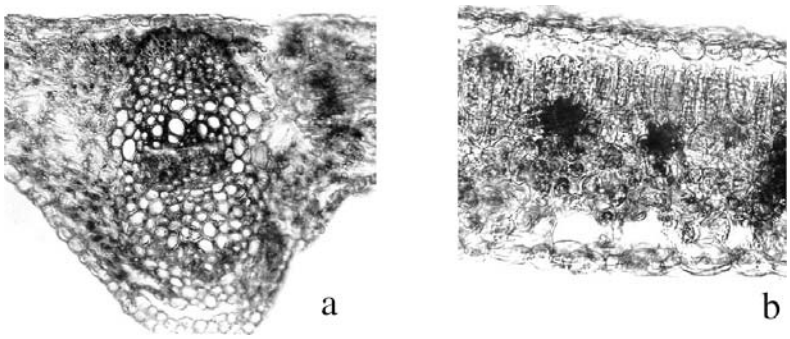

Fig. 2 — Leaflet cross sections: (a) main vein, (b) ¼ leaflet width

Tab. 3 — Leaflet anatomical characteristics (μm)

	Thickness			
	leaflet	mesophyll	palisade tissue	spongy tissue
X	257	198	64	137
min-max	219—306	158—235	51—76	101—173

	Adaxial epidermis cells			Abaxial epidermis cells			Palisade tissue cells	
	height	width	cuticle thickness	height	width	cuticle thickness	height	width
X	20.6	29.2	3.0	19.4	31.9	2.4	61.7	17.5
min-max	15.1—25.2	20.2—39.1	2.5—3.8	15.1—23.9	22.7—42.8	1.9—2.5	49.1—75.6	12.2—23.9

	Spongy tissue		Main vein		Main vein vascular bundle		Vessels	
	height	width	height	width	height	width	height	width
X	32.7	29.0	597	499	205	155	29.9	26.4
min-max	25.2—39.1	20.2—41.6	439—765	377—663	173—240	102—204	21.4—40.3	15.2—36.5

Stem is elliptical in transverse section with two wing-like expansions longer than stem diameter (Figure 3). Two opposite main ribs distinct, while intermediary far less distinct. Epidermis structured of a layer of almost spherical cells covered with a thick cuticle (Table 4). Primary cortex rather thin, composed of a few cell layers. Small, thin-walled cells filled with chloroplasts below

epidermis while deeper layers of primary cortex formed of larger cells. Subepidermally, developed collenchyma occurs only in larger ribs. Lacunae parallel to stem surface are found at cortex periphery.

The central cylinder is composed of parenchyma cells, which grow larger towards stem center. The largest central cells rupture to form a cavity. Collateral vascular bundles are arranged in a ring at the periphery of the central cylinder. They are of different size, those opposite to the ribs being larger. The number of bundles is around 20. In addition to these, there are two bundles located opposite to the wing-like expansions (cortical bundles). There are 6—12 bundles in each wing-like expansion. Above phloem, groups of sclerenchyma fibers more developed above larger bundles occur. Among xylem portions of vascular bundle cells of medullary rays with thicker and to some extent lignified walls are present. Together with xylem they form a continuous ring. Vessels are of a considerable width.

Tab. 4 — Stem anatomical characteristics (μm)

	Stem diameter		Length of stem wings	Number of vascular bundles	Number of vascular bundles in wings	Sclerenchyma tissue	
	larger	smaller				height	width
X	4163	3474	5483	20	9	135	476
min-max	3471—5850	2912—4602	4914—6526	15—30	6—12	102—153	408—581

	Phloem		Xylem		Vessels		Cylinder parenchyma cells	
	height	width	height	width	height	width	height	width
X	226	398	329	352	56.6	44.8	67.9	56.4
min-max	189—296	357—376	255—459	306—393	41.6—84.4	32.8—69.3	37.8—126	26.5—103.3

	Epidermis		
	height	width	cuticle thickness
X	22.8	28.6	6.1
min-max	18.9—31.5	21.4—36.5	5.0—6.3

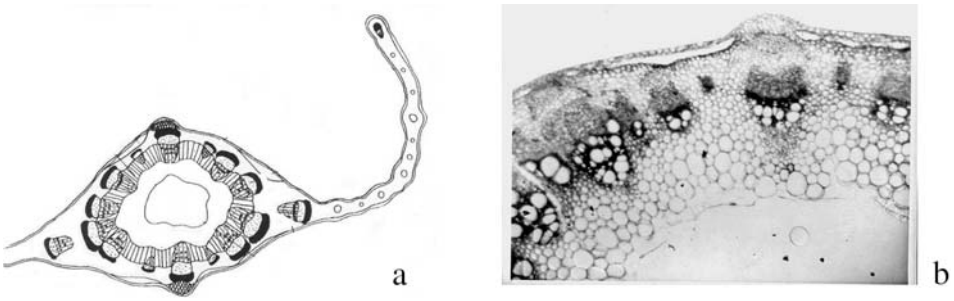

Fig. 3 — Stem cross section: a) schematic drawing, b) detail

Physiological characteristics

Of the surveyed elements, N, K, Ca, and Mg belong to essential, while Na to a group of useful elements. Their concentrations in plant tissue depend on plant age, cultivation conditions, plant organ, pest control, etc. The concentrations of these elements differ among organs.

