YU ISSN 0352-4906 UDK 5/6 (05)

# ЗБОРНИК

## МАТИЦЕ СРПСКЕ ЗА ПРИРОДНЕ НАУКЕ

## MATICA SRPSKA PROCEEDINGS FOR NATURAL SCIENCES

ЗБОРНИК МАТИЦЕ СРПСКЕ ЗА ПРИРОДНЕ НАУКЕ 99

# 99

NOVI SAD
2000


МАТИЦА СРПСКА
1826

МАТИЦА СРПСКА
ОДЕЉЕЊЕ ЗА ПРИРОДНЕ НАУКЕ

ЗБОРНИК
МАТИЦЕ СРПСКЕ ЗА ПРИРОДНЕ НАУКЕ

MATICA SRPSKA
DEPARTMENT OF NATURAL SCIENCES
PROCEEDINGS FOR NATURAL SCIENCES

Покренут 1951 / First published in 1951.

Published as *Научни зборник*, серија природних наука until the tenth issue (1955), as the Series for Natural Science from the eleventh issue (1956) — *Зборник за природне науке*, and under its present title since the sixty-sixth issue (1984)

*Главни уредници / Editors-in-Chief*

Miloš Jovanović (1951), Branislav Bukurov (1952—1969),
Lazar Stojković (1970—1976), Slobodan Glumac (1977—1996), Rudolf Kastori (1996—)

# 99

YU ISSN 0352-4906 UDK 5/6 (05)

# MATICA SRPSKA PROCEEDINGS FOR NATURAL SCIENCES

# 99

NOVI SAD
2000

# CONTENTS
# САДРЖАЈ

## ARTICLES AND TREATISES
## ЧЛАНЦИ И РАСПРАВЕ

*Зборник Матице српске за природне науке* издаје Матица српска
Излази двапут годишње
Уредништво и администрација: Нови Сад, Улица Матице српске 1
Телефон: 021/420-199
*Proceedings for Natural Sciences* published by Matica Srpska
Published twice a year
Editorial and publishing office: Novi Sad, Ul. Matice Srpske 1
21000 Novi Sad, Yugoslavia
Telefon: 021/420-199

---

The editors of the Matica srpska *Proceedings for Natural Sciences*
Completed the selection for Issue 99/2000 on July 9, 2001
Editorial Staff Secretary: Julkica Boarov
Managing editor: Dr. Slavka Gajin
English text proof-reader: Srđan Vraneš and Vera Vasilić
Technical design: Vukica Tucakov
Published in December 2000
Publish by: Mladen Mozetić, GRAFIČAR, Novi Sad
Printed by: „Ofset Ideal štampa", Novi Sad

---

Публиковање овог броја помогли су Војвођанска банка и
Научни институт за ветеринарство, Нови Сад.
The edition and printing of the Proceedings has been financially supported by the
Vojvođanska banka and Scientific Institute for Veterinary Medicine, Novi Sad.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 5—18, 2000

UDC 541.181.2

*Lazar M. Dovniković, Radovan B. Štulić*

Faculty of Engineering, University of Novi Sad
Cara Lazara 1, 21000 Novi Sad
Yugoslavia

# UNIFORM CONSTRUCTIONS OF THE RATIONAL 4th ORDER PARABOLAS

ABSTRACT: If some 4th order curves, which belong to the same class of the 4th order parabolas, have their joint property „hidden" in infinity they may have seemingly nothing in common. Namely, such curves have consecutive double points in infinity (the infinitely distant line is a tangent to the curve at those points) and their existence is proved by the birational quadratic transformation of a particular disposition of the elements of the transformation.

Thus, a circle can be transformed into the known parabolic lemniscate (also known as the lemniscate of Geronault) which has two isolated consecutive double points. By the same transformation the rectangular hyperbola (being the complement of the circle) is transformed into the complementary curve of the parabolic lemniscate. This curve has two real consecutive double points — crunodes which are coincident with acnodes of the parabolic lemniscate, all of them being in infinity. Those two complementary curves also have the third mutually coincident double point which is a crunode for the lemniscate and an acnode for its complement. Because of the three double points, such 4th order parabolas are rational, that is, they are of the genus zero.

In this paper, besides the parabolic lemniscate and its complement, some other 4th order parabolas obtained by the birational quadratic transformation, as well as their properties have been analyzed. Such curves are: 1) periform, 2) curve with an isolated point, 3) selftouching 4th order parabolas affine to the periform, 4) obliquely symmetric 4th order parabolas, 5) oblique selftouching 4th order parabolas and 6) orthogonally symmetric selftouching 4th order parabolas. Their projective complements form a group of curves: the infinitely distant line touches each of them at the selftouching point.

KEY WORDS: birational quadratic transformation, constructive geometry, fourth order parabolas

## INTRODUCTION

It has been shown [Dovniković, 1987], [Dovniković, 1990] that a birational quadratic transformation (Fig. 1) transforms a conic (point $M_o$) into a curve of the 4th order (point $M$) with three real double points $A$, $\overline{A}$, $V$. Therefore the curve is of the genus zero, that is, it is a rational algebraic curve. It means that any rational curve of the 4th order can be transformed into a

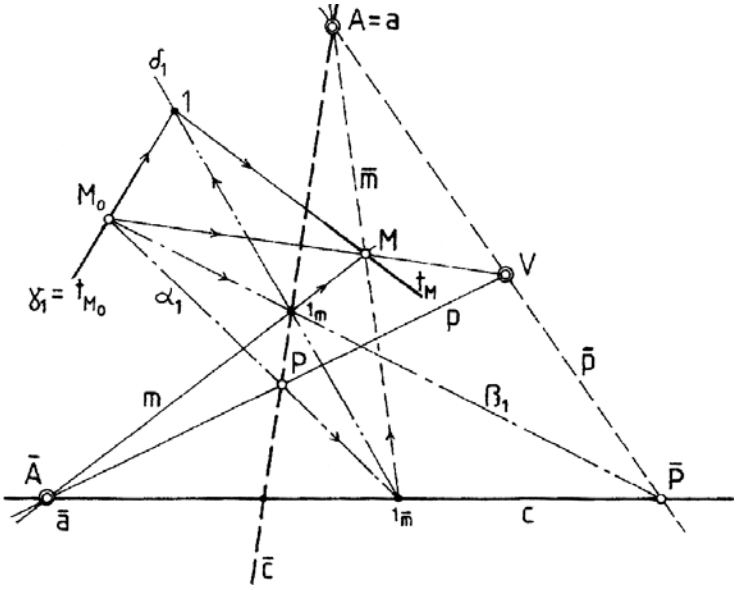



Fig. 1 — A birational quadratic transformation

conic by an inverse transformation whenever its double points are coincident with the fundamental points *P, $\bar{P}$, V* of the transformation. Now, the conic is only a part of a degenerate total curve of the 8th order. Thus, in order to transform a curve of the 4th order into a conic, it is necessary to know its three double points. Unfortunately these double points are seldom mentioned, particularly when they are isolated and in infinity (further on: ideal points). The knowledge of these points is certainly of the crucial importance not only for curve classification but for the geometrical construction, that is, in the synthesis of mechanisms for the mechanical derivation of motion along the curve. Therefore, if isolated double points are not known it is necessary to find a conic „intuitively" which is, according to the particular disposition of fundamental elements of the mapping, transformed into that very curve. Only then can it be stated that the curve has three double points *A, $\bar{A}$, V*. For example, it has been shown [D o v n i k o v i ć , 1989] that cubics of the Versier family have an ideal isolated double point, i.e., they belong to the rational cubic hyperbolic parabolas.

In this paper, it has been shown, in the same way, that the known curves such as parabolic lemniscate, periform, curves de Sluse, virtual parabolas of Cramer etc., have consecutive isolated double points, that is, they belong to the same group of the 4th order parabolas. Their projective complements form a group of curves which the infinitely distant line (further on: ideal line) touches each of them at the selftouching point. Since curves with two ideal isolated coincident double points at infinity have, in fact, the selftouching of imaginary branches of the curve, both groups of curves could be named as a class of selftouching 4th order parabolas.

In order to be a rational curve (i.e., of the genus zero) a selftouching 4th order parabola, because of two ideal coincident double points, must have its third double point in finiteness.

## RESULTS OF INVESTIGATION

### 1. *Parabolic lemniscate and its complement*

The „eight", obtained by the projecting of the known Viviani space curve (Fig. 2) is, in fact, a curve known as the parabolic lemniscate. From its deriva-

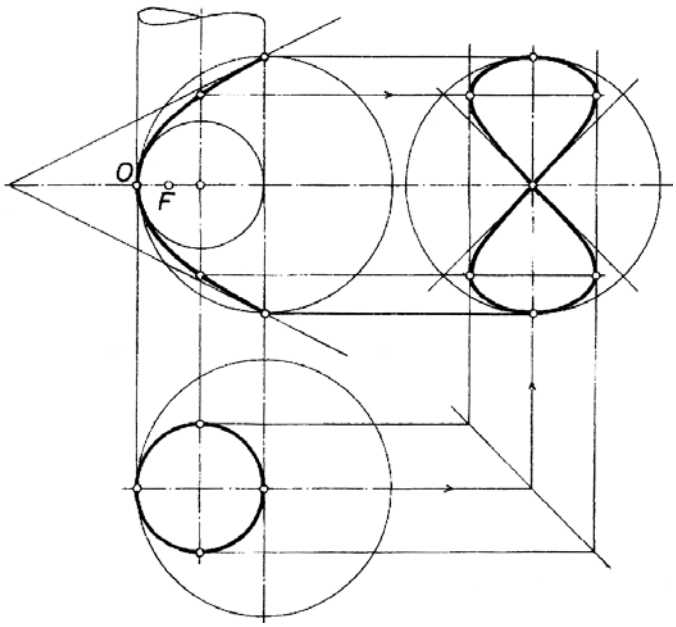



Fig. 2. — The Viviani space curve

tion, it is obvious that the curve has one crunode. However, the possibility of its derivation by the birational quadratic transformation (Fig. 3) proves that this curve has two more double points. Namely, the parabolic lemniscate is obtained by transforming the curve when $A$ is in the centre of the circle, axis $c$ is the tangent to the circle and $P$ is the ideal point of the normal to the line $c$, $V$ is the ideal point of the line $c$, which means that, apart from the double points $A$ and $V_\infty$, there is one more double point $\overline{A}$, the point of intersection between the line $p = PV$ and the first axis of mapping $c$ (compare with Fig. 1). Since $p$ is an ideal line, the point $\overline{A}$ is ideal point of the line $c$, i.e., it is coincident with the point $V$. Therefore, the parabolic lemniscate, apart from crunode $A$ has two ideal coincident acnodes $\overline{A} = V$. According to the principle of the projective complementariness [D o v n i k o v i ć , 1977], the complement

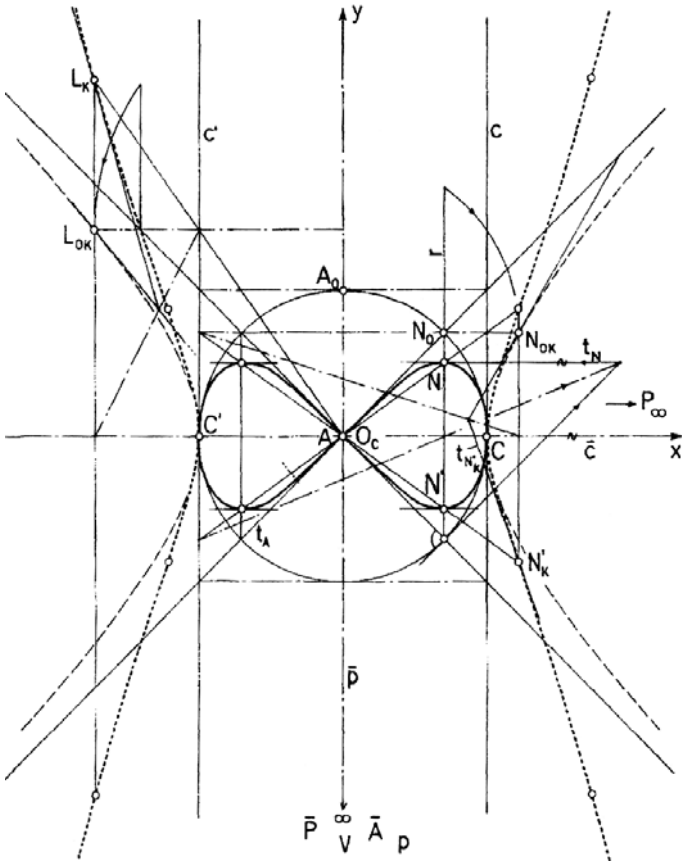



Fig. 3. — The parabolic lemniscate and its complement

of the parabolic lemniscate is obtained by mapping the rectangular hyperbola (the complement of the circle for the pole $V\infty$), and it has the same double points $A$, $\overline{A}$, $V$. However, for this curve (in Fig. 3 dotted curve) the point $A$ is acnode, while the ideal coincident double point $\overline{A} = V$ is now the touching point of two ideal branches of the curve; the ideal line $p = P\overline{A}V$ touches branches of the curve really, and it also touches imaginary branches of the parabolic lemniscate. At this coincident double point only the ideal line meets complementary curves at four points; all other lines meet them here at two consecutive points and two more, either real or conjugate — imaginary in finiteness.

Since $P$ is in infinity, the third axis of mapping $c'$, because of the $(APCC') = -1$, is symmetric to the axis $c$ [D o v n i k o v i ć , 1987]. Both these axes of the mapping are tangents to the circle-original, so that it osculates the lemniscate at its vertices [D o v n i k o v i ć , 1989]. Since the mentioned axes are the tangents to the rectangular hyperbola, the complementary 4th order parabola has the same curvature at the vertices as the lemniscate. Points and tangents of both curves are constructed according to the general principle

shown in Fig. 1 [D o v n i k o v i ć , 1987], [D o v n i k o v i ć , 1989]. (In Fig. 3, inflexional points and tangents to the complement of the lemniscate are constructed, but acceptable synthetic-geometric proof for the algorithm, saying that the inflexional point $N_k$' lies on the ray $AN'$ through the point $N'$ as the touching point of the tangent to the lemniscate constructed from $P$, has not been performed yet.)