In the surveyed species, the highest N was obtained in the flower, then in the pod and leaf, and the lowest in the stem. K concentration in plant organs showed the same variations as the N concentration. The highest P was recorded in the flower, then in the pod and stem, and the lowest in the leaf. The highest Ca was recorded in the leaf, the lowest in the stem. The highest Na was found in the leaf, the lowest in the flower.

Tab. 5 — Concentration of mineral elements in plant organs (mg%)

	N	P	K	Ca	Na
Leaf	3773	115	733	1336	77
Stem	2502	118	650	960	63
Flower	5942	363	1558	1116	28
Pod	4222	303	1225	1011	38

Tab. 6 — Concentration of photosynthetic pigments (mg/g dry weight) and rates of net photosynthesis and dark respiration (μmol O_2/gh)

	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids	Rate of net photosynthesis	Rate of dark respiration
Leaf	3.79	1.12	4.91	1.31	426.6	160.6
Stem	0.79	0.23	1.03	0.26		

Chlorophyll and carotenoid concentrations were higher in the leaf than in the stem. Chlorophyll a was ranking first, then carotenoid, and chlorophyll b. According to P e t r et al. (1971), the rates of net photosynthesis of pea leaves and stipules are almost identical. In our survey, the rate of net photosynthesis was 426.6 μmol O_2/gh, while the respiration rate amounted to 160.6 μmol O_2/gh.

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МОРФОЛОШКА, АНАТОМСКА И ФИЗИОЛОШКА СВОЈСТВА *LATHYRUS LATIFOLIUS* L. (*FABACEAE*)

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Резиме

Испитивање карактеристика дивљих сродника гајених биљака добија све више на важности због могућности уношења њихових квалитетних и пожељних особина, процесом оплемењивања, у гајене генотипове. Врсте рода *Lathyrus* су дивљи сродници гајеног грашка. У раду су приказани резултати морфолошких, анатомских и физиолошких анализа врсте *Lathyrus latifolius* L.

Установљено је да се неке од мерених вредности морфолошких карактера разликују у односу на податке из литературе, а подаци за ширину стабла са крилима, дужину дршке цвасти, дужину чашице и индекс листића нису нађени у коришћеној литератури. Детерминисане су две форме — f — *rotundifolium RCHB.* и f. *latifolius* BECK. Листићи су амфистоматични, са стомама анизоцитног типа, које су присутне у скоро подједнаком броју на оба епидермиса. Листић је дорзивентралне грађе, са једнослојним палисадним и вишеслојним сунђерастим ткивом. Стабло је на попречном пресеку округло до овално, са два дуга бочна криласта израштаја и присутним кортикалним снопићима. Анализом састава минералних елемената установљено је да су концентрације N, P и K највише у цвету, а Ca и Na у листу. Садржај пигмената је већи у листу него у стаблу. Интензитет фотосинтезе је два пута већи од интензитета дисања.

ЗАХВАЛНОСТ

Овај рад је део истраживања на пројекту број 1760 „Дивљи сродници гајених биљака: *Lathyrus* spp., *Trifolium* spp и *Allium* spp.”, финансираног од стране Министарства за науку, технологије и развој Републике Србије.

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NEW DATA ON HOVERFLIES DIVERSITY (*INSECTA: DIPTERA: SYRPHIDAE*) ON THE FRUŠKA GORA MOUNTAIN (SERBIA)

ABSTRACT: The hoverflies fauna of the Fruška Gora Mountain is well known on account of long-term investigations conducted on the location (1956—2002). Previously, the list of hoverflies had included 203 species (Vujić and Glumac, 1994). Based on new investigations, nomenclature changes and recently published data, the following results were obtained: three species have been recorded for the first time for southeastern Europe, one species for F.R. Yugoslavia and seven species for the Fruška Gora; seven previously published species have been replaced and seven excluded from the list of the hoverflies fauna of the Fruška Gora; 16 species with recently established junior synonyms and 12 species with new generic names have been noted. According to these results, the revised list of hoverflies of the Fruška Gora Mountain now consists of 210 species.

KEY WORDS: Diptera, Syrphidae, hoverflies fauna, the Fruška Gora Mountain

INTRODUCTION

The Fruška Gora is a low mountain, like an island surrounded by the flatlands of the Vojvodina Province (Serbia). The valleys of the Fruška Gora Mountain and their adjoining slopes harbor meadows and grain fields, vineyards and orchards. Dense deciduous forests cover its higher parts, above 300 meters. The great forest diversity enables the survival of many animal species, especially insects. This is also the case with hoverflies, which are generally numerous in the south European biome, including the prevalently deciduous forest that dominates the Fruška Gora Mountain.

Hoverflies are a well-known group of Diptera, with over 5000 species described until now. They occur in all kind of habitats, from seashores to high mountains, at the altitudes over 3000 meters. They are widely distributed thanks to their distinctive adaptive radiation, different types of larval development and heterogeneous ecological requirements of adults. Faunistic investiga-

tions of this interesting group of insects provide valuable data for many scientific disciplines like taxonomy, biogeography, ecology, evolution, environmental science, etc. Long-term investigations of the hoverflies fauna of the Fruška Gora Mountain, which resulted in the discovery and registration of many species from different zoogeographical regions, complete the biogeographical characteristics of this mountain and its history. Also, they points out the importance of the Fruška Gora Mountain as a refuge for many species (rare and endangered) and the necessity for permanent monitoring of its biodiversity.

The aims of this paper were to complete the faunal data of the Fruška Gora National Park and to serve as a reminder about the necessity of enforcing the biodiversity conservation policy in this national park which has been designated as an area of special value.

MATERIAL AND METHODS

A part of the material analyzed in this study had been collected on the Fruška Gora Mountain in 1956/57 is deposited in the Museum of Natural History in Belgrade (Serbia). The other part of the analyzed material was collected later and kept in the collection of the Department of Biology and Ecology, University of Novi Sad (Serbia).

For each species treated in Results and Discussion, information are given on current taxonomic status (with recently established junior synonyms and reference where it was previously published), new distribution and biological data. Following abbreviations are used: (S) — synonym; (R) — revised taxon; (E) — erroneous determination.

Standard methods of collection and preparation were used during this investigation (Vujić and Glumac, 1994).

RESULTS AND DISCUSSION

The analysis of the recently collected material (1992—2002) and the review of the previously published list (Vujić and Glumac, 1994) produced new data for the fauna of the Fruška Gora Mountain.

1. ***Anasimyia lineata*** (Fabricius, 1794)

Vujić and Glumac, 1994: as *Helophilus lineatus* (Fabricius, 787)

Remark: This species appears in recent literature as the genus *Anasimyia*, subgenus *Eurinomyia* (Ssymank et al., 1999).

2. ***Anasimyia interpuncta*** (Harris, 1776)

Eurinomyia transfuga Zett. by Glumac, 1959 (E)

Helophilus lunulatus Meigen, 1822 by Vujić and Glumac, 1994 (E)

Published records: Novi Ledinci, 10 May 1957. Two females (1 female det. by Glumac as *Eurinomyia transfuga*; 1 female det. by Glumac as *E. lunulata*; both specimens det. by Vujić as *Helophilus lunulatus*).

New data: Tekije, 5 May 1985. One male 5 females leg. Radišić; Karlovački rit, 13 April 1990. Two males leg. Vujić.

Remarks: The redetermination of material from the subgenus *Anasimyia* has shown that the specimens from the region of the Fruška Gora Mountain belong to a species previously unknown in southeastern Europe. The range of distribution of *A. interpuncta* covers the territory from Fennoscandia south to northern France and from Britain eastwards through central Europe into European parts of Russia (Speight, 2001). In Europe, the preferred environment of the species is wetland/open ground; close to standing water in fen and river floodplains. Flowers visited by adults include white umbellifers, *Caltha, Euphorbia, Ranunculus, Salix, Sorbus aucuparia* (Speight, 2001).

3. *Chalcosyrphus eunotus* (Loew, 1873)

New data: Vrdnik, 31 March 2002. One male leg. Vujić.

Remark: *C. eunotus* is generally a decreasing species in Europe, with noticeable decreases in population numbers (Speight and Castella, 2001). This species was collected in few locations in Serbia (the Vršac Mountains, the Kopaonik Mountain, the Vlašić Mountain, the Homolje Mountains) (Vujić and Radović, 1990; Milankov et al., 1995). This is the first record of this species on the Fruška Gora Mountain.

4. *Cheilosia aerea* Dufour, 1848

Cheilosia zetterstedti Becker, 1894 by Vujić and Glumac, 1994 (S)

Remark: Claussen and Thompson (1996) reinstated the old synonym *C. aerea* as valid and proposed *C. zetterstedti* as a new synonym. This change was published in Vujić (1996).

5. *Cheilosia alba* Vujić et Claussen, 2000

Cheilosia clama Claussen et Vujić by Vujić and Glumac, 1994 (E)

Remark: The specimen published by Vujić and Glumac (1994) as *Cheilosia clama* actually belongs to the recently described species *C. alba* (published in Vujić and Claussen, 2000). Besides the Fruška Gora Mountain, the species is known in only two other locations (one in Germany, another in Montenegro). Unfortunately, *C. alba* is probably extinct in the Fruška Gora Mountain (Vujić et al., 2002).

6. *Cheilosia latifrons* (Zetterstedt, 1843)

Cheilosia intonsa Loew, 1857 by Vujić and Glumac, 1994 (S)

Remark: *Cheilosia latifrons* is recognized as the senior synonym for *C. intonsa* (Speight and Lucas, 1992). This change was published in Vujić (1996).

7. *Cheilosia melanopa redi* Vujić, 1996

Cheilosia melanopa (Zetterstedt, 1843) by Vujić and Glumac, 1994 (R)

Remark: Vujić (1996) described a new subspecies based on materials from many locations on the Balkan Peninsula and with the exception of the Alps, Mediterranean and sub-Mediterranean parts. In relation to the nominal subspecies, *C. melanopa redi* appears at lower altitudes. On the Fruška Gora Mountain, large populations of this species were recorded in many locations on the mountain slopes.

8. *Cheilosia psilophthalma* Becker, 1894

New data: Paragovo, 20 March 1997. One male leg. Radenković.