If the axis $c$ is moved parallelly to $y$ into the position where either $a > r$ or $a < r$ is valid, the curves of Sluse $x^4 - r^2x^2 + a^2y^2 = 0$ [5] will be obtained, which are affine to the parabolic lemniscate, so that the complements of the curves of Sluse $x^4 - r^2x^2 - a^2y^2 = 0$ are affine to the complement of the lemniscate. In other words, the parabolic lemniscate and its complement are the special case of the curve of Sluse and its complement, when $a = r$.

## 2. *Periform and its complement*

If the point $A$ (Fig. 3) is moved away from the centre of the circle along the $x$ axis, the obtained „eight" will have unequal loops. By moving $A$ towards $C'$ the left loop will diminish and the tangents at the crunode $A$ will tend to $x$ axis. When $A$ comes to $C'$ the pair of tangents at $A$ will become coincident, that is, the double tangent at the cusp of the so called periform [A r t o b o -

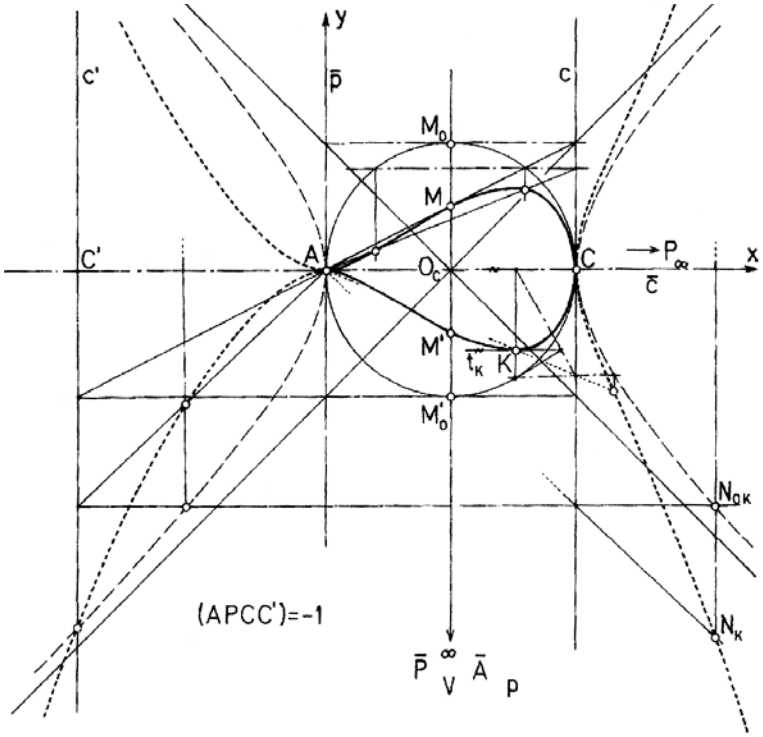



Fig. 4. — The periform and its complement

l e v s k i j , 1959], i.e., the pair of conjugate imaginary tangents at isolated double point $A$ will coincide at the real tangent at the contra-cusp of the periform's complement (Fig. 4). It is evident that the periform, same as the parabolic lemniscate, has, apart from the cusp at $A$, two more double points which are coincident in infinity. The periform is also called antiversier of circle, because the antiversier of circle — known as the Agnesi versier — is obtained when the points $A$ and $P$ interchange their position (since $P$ is on the circle, the antiversier is a 3rd order curve).

According to the principle of the projective complementariness the equation of the complement of periform $x^4 - 2rx^3 - 4r^2y^2 = 0$ is obtained from the equation of the periform [A r t o b o l e v s k i j , 1959] $x - 2rx^3 + 4r^2y^2 = 0$ by changing the sign in front of the member which contains the ray of projecting (y).

### 3. *Curve with an isolated point and its complement*

When the point $A$ on the $x$-axis is not on the circle, the 3rd order family of complementary curves is obtained, that is, a curve with an acnode and its

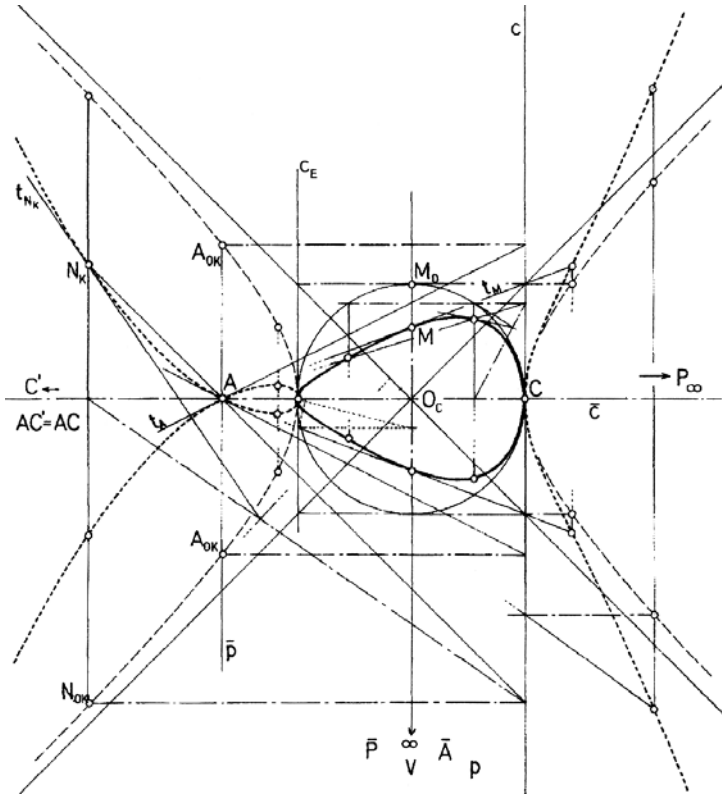



Fig. 5. — The curve with an isolated point and its complement

complement with crunode $A$ (Fig. 5). By moving away the point $A$ to infinity, a degeneration appears (since $P$ is in infinity) and the curve with an isolated point becomes a circle while the curve with the crunode becomes a rectangular hyperbola (which can be an indication that, in a certain real space, the point $A$ is selfsecting point of the hyperbola and the isolated point of the circle; the tangents at the crunode $A$ are asymptotes to the hyperbola and a real image of the isotropic asymptotes to the circle!). The curvature module of the complementary curves at the vertex $C$ is equal to the curvature of the initial circle, while, at another vertex, it is determined as a curvature of an ellipse which gives the same curvature where axis of mapping is the vertex tangent $c_E$ [D o v n i k o v i ć , 1989].

### 4. *Selftouching 4th order parabolas affine to periform*

If the axis $c$ is moved so that it becomes a passant to the circle (Fig. 6) or a secant to the circle (Fig. 7) we will get curves affine to the periform for the axis of affinity $\bar{c} = x$. In the same way their complements are affine to the complement of the periform. The curve in Figure 7 could be named antipseudoversier, since an interchange of points $A$ and $P$ gives a curve of the 3rd order, called pseudoversier. In Figure 7, an osculatory ellipse at the vertex $T$ of the curve is determined, which means that the anti-pseudoversier can be obtained by mapping that very ellipse, but, with respect to the axis $c_E$ which touches the ellipse. This ellipse is affine to the original circle wifh respect to the same affinity between the anti-pseudoversier (antiversier of ellipse) and the periform (antiversier of circle). It means that the position of the axis $c$ can be de-

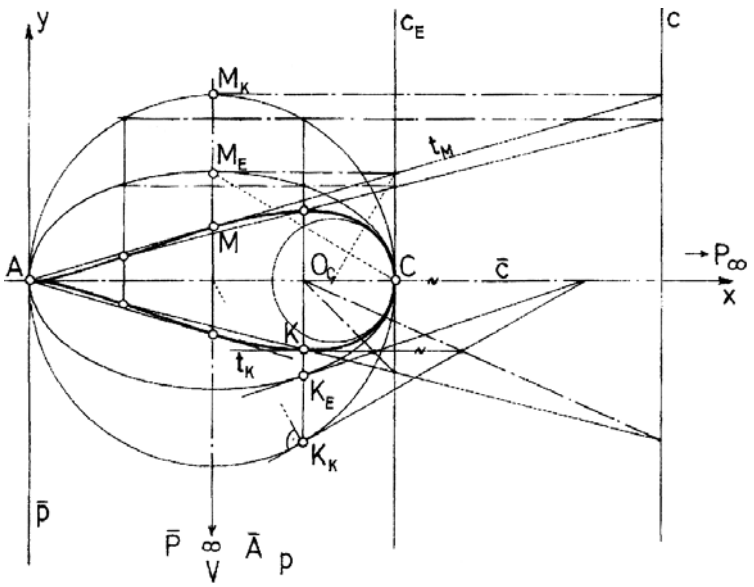



Fig. 6. — The selftouching 4th order parabola (axis $c$ passant to the circle)

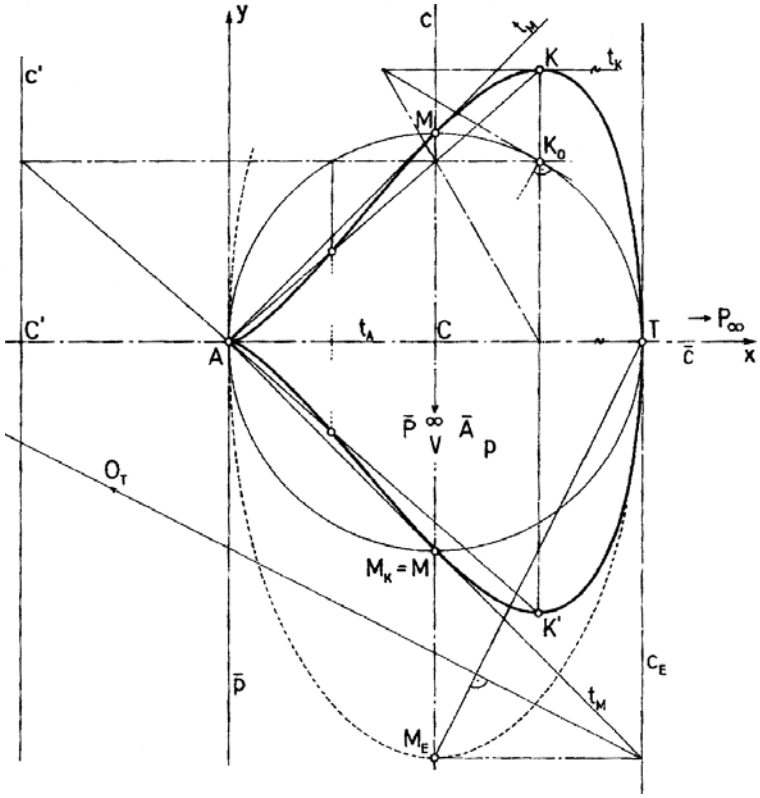



Fig. 7. — The selftouching 4th order parabola (axis $c$ secant to the circle)

termined after choosing the curvature (that is the osculatory ellipse) of the curve at the vertex $T$, which is in fact the determination of the point $M$ at which the curve meets the original circle.

The same affine relations are valid for the curve in Figure 6. It is affine „squeezed" periform which is obtained by the mapping of either the osculatory ellipse with respect to the tangent axis $c_E$ or of the circle for the moved axis $c$.

The fact that all antiversiers of ellipse can be obtained by the mapping of the circle with respect to a certain axis $c$ is of significant importance for the synthesis of mechanisms which derive those curves. Namely, in the total mechanism, ellipsographs can be replaced with the simple rotation. In case of complementary curves, it is similar, i.e., the mechanism has only a hyperbolograph for the rectangular hyperbola, and the varieties for the other (affine-related) hyperbolas can be obtained by parallel moving of the axis $c$.

## 5. *Obliquely symmetric selftouching 4th order parabolas*

If we move away the point $A$, i.e., the second axis of mapping $\bar{c}$ (since $P$ is in infinity), from the horizontal diameter of the circle, an obliquely symmet-

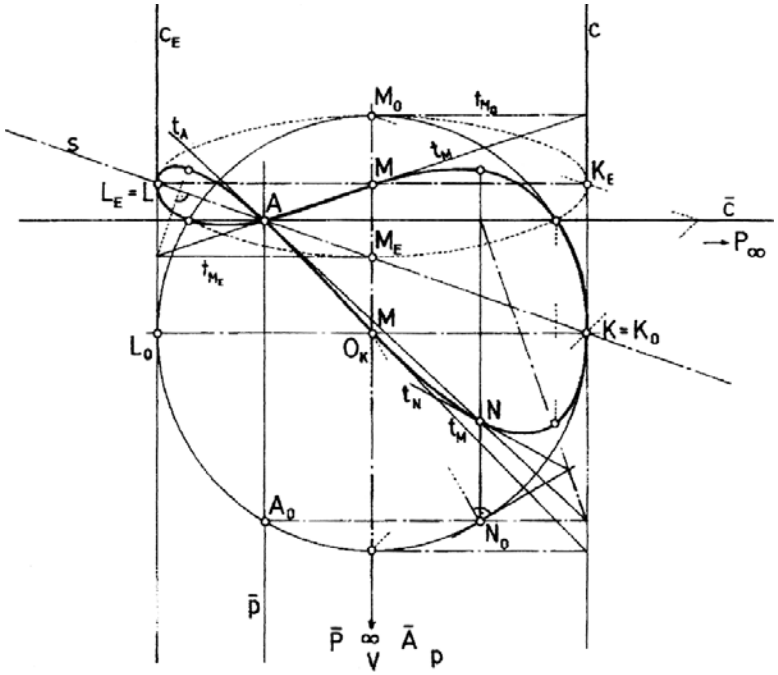



Fig. 8. — The obliquely symmetric „eight" of the parabolic type

ric „eight" of the parabolic type will be obtained (Fig. 8), as well as the corresponding complementary obliquely symmetric 4th order parabola (with corresponding isolated double point $A$) which is not drawn. The common axis of oblique symmetry of the complementary curves passes through their touching points $K$ and $L$ (points on the contour line of the projecting cylinder of the original circle, i.e., rectangular hyperbola). The curvature at the point $K = K_o$ is equal to the curvature of the original circle, while the curvature at the point $L$ is determined as the curvature of the osculatory ellipse of the curve at that point. This osculatory ellipse is determined as an ellipse which is mapped into the same 4th order parabola (the point $M$ is obtained by the mapping of either the point $M_o$ of the circle with respect to the axis $c$ or of the point $M_E$ of the ellipse with respect to the axis $c_E$, *so that the tangent* $t_M$ is the connecting line of the points where the tangent to the circle at $M_o$ i.e, the tangent to the ellipse at $M_E$ respectively meet axes $c$, and $c_E$). The osculatory ellipse at the point $L$ of the curve is affine to the osculatory circle at the point $K$ (the axis of affinity is $\bar{c}$).