Remark: Range of *C. psilophthalma* is uncertain, due to confusion, until recently, with *C. latigenis* Claussen et Kassebeer, *C. urbana* Meigen and *C. vujici* Claussen et Doczkal, but confirmed for southern Norway, Ireland, France (Vosges, the Alps), Poland, Switzerland, Greece, Montenegro, Serbia and Russia (Speight, 2001). In Serbia, *C. psilophthalma* has been recorded at low altitudes, on a few mountain slopes (the Vršac Mountains, the Suva planina, the Stara planina). This is the first record of this species on the Fruška Gora Mountain.

9. *Cheilosia ranunculi* Doczkal, 2000

Cheilosia albitarsis (Meigen, 1822) by Vujić and Glumac, 1994 (in part)

Cheilosia aff. *albitarsis* Doczkal, in litt. by Vujić, 1996

Remark: Two species are confused under the name *C. albitarsis* Meigen sensu auctt. Doczkal (2000) separated these two species and proposed the name *C. ranunculi* for the less widespread species. Both species are present on the Fruška Gora Mountain and they were previously separated by Vujić (1996) under the names *C. albitarsis* and *C.* aff. *albitarsis*. *C. ranunculi* occurs in dry grasslands from southern England to northern Spain and eastwards to Bulgaria (Doczkal, 2000). In Serbia, *C. ranunculi* appears sympatrically with *C. albitarsis* over the majority of the examined locations (Vujić, 1996).

10. *Cheilosia soror* (Zetterstedt, 1843)

Cheilosia rufipes (Preyssler, 1798) by Vujić and Glumac, 1994 (S)

Remark: On the basis of Peck's (1988) data, Vujić and Glumac (1994) cited *C. rufipes* as a senior synonym of *C. soror*. In recent literature this taxon appears under the name *C. soror* and it was accepted by Vujić (1996).

11. *Cheilosia urbana* (Meigen, 1822)

Cheilosia ruralis (Meigen, 1822) by Vujić and Glumac, 1994 (S)

Cheilosia praecox (Zetterstedt, 1843) by Vujić, 1996 (S)

Remark: To untangle the nomenclatural confusion of this species, Claussen and Speight (1999) reviewed 19 European species-group names and reinstated *C. urbana* as the valid senior synonym of *C. praecox*. The types of *C. ruralis* are not conspecific with *C. praecox* and actually the name *ruralis* was considered as junior synonym of *C. mutabilis* Fallen (Claussen and Speight, 1999).

12. *Doros profuges* (Fabricius, 1775)

Doros conopseus (Fabricius, 1775) by Vujić and Glumac, 1994 (S)

Remark: Based on a study of Linnean collection, Thompson et al. (1982) established that the name *D. conopseus* of the authors is an unjust emendation. The oldest available name for this species is *profuges* Harris. Vujić and Glumac (1994) overlooked this nomenclature change.

13. *Epistrophe flava* Doczkal et Schmid, 1994

Epistrophe melanostomoides (Strobl, 1880) by Vujić and Glumac, 1994 (R)

Remark: *E. flava* was recently described (Doczkal and Schmid, 1994) based on a revision of central European species of the genus *Epistrophe*. The only record of this species in southeastern Europe was published for the area of Obedska bara in Serbia (Vujić et al., 1998). The range of the species includes from Scandinavia south to the Pyrenees and from Belgium eastwards through central and southern Europe (Spain, Italy) into European parts of Russia; through Siberia to the Pacific coast (Speight, 2001). The preferred environment of the species is deciduous forest, from riverine gallery forest of *Populus/Salix alba* to dry *Quercus/Castanea* forest and on to humid *Fagus/Picea* forest (Speight, 2001).

14. *Epistrophella euchroma* (Kowalewsky, 1885)

Vujić and Glumac, 1994: as *Epistrophe euchroma* (Kowarz, 1885)

New data: Paragovo, 1987 (Malaise trap), one male; Karlovački rit, 21 April 1993, one female leg. Vujić.

Remark: This taxon appears in recent literature under the genus name of *Epistrophella* Dušek et Laska.

15. *Eristalis interrupta* (Poda, 1761)

Eristalis nemorum (Linneaeus, 1758) by Vujić and Glumac, 1994 (S)

Remark: It has been established that *E. nemorum* Linnaeus differs from *E. nemorum* of the authors. The valid name of this species is *interrupta* Poda (Thompson et al., 1982). This is another oversight of Vujić and Glumac (1994).

16. *Eristalis similis* Fallen, 1817

Eristalis pratorum Meiegn, 1822 by Vujić and Glumac, 1994 (S)

Remark: During a study of Norwegian material of *Eristalis*, Nielsen (1995) found that the correct name for the taxon previously called *E. pratorum* is *E. similis* Fallen.

17. *Eupeodes bucculatus* (Rondani, 1857)

Syrphus latilunulatus Collin, 1931 (S)

Metasyrphus luniger (Meigen, 1822) by Vujić and Glumac, 1994, in part (E)

Revised data: Hopovo, 11 April 1988, one male (*Metasyrphus luniger* in Vujić and Glumac, 1994).

New data: Vrdnik, 31 March 2002, one male leg. Vujić.