When the point $A$ is in the middle (on the axis of the projecting circular and hyperbolic cylinders) a centrally symmetric 4th order parabola with a pair of conjugate axes of oblique symmetry (Fig. 9) is obtained. Because of the symmetry, the curve has the same curvature at the points $K$ and $L$ since the osculatory „ellipse" at the point $L$ is now the circle affine to the original circle ($\bar{c}$ is the axis of that affinity), and because of the central symmetry of the curve, the tangents at the crunode $A$ are inflexional.

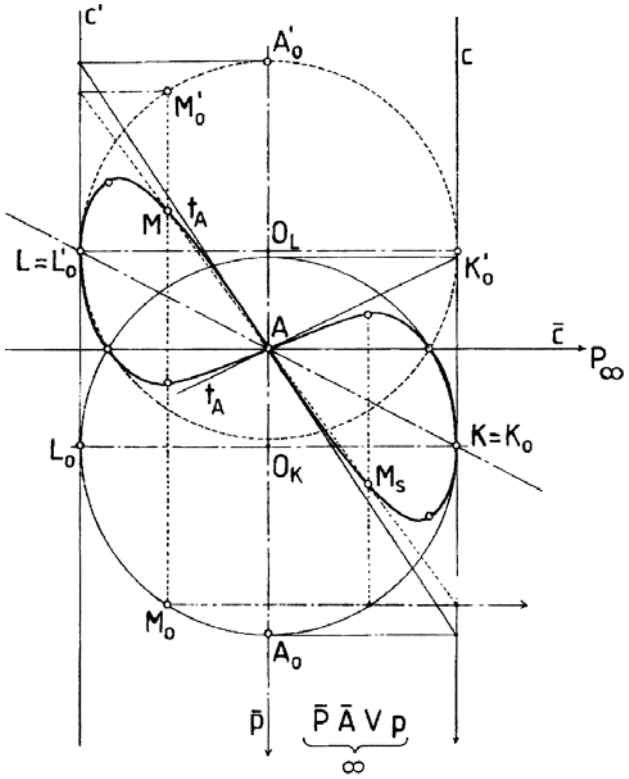



Fig. 9. — The centrally symmetric 4th order parabola

## 6. *Oblique selftouching 4th order parabolas*

Asymmetric selftouching 4th order parabolas are obtained when the axis $\bar{c}$ is not perpendicular to the axis $c$ and when the point $A$ is not in the middle. In Fig. 10 a pair of the complementary oblique 4th order parabolas with a cusp at $A$ are shown. In the case of oblique selftouching 4th parabolas, the crunode $A$ can be chosen so that one tangent at the crunode $A$ is inflexional ($A$ is chosen on the ray of mapping $A_oV$ while the tangent of the circle at the point $A_o$ is parallel to $c$).

When the point $A$ is in the middle, an obliquely symmetric curve is obtained again, but this time the axis of oblique symmetry is $\bar{p} = A\bar{V}\bar{P}$ (Fig. 11). The same curve is obtained by mapping the osculatory ellipse at the point $L$ with respect to the axis $c_E$ (the ellipse is affine to the circle with respect to the axis $\bar{c}$). The curvatures at the obliquely symmetric contour points $K$ and $L$ of the curve are the same, i.e., the radius of curvature at the point $L_E = L$ of the osculatory ellipse is equal to the radius of curvature of the osculatory circle at the point $K_o = K$.

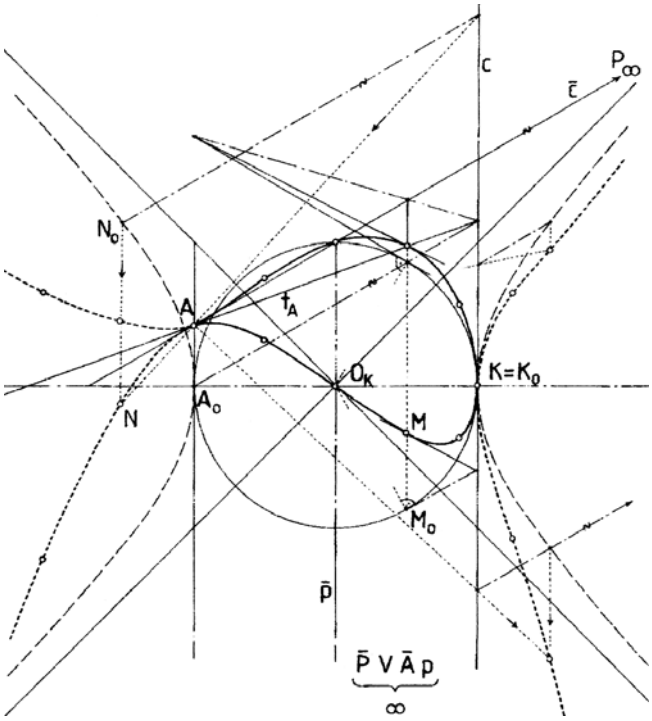



Fig. 10. — A pair of the complementary oblique 4th order parabolas

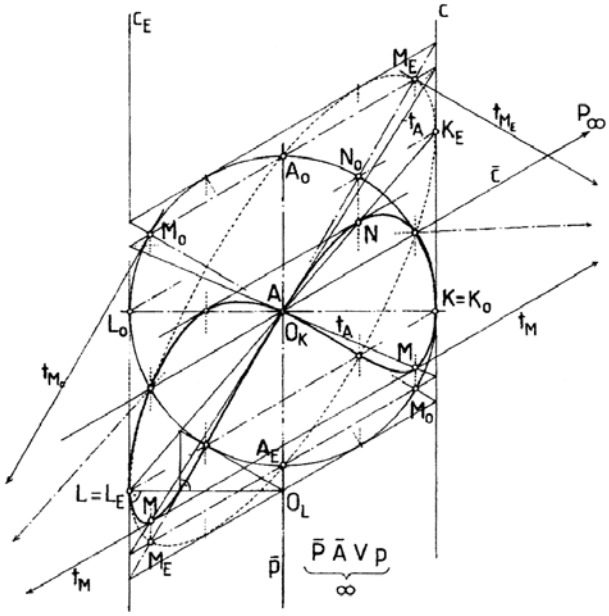



Fig. 11 — The obliquely symmetric curve

## 7. *Orthogonally symmetric selftouching 4th order parabolas*

The fact that all, either oblique or orthogonal, symmetric curves with respect to the axis $\bar{p}$ have the same curvature at the points $K$ and $L$ becomes obvious when the axis $\bar{c}$ is put through the point $L$ (Fig. 12), since the same or-

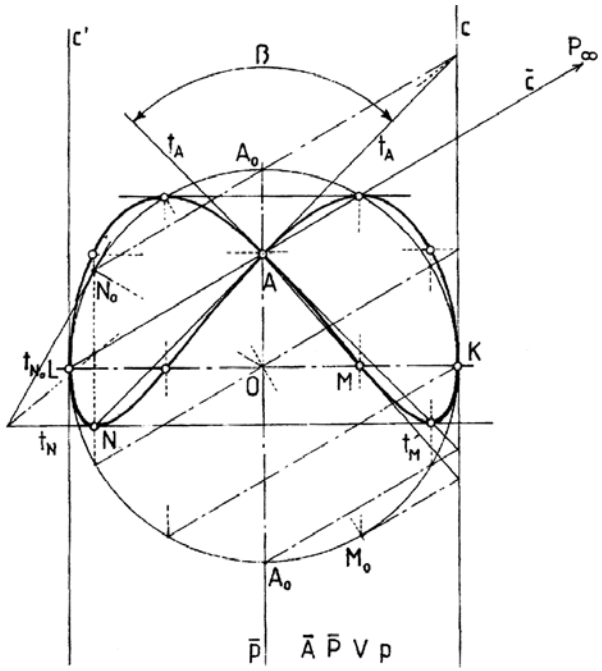



Fig. 12. — The orthogonally symmetric selftouching 4th order parabola

thogonally symmetric curve with the same osculatory circle at both points ($K$ and $L$) is obtained. The angle β between the tangents at the crunode $A$ of the orthogonally symmetric curves remains right even when the point moves along the axis of symmetry, i.e., even when the axis $\bar{c}$ changes the slope to the axis of symmetry (rotating about the point $L$).

By the affine either „squeezing" or „loosening" of the original circle, i.e., by the mapping of the ellipse of which $LK$ is either the great or the small axis, curves with an angle β either greater or smaller than the right angle are obtained. As in the case of the derivation of periform and its affine curves, each mapping of the ellipse can be replaced with the mapping of the circle by parallel moving of the axis $c$ (which is equally important both for constructive geometry and mechanism synthesis). It also enables us, for chosen $A$ and β in advance, to determine the position of the axis $c$ so that the circle of the diameter $LK$ can be mapped into the 4th order parabola.

The orthogonally symmetric parabolas constructed in this way comprise in their class seemingly different particular curves already known for two or three centuries, such as virtual parabolas of Vincentio i.e., of Cramer, curves

de Sluse, parabolic lemniscate, so that particular mechanisms for their derivation [3] can be replaced by a simpler uniform mechanism.

## CONCLUSION

The main results of the previous analysis could be drawn into three important facts. Namely, with the same disposition of the characteristic elements of the mapping (where $\overline{A}_\infty = V_\infty = \overline{P}_\infty$, $p_\infty = V_\infty\overline{A}_\infty P_\infty$, $c/\!/\overline{p} = AV_\infty\overline{P}_\infty$), which defines the class of rational selftouching 4th parabolas, the shape of these curves depends, first, on the fact whether they are obtained by the mapping of the circle or by the mapping of its complementary rectangular hyperbola; second, on the position of the double point $A$, and, third, on the position of the second axis of mapping $\overline{c} = AP_\infty$.

1. By the mapping of a circle (ellipse), 4th order parabolas are obtained whose conjugate imaginary branches touch each other at the consecutive double point lying on the infinitely distant line. By the mapping of a hyperbola, 4th order parabolas are obtained whose real branches touch each other at consecutive double point on the ideal line.

2. The position of the point $A$ defines whether the curve will have a crunode at that point, cusp of the 1st kind or isolated double point. Since this transformation is in fact the intersection of the hyperbolic paraboloid (because of $\overline{A}_\infty$) and the circular or hyperbolic cylinder, where the point $A$ is the image of the projecting generatrix of the hypar, the double point $A$ will be crunode, cusp, or isolated point, which depends on the position of the projecting generatrix with respect to the cylinder, i.e., whether it, respectively, pierces, touches or passes by the cylinder. When the point $A$ is on the common contour of the complementary cylinders the complementary curves, faced by their cusps, are obtained (Fig. 4, Fig. 10). When the projecting generatrix pierces one cylinder at real points then it passes by the other, so that the point $A$ is the crunode of the first curve and the isolated double point of the other (Fig. 3, Fig. 5).

3. Whether the curve will be centrally, orthogonally, obliquely symmetric or asymmetric, it depends both on the position of the second axis of the mapping $\overline{c} = AP$ and on the position of the point $A$ on it.

Removing the finite double point, selftouching parabolas would be of the genus one, but, because of the birationality of the transformation they cannot be obtained in this way. They can be derived as an intersection of the 2nd order cylinders which is to be presented in further investigations.

## REFERENCES


Artobolevskij, I. I. (1959): *Teorija mehanizmov dlja vosproizvedenija ploskih krivyh*, Izdateljstvo Akademii nauk SSSR, Moskva.

Довниковић, Л. (1977): *Нацртно геометријска обрада и класификација равних кривих 3. реда* (Summary in English), Зборник Матице српске за природне науке, 52, Нови Сад, p. 107—225.

Довниковић, Л. (1987): *Униформна конструктивна геометрија рационалних равних кривих* (Summary in English), Зборник Матице српске за природне науке, 73, Нови Сад, p. 165—207.

Довниковић, Л. (1989): *О верзијерама, псеудоверзијерама и њиховим комплементима* (Summary in English), Зборник Матице српске за природне науке, 76, Нови Сад, p. 5—19.

Dovniković, L. (1990): *Uniform Mechanisms for Rational Plane Curves*, Computer Graphics — a New Vision On Eng., Fourth International Conference on Engineering Graphics and Descriptive Geometry, Miami, Florida, June 1990, Published by ASEE, Washington, D.C. 20036, p. 125—131.

Савелов, А. А. (1979): *Равнинске кривуље* (Translation from Russian), Школска књига, Загреб.

## УНИФОРМНЕ КОНСТРУКЦИЈЕ РАЦИОНАЛНИХ ПАРАБОЛА 4. РЕДА

Лазар М. Довниковић, Радован Б. Штулић
Факултет техничких наука Универзитета Нови Сад,
Цара Лазара 1, 21000 Нови Сад, Југославија

Резиме

Ако је заједничко својство неких кривих 4. реда које припадају истој класи парабола 4. реда скривено у бесконачности, може се на први поглед уочити да оне немају ништа заједничко. Наиме, такве криве имају своје две консекутивне двоструке тачке у бесконачности (бесконачно далека права је тангента криве у те четири тачке) и њихово постојање се доказује бирационалном квадратном трансформацијом са тачно одређеним распоредом елемената трансформације.

Тако на пример, круг може, квадратном трансформацијом, бити пресликан у познату параболичку лемнискату која има две изоловане консекутивне двоструке тачке у бесконачности. Истом трансформацијом се правоугла хипербола (као комплемент круга) пресликава у криву комплементарну параболичкој лемнискати. И ова крива има дакле две реалне консекутивне двоструке тачке — које су као самододирне тачке поклопљене са изолованим тачкама параболичке лемнискате у бесконачности. Ове две комплементарне криве такође имају и трећу поклопљену двоструку тачку која је за лемнискату чвор а за комплемент изолована двострука тачка. Управо због три двоструке тачке криве 4. реда су рационалне, т.j. рода су нултога.

У овом раду су, осим параболичке лемнискате и њеног комплемента, анализиране још неке параболе 4. реда које се могу добити применом бирационалне квадратне трансформације. То су периформа, крива са изолованом тачком, самододирне параболе 4. реда афине периформи, косо симетричне параболе 4. реда, косе самододирне параболе 4. реда и ортогонално симетричне самододирне параболе 4. реда. Њихови комплементи чине групу кривих које бесконачно далека права додирује у самододирној тачки.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 19—23, 2000

UDC 633.11:547.962.6]:631.52

*Ljiljana B. Vapa, Dragana R. Obreht*

Institute of Biology, Faculty of Sciences
Trg Dositeja Obradovića 2
21000 Novi Sad, Yugoslavia

# HMW GLUTENIN COMPOSITION IN WHEAT CULTIVARS

ABSTRACT: The method of sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used to determine high molecular weight (HMW) glutenin subunits in wheat cultivars developed at the Institute of Field and Vegetable Crops in Novi Sad. Eleven alleles at *Glu-1* loci were determined: three alleles at *Glu-A1*, five at *Glu-Bl*, and two at *Glu-Dl* locus. Typical HMW glutenin compositions in wheat cultivars were 2*, 7+9, 5+10 and 1, 7+9, 2+12. Cultivars Gracija, Mina and Selekta were heterogeneous showing two to four different electrophoregrams on the gel.

KEY WORDS: electrophoresis, HMW glutenin, wheat

## INTRODUCTION

Glutenins are storage proteins located in the protein bodies of wheat endosperm. The synthesis of glutenins is carried out on the endoplasmatic reticulum during the formation of endosperm. Glutenin subunits can be divided into two groups: subunits of low molecular weight, to 70 000 daltons, and subunits of high molecular weight, from 70 000 to 136 000 daltons. In the allohexaploid bread wheat, *Triticum aestivum*, high molecular weight glutenins (HMW), are encoded by genes at three complex loci: *Glu-A1, Glu-B1* and *Glu-D1* located on the long arms of chromosomes 1A, 1B and 1D. It was shown that up to 60—65% of variability in bread making quality (BMQ) can be accounted for by differences in HMW glutenin composition (Payne, 1987; Payne et al., 1988; Rogers et al., 1989). The high molecular weight glutenin subunits, a main group of storage proteins of wheat, have been studied extensively in relation to BMQ in Yugoslav wheat cultivars (Vapa et al., 1995, Denčić and Vapa, 1996).

The aim of this paper was to analyze genetic variability of high molecular weight glutenin subunits by the method of SDS PAG electrophoresis in so-

me new wheat cultivars selected at the Institute of Field and Vegetable Crops in Novi Sad.

## MATERIAL AND METHOD

*Plant material*: Single grains of winter wheat cultivars selected at the Institute of Field and Vegetable Crops in Novi Sad were used in this study. Twenty grains per cultivar were analyzed.

*Electrophoresis*: HMW glutenins were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gel, in Tris--glycine buffer (pH 8.3) as described by Vapa and Savić (1988).

Glutenin loci, alleles and subunits were designated according to Payne and Lawrence (1983).

## RESULTS AND DISCUSSION

The storage proteins of 21 wheat cultivars were separated by SDS-PAG electrophoresis to determine their HMW glutenin subunits. At the *Glu-A1* locus subunits 1 and 2*, as well as null allele N, which did not form a visible band on the gel, were determined. Subunits 7+9, 7+8, 6+8, 7 and 14+15 were found at *Glu-B1* locus, subunits 2+12 and 5+10 at *Glu-D1* locus (Table 1).

Tab. 1. — Glutenin subunits composition and their frequency in wheat cultivars

| Cultivar | Glutenin subunits | | | % |
|---|---|---|---|---|
| | *Glu-A1* | *Glu-B1* | *Glu-D1* | |
| Sreća, Gracija | 1 | 7+9 | 5+10 | 9.1 |
| Ivanka | 1 | 7+8 | 5+10 | 4.5 |
| Dobra | 1 | 7 | 5+10 | 4.5 |
| Delta, Selekta, Sara, Sonja, Stamena | 2* | 7+9 | 5+10 | 22.7 |
| Bajka | 2* | 7+8 | 5+10 | 4.5 |
| Sirena | 2* | 14+15 | 5+10 | 4.5 |
| Anastasija, Prva, Zlatka, Super rana, Galija | 1 | 7+9 | 2+12 | 22.7 |
| Sofija | N | 7+9 | 5+10 | 4.5 |
| Senica | N | 6+8 | 5+10 | 4.5 |
| Kremna, Milena | N | 7+9 | 2+12 | 9.1 |
| Mina | N | 7 | 5+10 | 4.5 |

At *Glu-A1* locus, the highest frequency (43%) was obtained with subunit 1, at *Glu-B1* locus with subunits 7+9 (71%), and at *Glu-D1* locus with subunits 5+10 (67%) (Figure 1). The most frequent HMW glutenin compositions were 2*, 7+9, 5+10 and 1, 7+9, 2+12 (Table 1).

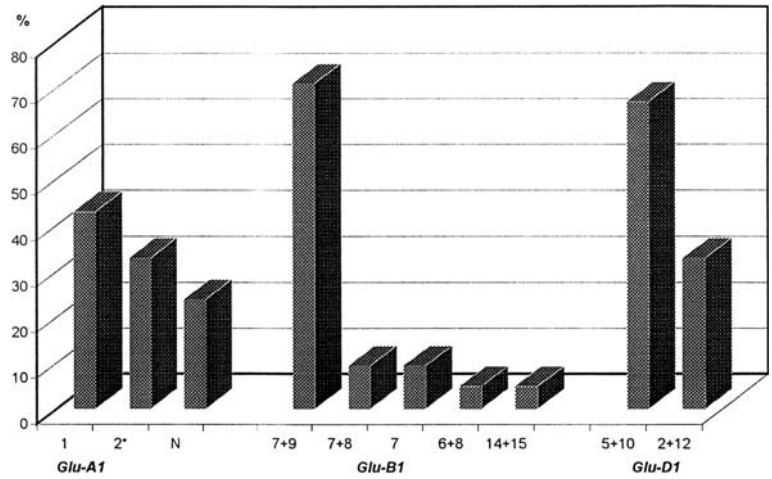



Fig. 1. — Frequency of HMW glutenin subunits in wheat cultivars

Subunit 2* and N allele (coded by the *Glu-A1* locus), subunits 7+9 (by the *Glu-B1* locus), and subunits 5+10 (by the *Glu-D1* locus) were most frequent in the Novi Sad wheat cultivars selected during the period 1987—92 (V a p a et al., 1995; V a p a et al., 1997). The obtained allelic variability of HMW glutenins is mostly due to the use of particular parents in the breeding process, or to the adaptive value of individual alleles to the environmental conditions under which the cultivars had been selected.

Presence of the most frequent glutenin subunits composition 2* 7+9 5+10 correlate with some components of BMQ, specially subunits 5+10 (coded by the *Glu-D1* locus) (P o g n a et al., 1987; V a p a et al., 1995).

The cultivars Gracija, Mina and Selekta were heterogeneous showing two to four different electromorphs in addition to the predominant pattern (Table 2). Occurrence of heterogeneity has been detected in hexaploid wheat cultivars grown in Italy at the frequency of 10.8%, in Canada 12.7%, in Yugoslavia 15% (V a p a , 1991), and in USSR 22% (M o r g u n o v et al., 1990). This heterogeneity makes it possible to improve BMQ by choosing biotypes with superior HMW composition as parents in breeding programs.

Tab. 2. — Heterogeneous wheat cultivars

| Cultivar | *Glu-A1* | *Glu-B1* | *Glu-D1* | (%) |
|---|---|---|---|---|
| Gracija | 1 | 7+9 | 5+10 | 85.7 |
| | 1 | 7+9 | 2+12 | 4.8 |
| | 1, 2* | 7+8/7+9 | 5+10 | 4.8 |
| | 2* | 7+8/7+9 | 5+10 | 4.8 |
| Mina | N | 7 | 5+10 | 65.0 |
| | N | 6+8 | 5+10 | 35.0 |
| Selekta | 2* | 7+9 | 5+10 | 77.8 |
| | 2* | 7 | 5+10 | 5.5 |
| | 1 | 7+8 | 2+12 | 11.1 |
| | 1 | 7 | 2+12 | 5.5 |

## CONCLUSION

The method of SDS-PAG electrophoresis was used for the analysis of the genetic variability of HMW glutenin subunits in wheat cultivars. Eleven alleles at *Glu-1* loci were determined, three at *Glu-A1*, five at *Glu-B1*, and two at *Glu-D1*. Typical HMW glutenin compositions in wheat cultivars were 2*, 7+9, 5+10 and 1, 7+9, 2+12 indicating good BMQ of the cultivars. The cultivars Gracija, Mina and Selekta were heterogeneous showing two to four different electrophoregrams.

## REFERENCES


Denčić, S., Vapa, Lj. (1996): *Effect of intra and inter-allelic variation in Glu-A1 and Glu-D1 loci on bread making quality in wheat*, Cereal Res. Commun. 24: 317—322.

Morgunov, A. I., Rogers, W. J., Sayers, E. J., Metakovsky, E. V. (1990): *The high molecular weight glutenin subunit composition of soviet wheat varieties*, Euphytica 51: 41—52.

Payne, P. I., Lawrence, J. G. (1983): *Catalogue of alleles for the complex gene loci, Glu-A1, Glu-B1 and Glu-D1, which code for HMW glutenin subunits in hexaploid wheat*, Cereal Res. Commun. 11: 29—35.

Payne, P. I. (1987): *Genetics of wheat storage proteins and the effect of allelic variation on bread making quality*, Ann. Rev. Plant Physiol. 38: 141—153.

Payne, P. I. Nightingale, M. A., Krattiger, A. F., Carillo, J. M. (1988): *Relationship between seed quality characteristics and HMW glutenin subunit composition determined using wheats grown in Spain*, J. Cereal Sci. 7: 229—235.

Pogna, N. E., Mellini, F., Dal Belin Peruffo, A. (1987): *Glutenin subunits of Italian common wheats of good BMQ and comparative effect of HMW glutenin subunit 2 and 5, 10 and 12 on flour quality*, pp 53—69. Proc. of Workshop in the Community Programme for Coordination of Agricultural Research, S. Angelo Lodigiano, Milano.

Rogers, W. J., Payne, P. I., Harrinder, K. (1989): *The HMW glutenins and gliadins compositions in German grown wheat varieties and their relationship with BMQ*, Plant Breed. 103: 80—89.

Vapa, Lj., Savic, M. (1988): *The HMW glutenin subunit composition of wheat varieties bred in Yugoslavia*, pp 1019—1023. In: T. E. Miller, R. M. D. Koebner (Eds) Proc. 7th Int. Wheat Genet. Symp. Cambridge, UK.

Вапа, Љ., Радовић, Д., Симић, Т. (1997): *Полиморфност Glu-1 локуса пшенице*, Зборник радова ПМФ, Серија биологија 26: 59—64.

Vapa, Lj., Denčić, S., Šoltes-Rak, E., Kevrešan, S. (1995): *Genetic variation in Glu-1 loci and bread making quality*, Cereal Res. Commun. 23(1—2): 161—166.

Vapa, Lj. (1991): *Allelic variation of Glu-1 loci in Yugoslav wheat cultivars*, pp 646—657. In: W. Bushuk and R. Tkachuk (Eds) *Gluten proteins 1990*, AACC, USA.

# КОМПОЗИЦИЈА ГЛУТЕНИНА ВЕЛИКЕ МОЛЕКУЛСКЕ МАСЕ СОРТИ ПШЕНИЦЕ

Љиљана Б. Вапа, Драгана Р. Обрехт
Институт за биологију, Природно-математички факултет,
Трг Доситеја Обрадовића 2, 21000 Нови Сад

Резиме

Циљ овог рада је био да се анализира варијабилност субјединица глутенина велике молекулске масе, методом SDS PAGE електрофорезе, код новопризнатих сорти пшенице селекционираних у Научном институту за ратарство и повртарство у Новом Саду.

Резултати истраживања су показали да у локусу *Glu-A1* постоје субјединице 1 и 2*, као и нулти алел N, који не даје видљиву траку на гелу. Субјединица 1 је имала највећу учесталост, 43%. У локусу *Glu-B1* одређене су субјединице 7+9, 7+8, 7, 6+8 и 14+15. Најучесталије (71%) су биле субјединице 7+9. У локусу *Glu-D1* нађене су субјединице 2+12 и 5+10. Учесталост субјединица 5+10 била је већа и износила 67%. Најучесталије комбинације глутенина велике молекулске масе биле су 2*, 7+9, 5+10 и 1, 7+9, 2+12. Сорте Селекта, Грација и Мина биле су хетерогене са два до четири електроморфа на гелу.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 25—35, 2000

UDC 635.656:546.48]:631.81.033

*Vojislav M. Mihailović*[2], *Novica M. Petrović*[1,2], *Ivana V. Arsenijević-Maksimović*[1,2], *Žarko S. Kevrešan*[1]

[1] Faculty of Agriculture Novi Sad
Trg D. Obradovića 8
21000 Novi Sad, Yugoslavia
[2] Institute of Field and Vegetable Crops Novi Sad
M. Gorkog 30
21000 Novi Sad, Yugoslavia

# CADMIUM TRANSLOCATION AND RETRANSLOCATION BY PEA (*Pisum sativum* L)

ABSTRACT: Under semi-controlled conditions, we studied cadmium (Cd) translocation and retranslocation in the aphylla pea cultivar Jezero at two stages of growth and development. The applied Cd concentrations were 0 (control), $10^{-7}$ and $10^{-5}$ M Cd and the age of plants at the time of treatment was 26 and 63 days. The application of Cd via the root lasted for 48 h. The plants were analyzed immediately after the treatment, 38 days later and at maturity. Dry matter mass and Cd contents were determined in individual plant organs. The results showed that dry mass had decreased noticeably depending on the Cd concentration used and plant age. The decrease of dry matter mass in both vegetative and generative plant organs was more significant when Cd was applied at flowering (63 day-old plants) than when the plants were younger (25 day-old). In all the treatments, the highest Cd content was found in the root. The translocation of Cd from the root to the aboveground vegetative and generative plant parts took place and it was relatively more intensive at the lower Cd concentration than at the higher one. The possibility that a minor amount of Cd was retranslocated from the leaves to the seeds and pods could not be ruled out either.