Remark: *E. bucculatus* was recently redescribed and reinstated as valid taxon name (Mazanek et al., 1998). The range of the species includes from Scandinavia south to the Pyrenees and from Belgium eastwards through central and southern Europe (Spain, Italy) into the European parts of Russia; through Siberia to the Pacific coast (Speight, 2001). The only record of this species for southeastern Europe was published for the Tisza basin (Šimić and Vujić, 1987) under the name *Postosyrphus latilunulatus* Collin (synonym of *E. bucculatus*).

18. *Eupeodes corollae* (Fabricius, 1794)

Vujić and Glumac, 1994: as *Metasyrphus corollae* (Fabricius, 1794)

Remark: This genus appears in recent publications under the name *Eupeodes* Osten-Sacken, 1877, instead of the junior synonym *Metasyrphus* Matsumura, 1917.

19. *Eupeodes (Lapposyrphus) lapponicus* (Zetterstedt, 1838)

Vujić and Glumac, 1994: as *Metasyrphus lapponicus* (Zetterestedt, 1838)

20. *Eupeodes latifasciatus* (Macquart, 1829)

Vujić and Glumac, 1994: as *Metasyrphus latifasciatus* (Macquart, 1829)

21. *Eupeodes lucasi* (Marcos-Garcia and Laska, 1983)

Metasyrphus nuba (Wiedemann, 1830) by Vujić and Glumac, 1994 (R)

Metasyrphus luniger (Meigen, 1822) by Vujić and Glumac, 1994, in part (R)

Revised data: Vujić and Glumac, 1994: all data of *Metasyrphus nuba*; Iriški Venac, 30 April 1957, one female det. by Glumac as *Syrphus luniger*; Glavica, 1 May 1988, one male det. by Vujić as *Metasyrphus luniger*.

Remark: Marcos-Garcia and Laska (1983) described *Metasyrphus lucasi* based on a series of females captured in Spain. An unknown male of this species was recently described (Marcos-Garcia et al., 2000). The range of *E. lucasi* includes Spain, French Pyrenees, Corsica, the Alps, the

Apennines, Sicily, the Czech Republic, Hungary, FR Yugoslavia, Macedonia, Greece (Marcos-Garcia et al., 2000). A revision of material from Yugoslavia has confirmed the presence of the species in this area. These records from the Fruška Gora Mountain are the first published data on the species in Yugoslavia.

22. *Eupeodes luniger* (Meigen, 1822)

Vujić and Glumac, 1994: as *Metasyrphus luniger* (Meigen, 1822)

New data: Rokov potok, 29 March 1998, one male leg. Radišić.

23. *Fagisyrphus cinctus* (Fallen, 1817)

Vujić and Glumac, 1994: as *Melangyna cincta* (Fallen, 1817)

Remark: There are three different approaches to the status of this species: a member of the taxon *Meligramma*, as subgenus of the genus *Melangyna* (Peck, 1988) or as a separate genus (Speight, 2001); a species belonging to the genus *Fagisyrphus* (Ssymank et al., 1999). The isolated morphological features of the species support the last position, as accepted in this paper.

24. *Helophilus trivittatus* (Fabricius, 1805)

Helophilus parallelus (Harris, 1776) by Vujić and Glumac, 1994 (S)

Remark: *H. parallelus* as valid name for this taxon was presented in Peck's catalogue (1988) and used by Vujić and Glumac (1994). In recent publications this synonym is not accepted.

25. *Lejogaster tarsata* (Megerle in Meigen, 1822)

Lejogaster splendida (Meigen, 1822) by Vujić and Glumac, 1994 (S)

Remark: Maibach et al. (1994a) established that *Chrysogaster tarsata* is a senior synonym of *Lejogaster splendida*. This nomenclature change was previously published for the material from the Fruška Gora Mountain (Vujić, 1999).

26. *Mallota cimbiciformis* (Fallen, 1817)

New data: Glavica, 18 August 2001, one female leg. Vujić.

Remark: The range of this species includes the territory from southern Fennoscandia south to the Pyrenees, central Spain and on into North Africa; from Britain east through most of Europe to central Siberia; northern Iran (Speight, 2001). The only previous record of the species in Serbia was published by Glumac (1955), from the region of Homolje. This is an extremely rare and threatened hoverfly on the Balkan Peninsula. The preferred environment of *M. cimbiciformis* is the forest; deciduous forest of *Fagus* and *Quercus* with overmature and senescent trees, and evergreen oak forest of *Q. suber* and *Q. ilex* (Speight, 2001).

27. *Melanogaster nuda* (Macquart, 1829)

Chrysogaster lucida (Scopoli, 1763) by Vujić and Glumac, 1994 (S)

Remark: Based on a revision of Linnaeus collection, Thompson et al. (1982) mentioned that the species *viduata* of the authors corresponded to *Chrysogaster lucida*. This datum was used by Vujić and Glumac (1994) in previous publication. Maibach et al. (1994a) established that the name *lucida* has been wrongly applied as a replacement name for *viduata* auct. nec L. The oldest corresponding name for this species is *nuda* (Maibach et al., 1994a), accepted by Vujić (1999) for the Balkan material.