KEY WORDS: pea, plant age, cadmium, plant organ mass, translocation, retranslocation.

## INTRODUCTION

The rates of uptake, accumulation, and translocation of heavy metals (HMs) in plants vary according to the physical-chemical properties of their ions as well as according to plant species and genotype involved. A number of authors have established for many species that HMs accumulate more intensively in the root than in the aboveground plant parts (Florijn and Van Beusichem, 1993; Gárate et al., 1993). HM translocation, and hence, HM distribution typically depend on the plant's capacity to build chelate complexes with the components of xylem sap (Kochian, 1991). In contrast to

the other HMs, cadmium and nickel are rapidly transported to the aboveground plant parts after uptake in many plant species (Verkleij and Schat, 1990; Petrović and Kastori, 1994). Cadmium differs from nickel in that it retranslocates poorly. Because of this, Cd content in seeds and fruits of some plant species is very low, even when these grow on soils highly contaminated by Cd (Leita et al., 1996).

Having in mind all of the above as well as the fact that Cd is transported to the aboveground plant parts primarily by transpiration (Barceló and Poschenrieder, 1990), i.e., that it depends on the morphological and anatomical structure of the aboveground plant parts, we thought it would be of interest to study Cd translocation and retranslocation in an aphylla pea genotype characterized by a small leaf area and, hence, a low transpiration activity.

## MATERIALS AND METHODS

The trials with the aphylla pea cultivar Jezero were conducted in a greenhouse under semi-controlled conditions. The seeds were germinated in vermiculite in a thermostat at 25°C. After germination, the young plants were transferred to 2 l pots containing 1/2 strength Hoagland's nutrient solution (Hoagland and Arnon, 1950), in further text: complete nutrient solution. In the greenhouse, day/night temperature was 25/15°C, photoperiod 16 h, and relative humidity 70—75%. The translocation and retranslocation of Cd and its effect on plant organ mass were studied both in the early and late stages of growth and development (Experiments A and B, respectively).

### *Experiment A*

After 25 days on a complete nutrient solution, a portion of plants was treated with 0 (control), $10^{-7}$ or $10^{-5}$ M Cd for 48 hours. Cadmium was applied as $CdCl_2$ dissolved in distilled water. Following the Cd treatment, the roots of intact plants were immersed in 20 mM $Na_2$-EDTA for 15 minutes to remove Cd adhering to root surfaces. Immediately afterwards, a portion of plants was taken for analysis, i.e., harvested and separated into roots and the aboveground parts (I), while the rest of them were returned onto the complete nutrient solution.

At full flowering, i.e., 38 days after the Cd treatment, another portion of plants was taken for analysis. The plants were separated into roots, aboveground parts (I), and newly-formed aboveground parts (II).

At maturity, the remaining plants were harvested and separated into roots, aboveground parts (l), aboveground parts (II), pods and grains.

### *Experiment B*

Pea plants were grown on the complete nutrient solution until full flowering, i.e., for 63 days. A portion of these plants was then treated with either 0

(control), $10^{-7}$ or $10^{-5}$ M Cd (prepared as described above) for 48 hours. As in the experiment A, the roots of intact plants were immersed in 20 mM $Na_2$-EDTA for 15 minutes. After 48 hours, a portion of the plants was taken for analysis having been separated into roots and aboveground parts. The remaining plants were transferred onto the complete nutrient solution and grown till maturity, when they were harvested and separated into roots, aboveground parts, pods, and grains.

The dry matter mass of individual organs was determined after oven drying at 60°C to constant mass. Cadmium content was measured by AAS using a Varian AA-10.

The results were statistically processed by the analysis of variance. Differences between the treatments were calculated using Duncan's multiple range test.

## RESULTS

### *Dry matter mass*

The dry matter mass of roots and shoots of plants harvested at flowering (analyzed 38 days after the treatment with Cd) decreased significantly relative

Tab. 1. — Effect of Cd on dry matter mass of pea (g. plant$^{-1}$). Plant age at the time of treatment with Cd was 25 days (A) and 63 days (B).

| Cd concentration [M] | Plant organ | | | | |
|---|---|---|---|---|---|
| | Root | Total above ground portion of the plant | | Pods | Grain |
| | | I* | II** | | |
| *EXPERIMENT (A)* | | | | | |
| *Flowering* | | | | | |
| 0 (Control) | 0.530 | 1.413 | 3.358 | — | — |
| $10^{-7}$ | 0.456 | 1.235 | 2.990 | — | — |
| $10^{-5}$ | 0.408 | 1.080 | 2.655 | — | — |
| Duncan 5% | 0.071 | 0.150 | 0.491 | — | — |
| *Maturity* | | | | | |
| 0 | 0.587 | 1.163 | 4.006 | 0.502 | 3.543 |
| $10^{-7}$ | 0.501 | 1.129 | 3.251 | 0.435 | 2.900 |
| $10^{-5}$ | 0.439 | 1.022 | 3.044 | 0.377 | 2.484 |
| Duncan 5% | 0.098 | 0.111 | 0.507 | 0.078 | 0.384 |
| *EXPERIMENT (B)* | | | | | |
| *Maturity* | | | | | |
| 0 | 0.586 | 1.168 | 4.057 | 0.545 | 3.460 |
| $10^{-7}$ | 0.450 | 1.050 | 3.444 | 0.400 | 2.442 |
| $10^{-5}$ | 0.390 | 0.839 | 3.047 | 0.300 | 1.675 |
| Duncan 5% | 0.070 | 0.246 | 0.547 | 0.083 | 0.424 |

* Above-ground portion of 25 days old plants
** Above-ground plant parts formed after the 25th day

to the control. The decrease depended on the Cd concentration applied as well as on the plant organ studied. Both $10^{-7}$ and $10^{-5}$ M Cd significantly reduced the dry matter mass of the plants. The largest drop was recorded in the aboveground plant parts (I) developed during the Cd treatment and the lowest in the newly formed aboveground parts (II) treated with $10^{-7}$ M Cd (Tab. 1).

At maturity, the dry matter mass of both vegetative and generative organs dropped considerably (Table 1). Contamination of plants with Cd in the earlier stages of growth and development had a particularly large effect on the dry matter mass of the grain and root. At this stage too, $10^{-5}$ M Cd brought about a larger decrease of total dry matter mass than $10^{-7}$ M.

At maturity, the dry matter mass of pea plants treated with Cd at flowering (Experiment B) was significantly reduced relative to that of control plants. The effect of $10^{-5}$ M Cd was particularly unfavorable, especially on the dry matter mass of the grain and root (Tab. 1).

The two Cd concentrations had no significant effect on the ratio: [root dry matter mass] / [shoot dry matter mass] in any phase of the study. The dry matter mass of the root and aboveground parts had been reduced nearly to the same amount (Tab. 1).

Table 1 shows that the phytotoxicity of Cd depends not only on its concentration in the nutrient substrate but also on the age of plant at the time of contamination, in other words, on the rate of its uptake, translocation, and retranslocation by plants, or plant metabolic activity.

### *Cd content, translocation, and retranslocation*

Forty-eight hours after the Cd treatment, the amount of Cd that had accumulated in the root was higher than that in the aboveground parts of the young pea plants. The Cd content in the plants treated with $10^{-5}$ M Cd was two times higher than the content in the plants treated with $10^{-7}$ M Cd. However, the translocation of Cd from the root to the aboveground plant parts during the treatment was somewhat greater in the lower Cd concentration treatment (Tab. 2).

At flowering, 38 days after the treatment with Cd, the percentage contribution of root Cd content to total Cd content of the plant was 48% for the $10^{-7}$ M Cd treatment and 62% for the $10^{-5}$ M one. This means that the translocation of Cd, i.e., its movement to the newly formed aboveground parts, was significantly higher in the treatment with $10^{-7}$ M Cd (Tab. 2).

At maturity, the contribution of root Cd content to total Cd content in the plant was 20.4% for the $10^{-7}$ M Cd treatment and as high as 51.2% for the $10^{-5}$ one. In the pea genotype under study, the retranslocation of Cd to the generative organs — the pod and grain — was noticeable. It depended significantly on Cd concentration in the nutrient substrate and was considerably more obvious in plants treated with the lower Cd concentration (Tab. 2).

In the 63 day-old plants treated with $10^{-5}$ M Cd at flowering, the total Cd content per plant after 48 h treatment was considerably higher than in either the plants treated with $10^{-7}$ M Cd or plants that were 25 days old at the

Tab. 2. — Translocation and retranslocation of Cd in pea at different stages of growth and development (μg Cd. organ$^{-1}$). Plant age at the time of treatment with Cd was 25 days (A) and 63 days (B).

| Cd concentration [M] | Plant organ | | | | |
|---|---|---|---|---|---|
| | Root | Total above ground portion of the plant | | Pods | Grain |
| | | I* | II* | | |
| *EXPERIMENT (A)* | | | | | |
| *After treatment with Cd* | | | | | |
| $10^{-7}$ | 17.33 | 6.35 | — | — | — |
| $10^{-5}$ | 34.26 | 10.80 | — | — | — |
| Duncan 5% | 5.55 | 1.21 | — | — | — |
| *Flowering* | | | | | |
| $10^{-7}$ | 10.62 | 7.74 | 3.75 | — | — |
| $10^{-5}$ | 24.71 | 10.72 | 4.30 | — | — |
| Duncan 5% | 3.19 | 1.39 | 0.76 | — | — |
| *Maturity* | | | | | |
| $10^{-7}$ | 4.22 | 6.12 | 7.15 | 3.07 | 0.14 |
| $10^{-5}$ | 23.27 | 8.22 | 7.21 | 5.57 | 0.21 |
| Duncan 5% | 3.21 | 1.42 | 0.53 | 0.65 | 0.03 |
| *EXPERIMENT (B)* | | | | | |
| *After treatment with Cd* | | | | | |
| $10^{-7}$ | 23.56 | 3.53 | 2.35 | — | — |
| $10^{-5}$ | 108.54 | 8.47 | 6.93 | — | — |
| Duncan 5% | 7.50 | 0.67 | 0.48 | — | — |
| *Maturity* | | | | | |
| $10^{-7}$ | 15.44 | 3.70 | 7.37 | 0.99 | 2.66 |
| $10^{-5}$ | 85.02 | 7.71 | 19.50 | 2.13 | 11.93 |
| Duncan 5% | 7.72 | 1.07 | 1.66 | 0.38 | 1.83 |

* Above-ground portion of 25 days old plants
** Above-ground plant parts formed after the 25$^{th}$ day

time of Cd treatment. The transport of Cd to the aboveground plant parts during the treatment was quantitatively higher at $10^{-5}$ M Cd and relatively higher at $10^{-7}$ M Cd. The results have shown that the transport of Cd from the root to the aboveground plant parts primarily depended on the Cd concentration in the substrate (Tab. 2).

At maturity, Cd content per plant was either 30.1 or 126.3 μg Cd/plant, depending in the first place on the Cd concentration applied. The translocation and retranslocation of Cd were also visibly dependent on Cd concentration in the nutrient medium. The transport of Cd from the root to the vegetative aboveground parts was more pronounced in the treatment with $10^{-7}$ M Cd. When retranslocation of Cd to the generative organs is considered, more Cd moved to the grain in Experiment B, while in Experiment A larger amounts of Cd were found to have moved to the pod (Tab. 2).

The results of the study suggest that Cd accumulation in pea depends not only on the concentration of the element in the substrate but also on the stage of plant growth and development at the time of Cd application. In the aphylla-type pea, Cd translocation and retranslocation are conspicuous. After comparing the Cd content in pea with those in other plant species, it can be concluded that the aphylla-type pea belongs to the group of plant species in which Cd uptake and accumulation are less pronounced.

## DISCUSSION

Contamination of nutrient substrate brings plant roots into direct contact with HMs, which could be one of the reasons why HMs have a greater inhibitory effect on root growth than on the growth of the aboveground parts of the plant (Kastori et al., 1997). Recently, HMs have been thought to affect plant growth and development mostly through phytohormones. Most HMs affect plant metabolism and auxin transport (Vangronsveld and Clijsters, 1994). Excess HM amounts inhibit both the division and elongation of root cells and thereby visibly reduce their growth (Wierzbicka, 1988; Jalil et al., 1994). Factors that inhibit root growth also inhibit biosynthesis and translocation of cytokinins (Marschner, 1995). Excess HMs affect not only the growth of the primary root but also the formation of lateral roots in general and the rate of formation of root hairs in particular, thereby affecting root area as well (Brune and Dietz, 1995). The extent of root mass reduction depends on the nature of HM, its concentration and, especially, the plant species under study (Petterson, 1976; Yang et al., 1996).

After taking up HMs by the root, many plant species transport them to a greater or lesser extent to the aboveground organs, where they disrupt plant metabolism (Lang et al., 1995). The inhibition of chlorophyll biosynthesis by Cd is specific (Parekh et al., 1990). Excess Cd reduces chlorophyll content in the PSI and PSII as well as in their antennae complexes (Lang et al., 1995). Cd promotes the activity of chlorophyllase in leaves (Jana and Choudhuri, 1984). This enzyme not only inhibits plant growth but also speeds up the aging process in plants (Lagriffoul et al., 1998).

In a number of species, HM accumulation is more intensive in the root than in the aboveground parts of the plant (Woolhouse, 1983). Studies carried out in sugar beet, maize, pea, and lettuce have shown that there is 10 to 20 times more Cd in the roots of these plant species than in their aboveground parts (Petrović et al., 1990; Florijn and Van Beusichem, 1993; Gárate et al., 1993). The high capacity of the root to accumulate HMs may be a way whereby the plant protects its aboveground parts from excess HM levels in the environment. In most cultivated plant, Cd concentrations above 10 mg/kg of dry matter are considered toxic (Page et al., 1981).

Cadmium differs from other HMs in that, after uptake, it is readily transported into the aboveground plant parts (Verkleij and Schat, 1990). Most authors think that the mechanisms of Cd uptake and ascendant transport are similar to those of the $Ca^{2+}$ ion, i.e., that these are passive processes (Jar-

vis et al., 1976). Opinions still vary on the Cd uptake and transport by plants (Girling and Peterson, 1981). According to Leita et al. (1996), most plant species take up and transport Cd by the xylem in the form of $Cd^{2+}$.

The mobility of Cd varies according to the species. For example, Cd is considerably more mobile in oil rape and pumpkin than in tomato, wheat, and oat (Pettersson, 1976). Studying 23 plant species, Jarvis et al. (1976) found notable differences among them not only in terms of the rate of Cd uptake but also in terms of the elements transport from the root to the aboveground parts. Furthermore, Florijn and Van Beusichem (1993) also found significant differences in Cd content and mobility among 19 maize inbred lines. The translocation of Cd from the root to the aboveground parts was about four times greater in *Triticum aestivum* L. than in *Triticum turgidum* L. var. *durum* (Hart et al., 1998).

Cd translocation in plants depends not only on the species but also on the available Cd concentration in the substrate (Cieslinski et al., 1996). In many species grown in the presence of high Cd concentrations, Cd translocation to the aboveground plant parts was found to be reduced as compared to the treatments in which plants were grown on a substrate with a lower Cd concentration. The results of our study support this finding. It is hypothesized that such conditions trigger internal detoxification mechanisms whereby the aboveground plant parts rid themselves of excess Cd (Baker et al., 1990).

Mechanisms that enable Cd accumulation in the fruit and seed have not been sufficiently clarified. Popelka et al. (1996) hold that *Arachis hypogae* L. transports Cd to the generative organs by the phloem, i.e., by retranslocation. There are also researchers who think that Cd retranslocates poorly and that its presence in the fruit and seed is negligible, even on highly contaminated soils (Leita et al., 1996).

Based on what science has learned thus far about the translocation and retranslocation of Cd into the generative organs of plants, it can be concluded that these two processes depend primarily on the plant genotype and the available Cd concentration in the substrate, while the influence of other factors is much less significant. The concentration of Cd in strawberry fruits depends both on the genotype used and the Cd level present in the nutrient medium (Cieslinski et al., 1996). The Cd content in the reproductive organs of 44 maize lines ranged from 0.08 to 3.70 mg/kg of dry matter (Hinsley et al., 1978). The Cd concentration in celery seeds was as much as 10 times higher than that found in the reproductive organs of wheat (Van Lune and Zwart, 1997). In the aboveground parts of *Papaver somniferum* L. plants, the highest Cd accumulation was in the seed, followed by the leaves, stem and, finally, the seed coat (Pavlikova et al., 1996).

Taking note of the results of the present study and the available literature data about Cd content, translocation, and retranslocation in other plant species, we can conclude that the aphylla-type pea belongs to the group of plants with a low capacity for Cd uptake and accumulation. The reason may be that this type of pea has a small leaf area and hence low transpiration. This is suggested by the results of Salt et al. (1995), who found a positive correlation between the rate of transpiration and the rate of Cd transport from the root to the

aboveground plant parts. Given that in our study it was the root Cd content that decreased the most after the treatment, it can be assumed that the larger part of Cd in the pods and seeds came from the root by translocation. While Cd retranslocation from leaves to generative organs could not be completely eliminated, the results showed that it had no significant effect on Cd accumulation in the pods and grains. Our results support the proposition that Cd retranslocates poorly from the leaf to the fruit. The knowledge that Cd is translocated from the root to the generative organs can be of importance for the production of biologically safe food.

## CONCLUSIONS

Using the water culture method, we studied the translocation and retranslocation of Cd in the aphylla-type pea (cultivar Jezero) at two stages of growth and development. Cadmium was applied as 0, $10^{-7}$ and $10^{-5}$ M $CdCl_2$. The following conclusions have been reached.

The reductions in plant dry matter mass depended significantly on Cd concentration and plant age. The decrease of dry matter mass of both vegetative and generative plant organs was more significant when Cd was applied at flowering (63 day-old plants) then when plants were young (25 day-old), especially in the treatment with the higher Cd concentration.

In all treatments, Cd content was highest in the root. Based on the Cd content per plant, it can be concluded that the aphylla-type pea belongs to the group of plants with a low capacity for Cd uptake and accumulation.

It was established beyond doubt that translocation of Cd from the root to the aboveground vegetative and generative plant parts did occur. The possibility that a minor amount of Cd was retranslocated from the leaves to the seeds and pods could not be completely eliminated, either. However, the results showed that this had no significant effect on Cd accumulation in the pods and grains.

The results of the study support the proposition that Cd retranslocates poorly from the leaf to the fruit. Nevertheless, the knowledge that Cd is translocated from the root to the generative organs can be important for the production of safe food.

## REFERENCES


Baker, A. J. M., Ewart, K., Hendry, G. A. F., Thorpe, P. C., Walker, P. L. (1990): *The evolutionary basis of cadmium tolerance in higher plants*. In: Barceló, J. (ed.): *Proc. 4th International Conference Environmental Contamination*, Barcelona, CEP Consultants, Edinburgh, pp. 23—29.

Barceló, J., Poschenrieder, C. (1990): *Plant water relations as affected by heavy metal stress: A review*, J. Plant. Nutr., 13, 1—37.

Brune, A., Dietz, K. J. (1995): *A comparative analysis of element composition of roots and leaves of barley seedlings grown in the presence of toxic cadmium, molybdenum, nickel and zinc concentrations*, J. Plant. Nutr., 18, 853—868.

Cieslinski, G., Neilsen, G. H., Hogue, E. J. (1996): *Effect of cadmium application and pH on growth and cadmium accumulation in roots, leaves and fruits of strawberry plants (Fragaria x ananassa Duch.)*, Plant. Soil, 180, 267—276.

Florijn, P. J., Van Beusichem, M. (1993): *Uptake and distribution of cadmium in maize inbred lines*, Plant. Soil, 150, 25—32.

Gárate, A., Ramos, I., Manzanares, M., Lucena, J. J. (1993): *Cadmium uptake and distribution in three cultivars of Lactuca sp.*, Bull. Environ. Contam. Toxicol., 50, 709—716.

Girling, C. A., Peterson, P. I. (1981): *The significance of the cadmium species in uptake and metabolism of cadmium in crop plants*, J. Plant. Nutr., 3, 707—720.

Hart, J. J., Welch, M. R., Norvell, W. A., Sullivan, L. A., Kochian, L. V. (1998): *Characterization of cadmium binding, uptake and translocation in intact seedlings of bread and durum wheat cultivars*, Plant. Physiol., 116, 1413—1420.

Hinsley, T. D., Alexander, D. E., Ziegler, E. C., Barrett, G. L. (1978): *Zinc and cadmium accumulation by corn inbreds grown on sludge-amended soil*, Agron. J., 70, 425—428.

Hoagland, D. R., Arnon, D. I. (1950): *The water culture method for growing plants without soil*, California Agric. Exp. Stat. Circ., 347, 1—32.

Jalil, A., Selles, F., Clarke, M. J. (1994): *Growth and cadmium accumulation in two durum wheat cultivars*, Commun. Soil Sci. Plant Anal., 25, 2597—2611.

Jana, S., Choudhuri, A. M. (1984): *Synergistic effects of heavy metal pollutants on senescence in submerged aquatic plants*, Water, Air, Soil Pollut., 21, 351—358.

Jarvis, S. C., Jones, P. H. L., Hopper, J. M. (1976): *Cadmium uptake from solution by plants and its transport from roots to shoots*, Plant Soil, 44, 179—191.

Kastori, R., Petrović, N., Arsenijević-Maksimović, I. (1997): *Heavy metals and plants*. In: Kastori R. (ed.): *Heavy Metals in the Environment*, Institute of Field and Vegetable Crops, Novi Sad, Feljton, Novi Sad, Yugoslavia, pp. 195—258 [In Serbian].

Kochian, L. V. (1991): *Mechanisms of micronutrient uptake and translocation in plants*. In: Mortvedt, J. J. (ed.): *Micronutrients in Agriculture*, Soil Sci. Soc. Amer. Madison, WI, pp. 251—270.

Lagriffoul, A., Mocquot, B., Mench, M., Vangronsveld, J. (1998): *Cadmium toxicity effects on growth, mineral and chlorophyll contents, and activities of stress related enzymes in young maize plants (Zea mays L.)*, Plant Soil, 200, 241—250.

Lang, F., Sarvari, T., Szigeti, Z., Fodor, F., Cseh, K. (1995): *Effects of heavy metals on the photosynthetic apparatus in cucumber*. In: Mathis, P. (ed.): *Photosynthesis: From Light to Biosphere*, Vol. IV. Kluwer Academic Publishers, Netherlands, pp. 533—536.

Leita, L., De Nobili, M., Cesco, S. (1996): *Analysis of intercellular cadmium forms in roots and leaves of bush bean*, J. Plant. Nutr., 19, 527—533.

Marschner, H. (1995): *Mineral Nutrition in Higher Plants*, Academic Press, London, England.

Page, A. L., Bingham, T. F., Chang, C. A. (1981): *Cadmium*. In: Leep, N. W. (ed.): *Effect of Heavy Metal Pollution on Plants: Effects of Trace Metals on Plant Function*, Vol 1. Applied Science Publishers, Ripple Road, Barking, Essex, England, pp. 77—109.

Parekh, D., Puranik, R. M., Srivastava, H. S. (1990): *Inhibition of chlorophyll biosynthesis by cadmium in greening maize leaf segments*, Biochem. Physiol. Pflanz., 186, 239—242.

Pavlikova, D., Vanek, V., Szakova, J., Balik, J. (1996): *The accumulation and distribution of cadmium, zinc and arsenic by poppy*, Proc. 4th ESA Congress, Vol. 2. Veldhoven-Wageningen, Netherlands, pp. 710—711.

Petrović, N., Kastori, R. (1994): *Uptake, distribution and translocation of nickel ($^{63}Ni$) in wheat*, J. Food Physics 1, 71—73.

Petrović, N., Kastori, R., Rajčan, I. (1990): *The effect of cadmium on nitrate reductase activity in sugar beet*. In: Van Beusichem, L. M. (ed.): *Plant nutrition — physiology and applications*, Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 107—109.

Pettersson, A. (1976): *Heavy metal ion uptake by plants from solutions with metal ion, plant species and growth period variations*, Plant Soil 45, 445—459.

Popelka, J. C., Schubert, S., Schulz, R., Hansen, A. P. (1996): *Cadmium uptake and translocation during reproductive development of peanut (Arachis hypogaea L.)*, Angew. Bot., 7, 140—143.

Salt, D. E., Prince, C. R., Pickering, I. J., Raskin, I. (1995): *Mechanisms of cadmium mobility and accumulation in Indian mustard*, Plant Physiol., 109, 1427—1433.

Van Lune, P., Zwart, K. B. (1997): *Cadmium uptake by crops from the subsoil*, Plant Soil 189, 231—237.

Vangronsveld, J., Clijsters, H. (1994): *Toxic effects of metals*. In: Farago, E. M. (ed.): *Plants and the Chemical Elements. Biochemistry, Uptake, Tolerance and Toxicity*, VCH Verlagsgesellschaft, Weinheim, Germany, pp. 149—177.

Verkleij, J. A. C., Schat, H. (1990): *Mechanisms of metal tolerance in higher plants*. In: Shaw, J. A. (ed.): *Heavy Metal Tolerance in Plants: Evolutionary Aspects*, CRC Press, Inc., Boca Raton, FL, pp. 179—191.

Wierzbicka, M. (1988): *Mitotic disturbances induced by low doses of inorganic lead*, Caryologia, 41, 143—160.

Woolhouse, H. W. (1983): *Toxicity and tolerance in responses of plants to metals*. In: Lange, L. O., Nobel, P. S., Osmond, C. B., Ziegler, H. (eds.): *Encyclopedia of Plant Physiology*, III Plant Ecology, Vol. 12C, Springer-Verlag, Berlin Heidelberg New York Tokyo, pp. 245—300.

Yang, X., Baligar, V. C., Martens, D. C., Clark, R. B. (1996): *Cadmium effects on influx and transport of mineral nutrients in plant species*, J. Plant. Nutr., 19, 643—656.