28. *Meligramma guttata* (Fallen, 1817)

Vujić and Glumac, 1994: as *Melangyna guttata* (Fallen, 1817)

Remark: *Meligramma* is frequently cited as subgenus of the genus *Melangyna* (Peck, 1988). It appears as a separate genus in recent publications (Ssymank et al., 1999; Speight, 2001).

29. *Meligramma trianguliferа* (Zetterstedt, 1843)

Vujić and Glumac, 1994: as *Melangyna trianguliferа* (Zetterstedt, 1843)

New data: Glavica, 1 May 1988, one male leg. Vujić; 30 April 1994, one female leg. Vujić; Stari Ledinci, 19 April 1988, one male leg. Vujić.

30. *Merodon recurvus* (Strobl, 1898)

Merodon strobli Bradescu, 1986 by Vujić and Glumac, 1994 (S)

Remark: Dirickx (1994) revised the status of the names *recurvus* and *strobli* and reinstead the older name as valid for this taxon.

31. *Microdon analis* (Macquart, 1842)

Microdon latifrons Loew, 1857 by Vujić and Glumac, 1994 (S)

Remark: Speight (1994) established *M. latifrons* as a junior synonym of *M. analis*. The central European taxa of the genus *Microdon* have been recently revised by Doczkal and Schmid (1999).

32. *Myolepta dubia* (Fabricius, 1805)

Myolepta luteola (Gmelin, 1790) by Vujić and Glumac, 1994 (S)

Remark: Thompson and Pont (1994) demonstrated that the name *luteola* is not applicable for this species and introduced *dubia* of Fabricius as the valid replacement.

33. *Parasyrphus punctulatus* (Verrall, 1873)

Parasyrphus macularis (Zetterstedt, 1843) by Vujić and Glumac, 1994 (E)

Revised records of *P. macularis* in Vujić and Glumac, 1994: *P. punctulatus*: Glavica, 19 April 1988, one male; 25 March 1989, one male, one female; 5 April 1989, four males; Hopovo, 25 March 1989, one male; Stari

Ledinci, 26 March 1989, three males; 5 April 1989, one male. *P. annulatus* (Zetterstedt, 1838): Andrevlje, 22 April 1988, one male.

New data: Glavica, 5 April 1989, one female leg. Vujić; 4 April 1995, one male, one female leg. Vujić.

Remark: There is considerable confusion about *P. macularis* in the literature. It is extremely similar to *P. punctulatus* (Speight, 2001). Revision of material from Serbia has shown the presence of both species in this area, but *P. punctulatus* is found only on the Fruška Gora Mountain. Previous record of this species in Serbia was published by Kula (1985), for the vicinity of Belgrade. Its range occupies the area from Fennoscandia south to the Pyrenees; from Ireland eastwards through northern and central Europe (plus northern Italy) into European parts of Russia and on through Siberia to the Pacific coast (Japan) (Speight, 2001). The preferred environment of the species is the forest; deciduous and coniferous forests and conifer plantations; *Quercus/Fraxinus* and *Betula/Salix/Alnus* forests and woodlands and forests and plantations of *Pinaceae* or *Larix*; plus suburban gardens and orchards with mature trees (Speight, 2001).

34. *Parhelophilus frutetorum* (Fabricius, 1775)

Vujić and Glumac, 1994: as *Helophilus frutetorum* (Fabricius, 1775)

Revised records: Petrovaradinski rit, 23 May 1985, two males; Glavica, 18 May 1988, one male (*Helophilus versicolor* in Vujić and Glumac, 1994).

Remark: Peck (1988) regards *Parhelophilus* as a subgenus of the genus *Helophilus*. In recent publications this taxon appears separately (Ssymank et al, 1999; Speight, 2001).

35. *Parhelophilus versicolor* (Fabricius, 1794)

Vujić and Glumac, 1994: as *Helophilus versicolor* (Fabricius, 1794)

Revised records: Sremska Kamenica, 25 June 1978, one female; Iriški Venac, 26 July 1983, one female; Petrovaradinski rit, 2 May 1985—23 June 1986, three males, nine females.

36. *Platycheirus albimanus* (Fabricius, 1781)

Platycheirus cyaneus (Muller, 1764) by Vujić and Glumac, 1994 (S)

Remark: In Peck's catalogue (1988) this species was referred to as *P. cyaneus*. That was accepted by Vujić and Glumac (1994), but Vockeroth (1990) provided a convincing argument as to why the name should revert to *albimanus*.

37. *Platycheirus europeus* Goeldlin, Maibach et Speight, 1990

Platycheirus clypeatus (Meigen, 1822) by Vujić and Glumac, 1994 (in part) (R)

Revised records: Mutalj, 13 July 1982, one female (*P. clypeatus* in Vujić and Glumac, 1994); Hopovo, 2 August 1983, one female (*P. clypeatus* in Vujić and Glumac, 1994).