# ТРАНСЛОКАЦИЈА И РЕТРАНСЛОКАЦИЈА КАДМИЈУМА У ГРАШКУ (*Pisum sativum* L)

Војислав М. Михаиловић[2], Новица М. Петровић[1, 2], Ивана В. Арсенијевић-Максимовић[1, 2] и Жарко С. Кеврешан[1]
[1] Пољопривредни факултет Нови Сад, Трг Д. Обрадовића 8, 21000 Нови Сад, Југославија
[2] Научни институт за ратарство и повртарство Нови Сад, М. Горког 30, 21000 Нови Сад, Југославија

Резиме

Проучавана је транслокација и ретранслокација кадмијума (Cd) у афила типу грашка сорте Језеро, у два стадијума раста и развића, у полуконтролисаним условима. Примењене концентрације Cd биле су 0 (контрола), $10^{-7}$ и $10^{-5}$ M Cd, а старост биљака у време третмана била је 26 и 63 дана. Третмани Cd преко корена трајали су по 48 h. Биљке су анализиране непосредно након третмана, 38 дана касније и у фази зрелости. Сува маса биљака и садржај Cd одређени су у појединачним биљним органима. Резултати су показали да се маса суве материје значајно смањила у зависности од примењене концентрације Cd и старости биљака. Смањење масе суве материје и вегетативних и генеративних органа било је значајније када је Cd примењиван у фази цветања (63 дана старе биљке) него када су биљке биле млађе (25 дана старе). Код свих третмана, највиши садржај Cd утврђен је у корену. Такође, дошло је до транслокације Cd из корена у надземне вегетативне и генеративне биљне органе и овај процес је био релативно интензивнији када је примењена концентрација Cd била нижа. Могућност да је дошло до ретранслокације минималних количина Cd из листова у махуне такође не може бити искључена.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 37—44, 2000

UDC 631.461.5:633.18

*Aleksandra V. Drobac, Zorica B. Svirčev, Olga V. Petrović*

Institute of Biology, University of Novi Sad
Trg Dositeja Obradovića 2
21000 Novi Sad, Yugoslavia

# CO-CULTIVATION OF $N_2$-FIXING HETEROCYSTOUS *CYANOBACTERIA* AND RICE CULTIVARS IN LIQUID AND SAND CULTURES

ABSTRACT: A large variety of cyanobacterial species capable of fixing atmospheric nitrogen have been identified. In this paper we present the data obtained during the co-cultivation of 12 cyanobacterial isolates (*Nostoc* and *Anabaena* strains), previously isolated from different soil types of the Vojvodina Province (Yugoslavia), and four rice (*Oryza sativa*) cultivars in liquid and sand cultures. The level of cyanobacterial influence on rice, considering the contribution to plant growth and development, as well as the N content of the rice plant were practically identical in the liquid and sand media. Also, diverse cyanobacterial influences were observed on the length, dry weight and N content of roots and shoots during the co-cultivation of rice cultivars and cyanobacteria in sand culture. The production and liberation of N compounds by cyanobacteria seem to be related to specific growth conditions created by the co-cultivation with a specific rice cultivar. Not only the nitrogen fixation, but also biologically active substances of cyanobacterial origin probably have an important role in co-cultivation.

KEY WORDS: *Anabaena*, co-cultivation, $N_2$ fixation, *Nostoc*, rice

## INTRODUCTION

Since it had been found that the natural fertility of flooded rice soils is associated with the diffusion of blue-green algae in them, and use of these microorganisms as biofertilizers in the production of rice suggested, many studies have been conducted on increasing $N_2$ fixation in rice fields by inoculation with specific cyanobacterial strains (Venkataraman, 1972). While the extracellular release of nitrogen compounds and the release of certain biostimulative substances are obvious ways by which the *Cyanobacteria* may benefit the higher plants, Rai (1990) demonstrated how plants may aid the growth of cyanobacteria and increase their $N_2$ fixation during co-cultivation. Paddy fields desiccation at the end of the cultivation cycle is followed by de-

ath of algal biomass and release of its content, which additionally fertilize the soil with nitrogen compounds. The contribution of cyanobacteria to $N_2$ fixation is 25—30 kg/ha of soil. In addition to yield increase, it reduces the need for artificial fertilizer by 60% (Venkataraman, 1972). Biostimulative substances — hormones and vitamins produced by cyanobacteria are also responsible for the improved growth and development of higher plants (Rodgers et al., 1979).

Recently, a large number of authors (Spiller and Gunasekaran, 1990; Obreht, 1992; Svirčev et al., 1996) have dealt with possible application of cyanobacteria as natural fertilizers during co-cultivation with agriculturally important plants. Since the rice field ecosystem is a favorable environment for the growth of *cyanobacteria*, rice seems to be the most suitable plant for such experiments.

Four rice cultivars were co-cultivated with 12 cyanobacterial strains (*Nostoc* and *Anabaena*), in order to determine the effects of these microorganisms on the growth of rice plants in liquid and sand cultures.

## MATERIALS AND METHODS

Four rice cultivars (*Oryza sativa*), marked $OS_4$, 9909, 36959 and 353, have been donated by the Manila University, the Philippines. Twelve cyanobacterial strains, *Nostoc* strains ($2S_9B$, $LBG_1$, $S_1$, 2, $S_2$) and *Anabaena* strains ($C_5$, 14, $LC_2B$, $2S_6B$, $S_8B$, $C_3$, $C_4$) have been taken from the algal collection of the microbiology laboratory, Institute of Biology, Novi Sad. These strains are described in detail in the paper of Obreht (1992).

Cyanobacteria were maintained in BG11 liquid culture (Rippka et al., 1979) in shake flasks, at room temperature and at the light flux density of 50 $\mu molm^{-2}s^{-1}$.

The co-cultivation of plant seedlings and $N_2$-fixing cyanobacteria was carried out in liquid and sand cultures, at 24°C, with continuous illumination at the photon fluence rate of 40 $\mu molm^{-2}s^{-1}$. The sand used for sand cultures was previously washed with water and sterilized. Co-cultivation was conducted in a medium without combined nitrogen, and the cultivation of control groups of plants was carried out in media with and without combined nitrogen. Each vessel with corresponding liquid or sand medium contained 8—10 plants, and each treatment consisted of five replicates. During the 24 days of cultivation, 200 ml of appropriate medium (BG11—N or BG11+N) were added to the experimental vessels and otherwise watered with distilled water.

During the first part of the experiment, the rice cultivars 36959 and 353 were cultivated in sand and liquid cultures with or without nitrogen, as control plants, and with five different cyanobacterial strains ($2S_9B$, $LBG_1$, $C_5$, $LC_2B$, $2S_6B$), selected on the basis of previous investigation data (Obreht, 1992; Svirčev et al.,1996). Further on in the experiment, the rice cultivars $OS_4$ and 9909 were cultivated only in sand culture with or without nitrogen, and co-cultivated with the 12 cyanobacterial strains.

After 24 days of plant cultivation and co-cultivation with cyanobacteria in liquid and sand cultures, the length, dry weight and N-content of the aboveground plant parts (shoots) and roots were measured. Nitrogen content of dry matter was determined using Kjeldahl method (Bremner and Mulvaney, 1982).

Experimental data were subjected to the analysis of variance (LSD-test).

## RESULTS

### *Co-cultivation of rice and cyanobacteria influenced by the type of the medium*

Tables 1 and 2 show the results of the measurements of the length, dry weight and N content in roots and aboveground parts (shoots) of the rice cultivars 36959 and 353, when grown in the presence and absence of N, and when co-cultivated with the cyanobacterial strains, in liquid and sand cultures. The levels of cyanobacterial influence on the rice regarding the contribution to plant growth, development and N content were practically identical in the liquid and sand media.

Tab. 1. — Length, dry weight and nitrogen content of shoots and roots of rice cv. 36959 co-cultivated with cyanobacteria in liquid and sand cultures

| Plant organ | CB strain | Liquid culture | | | Sand culture | | |
|---|---|---|---|---|---|---|---|
| | | Length [cm] | Dry weight [mg/plant] | N [%] | Length [cm] | Dry weight [mg/plant] | N [%] |
| Shoot | Control +N | 22,44** | 96,1** | 156** | 21,29** | 71,0** | 140** |
| | Control –N | 16,37 | 53,5 | 100 | 15,28 | 33,3 | 100 |
| | $2S_9B$ | 19,97** | 72,7** | 144** | 17,94** | 52,4** | 132** |
| | $LBG_1$ | 19,67** | 71,6** | 141* | 19,82** | 54,2** | 133** |
| | $C_5$ | 16,34 | 48,6*↓ | 99 | 15,38 | 36,2* | 113 |
| | $LC_2B$ | 19,65** | 66,3** | 140* | 18,62** | 43,0** | 128** |
| | $2S_6B$ | 16,97 | 60,2* | 102 | 16,23 | 39,0* | 103 |
| Root | Control +N | 21,95** | 41,8** | 138** | 23,21** | 47,8** | 151** |
| | Control –N | 23,66 | 19,3 | 100 | 25,14 | 17,3 | 100 |
| | $2S_9B$ | 21,61* | 39,0** | 133** | 23,61** | 40,0** | 130** |
| | $LBG_1$ | 22,18 | 32,4** | 131** | 24,18 | 34,1** | 142** |
| | $C_5$ | 25,72* | 19,1 | 85*↓ | 28,72** | 19,1* | 99 |
| | $LC_2B$ | 21,21* | 33,3** | 116* | 25,22 | 33,3** | 107 |
| | $2S_6B$ | 23,28 | 23,4* | 88 | 26,14 | 28,3* | 91 |

* Statistical significance of the tested parameters compared with the control $–NO_3$ at the confidence level of 0.05

** Statistical significance of the tested parameters compared with the control $–NO_3$ at the confidence level of 0.01

↓ Inhibiting influence of cyanobacteria

NOTE: The results of N content measurements are presented in relative values with reference to control $-NO_3$.

Tab. 2. Length, dry weight and nitrogen content of shoots and roots of rice cultivar 353 co-cultivated with cyanobacteria in liquid and sand cultures

| Plant organ | CB strain | Liquid culture | | | Sand culture | | |
|---|---|---|---|---|---|---|---|
| | | Length [cm] | Dry weight [mg/plant] | N [%] | Length [cm] | Dry weight [mg/plant] | N [%] |
| Shoot | Control +N | 31,73** | 116,53** | 210** | 32,71** | 151,4** | 148** |
| | Control –N | 18,50 | 46,6 | 100 | 16,49 | 36,3 | 100 |
| | $2S_9B$ | 28,39** | 86,1** | 191** | 30,28** | 86,1** | 138** |
| | $LBG_1$ | 19,35 | 49,2 | 193** | 19,04** | 38,3* | 141** |
| | $C_5$ | 17,62*↓ | 32,6**↓ | 100 | 18,60* | 26,7*↓ | 97 |
| | $LC_2B$ | 25,41** | 73,9** | 194** | 21,51** | 39,7** | 138** |
| | $2S_6B$ | 19,96 | 45,8 | 141* | 19,19** | 35,3 | 110 |
| Root | Control +N | 20,41** | 66,4** | 207** | 21,42** | 86,4** | 184** |
| | Control –N | 27,91 | 24,9 | 100 | 27,30 | 29,2 | 100 |
| | $2S_9B$ | 25,65* | 57,7** | 131* | 24,17* | 52,7** | 136* |
| | $LBG_1$ | 28,98 | 28,2* | 132* | 29,20* | 29,7 | 122* |
| | $C_5$ | 34,34** | 17,3**↓ | 77*↓ | 33,33** | 25,3*↓ | 102 |
| | $LC_2B$ | 23,95** | 53,4** | 128* | 22,97** | 34,2* | 126* |
| | $2S_6B$ | 27,16 | 33,2** | 90 | 27,21 | 33,7* | 94 |

* Statistical significance of the tested parameters compared with the control $-NO_3$ at the confidence level of 0.05

** Statistical significance of the tested parameters compared with the control $-NO_3$ at the confidence level of 0.01

↓ Inhibiting influence of cyanobacteria

NOTE: The results of N content measurements are presented in relative values with reference to control $-NO_3$.

### *Cyanobacterial contribution to the growth and N content of rice grown in sand*

Cyanobacterial strains $2S_9B$ and $LBG_1$ positively affected the growth and N content of roots (with decrease of its length) and shoots of the rice cv. 36959, which was co-cultivated with five different cyanobacterial strains in sand culture (Table 1). The same effect was displayed with strain $LC_2B$ in the case of shoots. Strain $C_5$ increased the root length highly significantly. Cyanobacterial strains $2S_9B$, $LC_2B$ and $LBG_1$ exhibited the highest influence on the rice cultivar 353 (Table 2), regarding all of the tested parameters. Strain $C_5$ provoked significant decreases of the dry weight of plants, as well as a high increase of the root length.

According to the data obtained for the rice cultivar $OS_4$ grown in sand medium (Table 3), almost all cyanobacterial strains increased the dry weight and N content of roots, but reduced their length. Cyanobacterial strain $LC_2B$ had the highest stimulative effect on the tested parameters of rice shoots.

Strain $S_1$ increased the tested parameters of shoots and roots, except the root length, which was reduced. Strains 14 and $C_5$ caused significant reductions of the dry weight of shoots. Strain $C_5$ also reduced the root length.

Cyanobacterial strain $2S_9B$ exhibited outstanding influence on the tested parameters of the rice cv. 9909 (Table 4), causing highly significant reductions of the root length and increases of the other parameters as compared with the control without N. Strains $C_5$ and 2 had high stimulative effects on the roots length. Strain $C_5$ caused highly significant increases of the dry weight, while strain $C_3$ decreased this parameter. Strain $LBG_1$ caused highly significant increases in all tested parameters of the shoot.

Tab. 3. — Length, dry weight and nitrogen content of shoots and roots of rice cultivar $OS_4$ co-cultivated with cyanobacteria in sand culture

| CB strain | Shoot | | | Root | | |
|---|---|---|---|---|---|---|
| | Length [cm] | Dry weight [mg/plant] | N [%] | Length [cm] | Dry weight [mg/plant] | N [%] |
| Control +N | 15,3** | 30,0** | 153** | 16,6** | 35,2** | 122** |
| Control –N | 13,0 | 24,3 | 100 | 20,5 | 13,7 | 100 |
| $2S_9B$ | 15,2** | 27,4** | 129** | 18,5* | 33,7** | 116* |
| $LBG_1$ | 14,2 | 24,7 | 137* | 16,9** | 20,0** | 118** |
| $C_5$ | 14,5 | 21,3*↓ | 98 | 17,1** | 16,5 | 102 |
| 14 | 14,2 | 21,5*↓ | 134** | 22,2* | 20,8** | 113* |
| $LC_2B$ | 19,0** | 32,2** | 138** | 20,8 | 25,4** | 114* |
| $2S_6B$ | 14,8* | 27,4** | 128** | 18,2* | 32,1** | 102 |
| $S_1$ | 15,0** | 25,4 | 119* | 14,5**↓ | 25,5** | 117** |
| 2 | 15,7** | 24,3 | 113 | 17,9** | 25,7** | 111* |
| $S_2$ | 15,8** | 25,7 | 110 | 19,0* | 25,0** | 108 |
| $S_8B$ | 15,8** | 25,6 | 125* | 17,4** | 32,0** | 114* |
| $C_3$ | 14,8* | 27,3** | 130** | 17,1** | 31,3** | 113* |
| $C_4$ | 16,3** | 26,2* | 111 | 20,2 | 31,2** | 113* |

* Statistical significance of the tested parameters compared with the control $–NO_3$ at the confidence level of 0.05

** Statistical significance of the tested parameters compared with the control $–NO_3$ at the confidence level of 0.01

↓ Inhibiting influence of cyanobacteria

NOTE: The results of N content measurements are presented in relative values with reference to control $–NO_3$.