Remark: Three new European species of the genus *Platycheirus*, closely related to *P. clypeatus*, were recently described (Goeldlin et al., 1990). Revision of specimens from *clypeatus* group collected on the Fruška Gora Mountain has shown the presence of two taxa among material previously determined and published as *P. clypeatus* (Vujić and Glumac, 1994): *P. europeus* and *P. occultus*. Both species have been unknown for southeastern Europe until now. The known range of *P. europeus* includes Sweden, Finland, Denmark, Britain, Germany, Netherlands, Belgium, France (the Cote d'Or, Vosges, the Alps and the Pyrenees), the Czech Republic, Hungary, the Swiss Plateau (the low-altitude plains between the Jura and the Alps), Austria, Spain and Italy (Speight, 2001). The preferred environment of the species is wetland/open ground; in particular, brook floodplains and wet flushes in montane grassland, grassy glades beside streams or flushes in forest in the *Carpinus/Quercus* zone up into the *Fagus/Picea* zone (including humid *Pinus*) and in humid unimproved grassland (Speight, 2001).

38. *Platycheirus occultus* Goeldlin, Maibach et Speight, 1990

Platycheirus clypeatus (Meigen, 1822) by Vujić and Glumac, 1994 (in part) (R)

Revised records: Karlovački rit, 22 March 1990, one male (*P. clypeatus* in Vujić and Glumac, 1994); Glavica, 25 March 1990, one male (*P. clypeatus* in Vujić and Glumac, 1994).

New data: Ljuba, 5 April 1990, one female leg. Vujić.

Remark: *P. occultus* is extremely similar to *P. clypeatus* and *P. europaeus*, from which in nearly all cases it may be distinguished using the keys of Speight and Goeldlin (1990). The range is not yet adequately known, so far it has been recorded in Sweden, Norway, Denmark, Ireland, Britain, northern Germany, France, lowland parts of Switzerland, Liechtenstein (the Rhine valley), Spain and northern Italy (Speight, 2001). The above records from the Fruška Gora Mountain are first for southeastern Europe.

The preferred environments of the species are wetland; fen and the periphery of raised bog, coastal marsh and dune slacks, humid, seasonally-flooded, unimproved grassland, moorland (Speight, 2001).

39. *Rhingia rostrata* (Linnaeus, 1758)

New data: Andrevlje, 27 May 1981, one female leg. Vujić; Paragovo—Zmajevac, 21 August 1981, one male leg. Vujić.

Remark: A revision of undetermined material from the Fruška Gora Mountain has indicated the presence of *R. rostrata* in this area. The only previous record of this species in Serbia was published by Kula (1985) for the Avala Mountain. The range of *R. rostrata* occupies the area from southern Finland and Denmark south to northern Spain; from Britain (Wales, southern England) eastwards through central Europe into European parts of Russia, the Caucasus and western Siberia (Speight, 2001). The preferred environment of species

is the forest; deciduous forest (*Quercus, Fraxinus/Fagus*) and scrub with a rich, tall-herb ground flora (Speight, 2001).

40. ***Riponnensia splendens*** (Meigen, 1822)

Vujić and Glumac, 1994: as *Orthoneura splendens* (Loew, 1843)

Remark: Based on a revision of the genera of the tribe *Chrysogasterini*, Maibach et al. (1994b) described the genus *Riponnensia* with four species, including *Riponnensia splendens*. This change of taxonomic status for the species from the Fruška Gora Mountain was published recently (Vujić, 1999).

41. ***Temnostoma meridionale*** Krivosheina et Mamaev, 1962

Temnostoma vespiforme (Linneaus, 1758) by Vujić and Glumac, 1994 (E)

Remark: This species was recently recognized in southeastern Europe (Vujić et al., 1998). Its range includes Finland, Estonia, Latvia, Germany, central and southwestern France (including the Pyrenees), the Czech Republic, Romania, the Ukraine, European parts of Russia and the Caucasus (Speight, 2001). In Serbia, *T. meridionale* is presently known only in the location of Obedska bara and on the Fruška Gora Mountain.

42. ***Xanthogramma festiva*** (Linnaeus, 1758)

Xanhogramma citrofasciatum (De Geer, 1776) by Vujić and Glumac, 1994 (S)

New data: Glavica, 4 May 1994, two males leg. Radenković.

Remark: Based on a study of Linnean collection, Thompson et al. (1982) established that the type specimen of *Musca festiva* belongs to the genus *Xanthogramma* and not to *Chrysotoxum*. The name *citrofasciatum* became a junior synonym of *X. festiva*. Vujić and Glumac (1994) omitted this nomenclature change.

43. ***Xylota abiens*** Meigen, 1822

Record: Čortanovci, 19 March 1994, one female leg. Vujić (published in Vujić and Milankov, 1999).

Remark: There are few records of this species in Serbia (the Vršac Mountain, the Fruška Gora Mountain and the location of Bosilegrad) (Milankov et al., 1995; Vujić and Milankov, 1999).

44. ***Xylota segnis*** (Linnaeus, 1758)

Xylota florum (Fabricius, 1758) by Vujić and Glumac, 1994 (E)

Remark: A study of the specimen published as *X. florum* in Vujić and Glumac (1994) showed that it belongs to *X. segnis*. Based on that, *X. florum* must be excluded from the list of species present on the Fruška Gora Mountain.

Excluded and replaced names

Cheilosia clama Claussen et Vujić = *C. alba* Vujić et Claussen, 2000 (E)

Cheilosia zetterstedti Becker, 1894 = *C. aerea* Dufour, 1848 (S)

Cheilosia rufipes (Preyssler, 1798) = *C. soror* (Zetterstedt, 1843) (S)

Cheilosia ruralis (Meigen, 1822) = *C. urbana* (Meigen, 1822) (S)

Chrysogaster lucida (Scopoli, 1763) = *C. nuda* (Macquart, 1829) (S)

Doros conopseus (Fabricius, 1775) = *D. profuges* (Fabricius, 1775) (S)

Epistrophe melanostomoides (Strobl, 1880) = *E. flava* Doczkal et Schmid, 1994 (R)

Eristalis nemorum (Linneaeus, 1758) = *E. interrupta* (Poda, 1761) (S)

Eristalis pratorum Meigen, 1822 = *E. similis* Fallen, 1817 (S)

Metasyrphus nuba (Wiedemann, 1830) = *Eupeodes lucasi* (Marcos-Garcia et Laska, 1983) (R)

Helophilus lunulatus Meigen, 1822 = *Anasimyia interpuncta* (Harris, 1776) (E)

Helophilus parallelus (Harris, 1776) = *H. trivittatus* (Fabricius, 1805) (S)

Lejogaster splendida (Meigen, 1822) = *L. tarsata* (Megerle in Meigen, 1822) (S)

Merodon strobli Bradescu, 1986 = *M. recurvus* Strobl, 1898 (S)

Myolepta luteola (Gmelin, 1790) = *M. dubia* (Fabricius, 1805) (S)

Parasyrphus macularis (Zetterstedt, 1843) = *P. punctulatus* (Verrall, 1873) (E)

Platycheirus clypeatus (Meigen, 1822) = *P. europeus* Goeldlin, Maibach et Speight, 1990 and *P. occultus* Goeldlin, Maibach et Speight, 1990 (R)

Platycheirus cyaneus (Muller, 1764) = *P. albimanus* (Fabricius, 1781) (S)

Temnostoma vespiforme (Linneaus, 1758) = *T. meridionale* Krivosheina et Mamaev, 1962 (E)

Xanhogramma citrofasciatum (De Geer, 1776) = *X. festiva* (Linnaeus, 1758) (S)

Xylota florum (Fabricius, 1758) = *X. segnis* (Linnaeus, 1758) (E)

Species under revision

Three species groups are still subject of study and contain species with unsolved taxonomic status and uncertain names. These are: *Cheilosia canicularis* (Panzer, 1801), a group with two species; *Merodon avidus* (Rossi, 1790), a complex and *Paragus bicolor* (Fabricius, 1794), a complex (the last two groups each have one species recorded on the Fruška Gora Mountain).

CONCLUSION

Based on new investigations, nomenclature changes and recently published data, the following data of special importance for hoverflies diversity on the Fruška Gora Mountain should be stated:

— three species have been recorded for the first time in southeastern Europe: *Anasimyia interpuncta*, *Platycheirus europeus* and *P. occultus*;

— this is the first published record of *Eupeodes lucasi* for FR Yugoslavia;

— seven species have been found for the first time on the Fruška Gora Mountain: *Chalcosyrphus eunotus*, *Cheilosia psilophthalma*, *C. ranunculi*, *Eupeodes bucculatus*, *Mallota cimbiciformis*, *Rhingia rostrata* and *Xylota abiens*;

— on the basis of material revision and redetermination, seven taxa from the previously published list of hoverfly species of the Fruška Gora Mountain have been replaced: *Cheilosia alba* (instead *C. clama*); *Epistrophe flava* (instead of *E. melanostomoides*); *Eupeodes lucasi* (instead of *E. nuba*); *Parasyrphus punctulatus* (instead of *P. macularis*); *Platycheirus europeus* and *P. occultus* (instead of *P. clypeatus*); *Temnostoma meridionale* (instead of *T. vespiforme*);

— seven species were excluded from the hoverfly fauna of the Fruška Gora Mountain: *Anasimyia lunulata*, *Cheilosia clama*, *Epistrophe melanostomoides* (syn. of *E. melanostoma* Zett.), *Eupeodes nuba*, *Parasyrphus macularis*, *Platycheirus clypeatus*, *Temnostoma vespiforme* and *Xylota florum*;

— 16 species with recently established junior synonyms and 12 species with new generic names have been mentioned;

— presently, the above mentioned results complete the list of 210 hoverfly species registered on the Fruška Gora Mountain.

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НОВИ ПОДАЦИ О ДИВЕРЗИТЕТУ ОСОЛИКИХ МУВА (*Insecta: Diptera: Syrphidae*) ФРУШКЕ ГОРЕ (СРБИЈА)

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Резиме

Фауна осоликих мува Фрушке горе је добро проучена захваљујући дугогодишњим истраживањима (1956—2002). Досад су биле регистроване 203 врсте (Вујић и Глумац, 1994). На бази нових истраживања, номенклатурних промена и скорије публикованих података, добијени су следећи резултати: три врсте су регистроване по први пут за југоисточну Европу, једна врста за С. Р. Југославију, а седам врста за Фрушку гору; седам претходно публикованих врста је замењено новима, а седам је искључено са листе фауне Фрушке горе; за 16 врста су коришћени недавно установљени синоними, а за 12 нова генеричка имена. На бази ових резултата, новоустановљена листа броји 210 врста осоликих мува за Фрушку гору.