Tab. 4. — Length, dry weight and nitrogen content of shoots and roots of rice cultivar 9909 co-cultivated with cyanobacteria in sand culture

| CB strain | Shoot | | | Root | | |
|---|---|---|---|---|---|---|
| | Length [cm] | Dry weight [mg/plant] | N [%] | Length [cm] | Dry weight [mg/plant] | N [%] |
| Control +N | 19,5** | 29,0** | 132** | 23,0** | 32,5** | 182** |
| Control –N | 15,9 | 25,8 | 100 | 27,2 | 26,0 | 100 |

| | | | | | | |
|---|---|---|---|---|---|---|
| $2S_9B$ | 17,6* | 35,0** | 119* | 23,4** | 34,7* | 155** |
| $LBG_1$ | 20,7** | 40,0** | 121** | 23,2** | 27,7 | 165** |
| $C_5$ | 17,8* | 28,7** | 98 | 32,1** | 35,8** | 120 |
| 14 | 16,7 | 25,3 | 106 | 26,4 | 26,7 | 172** |
| $LC_2B$ | 18,9** | 29,8** | 115* | 28,8 | 30,8* | 158* |
| $2S_6B$ | 18,7** | 34,0** | 116* | 24,5** | 39,3** | 121 |
| $S_1$ | 16,3 | 26,3 | 97 | 29,9* | 28,0* | 101 |
| 2 | 16,4 | 24,3 | 104 | 34,3** | 24,2 | 95 |
| $S_2$ | 17,0 | 29,2** | 115* | 28,5 | 27,5 | 105 |
| $S_8B$ | 16,8 | 27,2 | 105 | 26,4 | 32,5** | 128* |
| $C_3$ | 15,6 | 22,7**↓ | 100 | 28,0 | 23,1*↓ | 107 |
| $C_4$ | 16,9 | 26,3 | 104 | 25,6 | 24,3 | 101 |

* Statistical significance of the tested parameters compared with the control $-NO_3$ at the confidence level of 0.05
** Statistical significance of the tested parameters compared with the control $-NO_3$ at the confidence level of 0.01
↓ Inhibiting influence of cyanobacteria
NOTE: The results of N content measurements are presented in relative values with reference to control $-NO_3$.

## DISCUSSION

Unlike the other agricultural plants such as wheat, corn or sugarbeet, in which the influence of cyanobacterial strains was stronger during the co-cultivation in liquid than in sand culture (S v i r č e v et al., 1996), the co-cultivation of rice and cyanobacteria produced similar results in both media. However, this was expected since the cultivation of rice requires high medium humidity (sand), and such cultivation conditions provide appropriate environment for the growth of cyanobacteria. In liquid medium, which is usually not the real environment, plant roots easily find homogenously dissolved nutritious substances. Our results show that in the case of rice, sand humidity was sufficient to enable cyanobacteria to express (or not) their $N_2$-fixing ability and the ability of excretion of nitrogen compounds in almost the same intensity as the liquid medium. This explains why the second part of our experiment was performed in sand culture only.

Generaly, the two *Nostoc* strains, $2S_9B$ and $LBG_1$, had the highest influence on the growth of all rice cultivars involved in co-cultivation. Strain $2S_9B$ significantly increased the tested parameters of all four co-cultivated rice cultivars, as compared with the plants cultivated in the nitrogen-free medium. The only exception was root length, for which a decrease was observed. It is known that plant cultivation in nitrogen-free media leads to the elongation of roots. In light of this statement, the evident decrease of root length observed during the co-cultivation should be interpreted not only as a sign of inhibition but also as an indicator of liberation of nitrogen compounds of cyanobacterial origin into the environment. Similar effects of this strain on the other agricul-

turally important plants have already been observed (Svirčev et al., 1996). Strain $2S_9B$ is emphasized as a strain having high potentials for application as a biofertilizer.

In our experiment, we noticed that the different levels of cyanobacterial influence on the tested parameters of the rice were uniformly distributed between the *Nostoc* and *Anabaena* strains. *Anabaena* strain $LC_2B$ had similar influences on co-cultivation participants as the *Nostoc* srains indicated above. *Anabaena* strain 14, although it significantly increased the N content of the rice cv. $OS_4$, also caused it extend its roots above the values of the control plants grown in the N-free medium. Such elongation of plant roots, in spite of the presence of liberated N in the medium indicated by the highly significant increase of N concentration in plants, could point to the production and presence of biologically active compounds (BACs). Cyanobacteria have been previously shown to produce compounds that stimulate the growth of plants. The following cyanobacteria or cyanobacterial extracts have been reported to stimulate plant growth: *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp., *Plectonema* sp. and *Nodularia* sp. (Rodgers et al., 1979). Cyanobacterial stimulation and inhibition of fungal growth was also reported (Szigeti et al., 1998).

Significant elongation of plant roots when co-cultivated with strain $C_5$ can also be attributed to the presence of BAC. This happened with all rice cvs. except $OS_4$, in which root shortening occurred, although no increase in the N content of plants could be observed. This phenomenon can also be attributed to the presence of BACs, those exerting the inhibiting effect. It is known that the higher plants influence the growth of cyanobacteria during co-cultivation (Rai, 1990). This seems to explain how the different cyanobacteria influence, either stimulatively or inhibitively, the parameters of different rice cultivars.

## REFERENCES


Bremner, J. M., Mulvaney, C. S. (1982): *Nitrogen-Total*. In: A. L. Page, R. H. Miller, D. R. Kenney (Eds.), *Methods of Soil Analysis* (Part 2) — Chemical and Microbiological Properties, Wisconsin USA, 194—248.

Обрехт, З. (1992): *Морфолошка и екофизиолошка својства азотофиксирајућих земљишних цијанобактерија и могућност њихове примене као биофертилизатора*, докторска дисертација. ПМФ, Универзитет у Новом Саду, Нови Сад.

Rai, A. N. (1990): *Cyanobacteria in Symbiosis*. In: Rai, A. N., Eds, *CRC Handbook of Symbiotic Cyanobacteria*, CRC Press, Boca Raton, FL, 1—2.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., Stanier, R. Y. (1979): *Generic assignments, strain histories and properties of pure cultures of cyanobacteria*, J. G. Microbiol. 111: 1—61.

Rodgers, G. A., Bergman, B., Henriksson, E., Urdis, M. (1979): *Utilization of blue-green algae as biofertilizers*, Plant Soil 52: 99—107.

Spiller, H., Gunasekaran, M. (1990): *Ammonia-excreting mutant strain of the cyanobacterium Anabaena variabilis supports growth of wheat*, Appl. Microbiol. Biotechnol., 33: 477—480.

Svirčev, Z., Gajin, S., Krstić, B., Tamaš, I., Nenin, P., Drobac, A. (1996): *Co-cultivation of $N_2$-fixing cyanobacteria and some agricul-*

*turally important plants in liquid and sand cultures*, Proceedings of 1st Conference on „Progress in Plant Sciences from Plant Breeding to Growth Regulation", Mosonmagyarovar, Hungary, 207—212.

Szigeti, J., Ordog, V., Kuptsova, E., Lukavsky, J., Svirčev, Z. (1998): *Stimulation and inhibition of fungal growth by microalgae and cyanobacteria*, 2nd Conference on Progress in Plant Sciences from Plant Breeding to Growth Regulation, Mosonmagyarovar (Hungary), 1998. Abstract Book: 95.

Venkataraman, G. S. (1972): *Algal biofertilizers and rice cultivation*, Today and Tomorrow's Printers, Faridabad, India.

## КО-КУЛТИВАЦИЈА $N_2$-ФИКСИРАЈУЋИХ ХЕТЕРОЦИСТНИХ *CYANOBACTERIA* И ПИРИНЧА У ВОДЕНИМ И ПЕШЧАНИМ КУЛТУРАМА

Александра В. Дробац, Зорица Б. Свирчев, Олга В. Петровић
Институт за биологију, Трг Доситеја Обрадовића 2,
ПМФ Нови Сад, 21000 Нови Сад, Југославија

Резиме

Велик број врста цијанобактерија способан је да фиксира слободан азот из ваздуха. Хетероцистне филаментозне цијанобактерије већ вековима имају улогу биофертилизатора на пиринчаним пољима. Овај рад приказује резултате добијене током ко-култивације 12 цијанобактеријских изолата из родова *Nostoc* и *Anabaena*, претходно изолованих из различитих земљишта Војводине (Југославија) и четири линије пиринча, у воденим и пешчаним културама. Резултати показују да је ниво цијанобактеријског утицаја и доприноса расту и развоју пиринча, као и укупној количини N, практично исти у воденим и пешчаним културама. Такође је примећен различит утицај цијанобактерија на дужину, суву масу и садржај азота корена и надземног дела различитих линија пиринча гајених у пешчаним културама. Продукција и ослобађање азотних једињења од стране цијанобактерија вероватно је повезано са специфичним условима које ствара култивација у здруженој култури са специфичном линијом пиринча. Поред азотофиксације, и екскреција биолошки активних супстанци цијанобактеријског порекла вероватно има важну улогу у ко-култивацији.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 45—53, 2000

UDC 631.461.5:633.63
631.461.5:633.18
631.461.5:633.11

*Jelica B. Simeunović, Zorica B. Svirčev, Slavka K. Gajin*

Institute of Biology, University of Novi Sad
Trg Dositeja Obradovića 2
21000 Novi Sad, Yugoslavia

# EFFECT OF $N_2$-FIXING *CYANOBACTERIA* ON HIGHER PLANTS GROWN IN SAND

ABSTRACT: The results of the co-cultivation of cyanobacterial isolates (*Nostoc* and *Anabaena* strains) and some higher plant species of agricultural importance: sugar beet (*Beta vulgaris*), wheat (*Triticum vulgare*) and rice (*Oryza sativa*) are presented. The experiment was performed in sand culture in order to determine the interactive effect of the participants. During the co-cultivation of the cyanobacterial isolates and plants, diverse effects were observed on the plant length, dry weight and nitrogen content of the roots and plant shoots. Also, in certain cases of rice, penetration of cyanobacteria into the roots cells was noticed. Our results indicate statistically significant differences in the tested parameters and in the contribution of the $N_2$-fixing cyanobacteria to the plants during the co-cultivation with cyanobacteria, as compared with the control plants. The influence of the cyanobacteria on the plants grown in sand culture seems to be very specific and the kind of relations depends on both the strain and plant species.

KEY WORDS: Co-cultivation, $N_2$-fixing cyanobacteria, rice, sand culture, sugar beet, wheat

## INTRODUCTION

Cyanobacteria are microorganisms of special interest because many species of them fix $N_2$ via the enzyme nitrogenase. This ability makes these microorganisms potentially valuable sources of nitrogen biofertilizer. Free extant cyanobacteria constitute a major group of $N_2$-fixing microorganisms in rice fields and have a potencial as biofertilizer in rice cultivation. (Venkataraman, 1972; Roger and Kulasooriya, 1981). Investigation on the practical utilization of cyanobacteria in rice fields has focused mainly on inoculation with cyanobacteria inocula produced in soil (Venkataraman, 1981). The extracellular release of nitrogen compounds and the release of certain biostimulative substances are obvious ways in which cyanobacteria may

benefit the higher plants. On the other hand, some studies have shown how plants may aid the growth and increase of $N_2$-fixation by cyanobacteria during co-cultivation (Rai, 1990).

A beneficial effect of an ammonia-excreting mutant of *Anabaena variabilis* on the growth of wheat has been demonstrated (Spiller and Gunasekaran, 1990). Both the nitrogenase activity of the mutant strain and plant growth are enhanced by direct associations of the cyanobacterium with the plant roots. A positive influence of $N_2$-fixing cyanobacteria on the growth and nitrogen content of wheat seedlings grown hidroponically with their roots colonized by soil isolates of cyanobacteria has been noticed (Obreht et al., 1993).

This paper describes the effect of co-cultivation of three cyanobacterial strains (*Nostoc* and *Anabaena*) when they were associated with roots of three higher plant species of agricultural importance: rice, sugar beet and wheat.

## MATERIALS AND METHODS

### *Organisms*

Three filamentous nitrogen-fixing cyanobacteria (*Nostoc* and *Anabaena*) were used for the investigation. *Nostoc* strain 2S9B was isolated from solonetz, *Anabaena* strain LC2 from black meadow and *Anabaena* strain Č5 from chernozem soils in the Province of Vojvodina, Yugoslavia (Gantar et al., 1991). The strains were selected from our previous investigations (Gantar et al., 1995). Sugar beet (*Beta vulgaris*) and wheat (*Triticum vulgare*) cultivars were supplied by the Institute of Field and Vegetable Crops, Faculty of Agriculture, University of Novi Sad, Yugoslavia. The rice cultivar (*Oryza sativa*) was obtained from officials of Manila University, the Philippines.

### *Growth conditions*

Cyanobacteria were maintained in the BG-11 (Rippka et al., 1979) liquid culture, kept in flasks at room temperature and light flux density of 50 μmol $m^{-2}s^{-1}$. Cyanobacterial contribution to the growth of plants was studied in the sand that had been thoroughly washed with water and sterilized. The sand was placed into pots lined with plastic bags to allow the retention of 1 cm deep surface layer of cyanobacterial culture in nitrogen — free medium. The pots were incubated at 24°C and shaded from excessive light for 7 days, during which time an abundant cyanobacterial mat developed on the surface of the sand. The liquid was then drained from the pots by puncturing the plastic bags and plant seeds were placed in the sand. During the subsequent 24-day cultivation period, the sand was watered three times with nitrogen-free medium but was otherwise kept moist by adding distilled water. Control pots without cyanobacteria were watered with nitrogen or without nitrogen medium. Each pot contained 10 plants and each treatment consisted of five replicates.

### *Plant measurements*

After 24 days of plant cultivation and co-cultivation with cyanobacteria in sand culture, preparations were made for measurements of the length, dry weight and nitrogen content in plants. After harvesting the plants, roots were washed free from sand and excised from shoots. The lengths of aboveground parts (shoots) and roots were measured. After that the aboveground parts and roots were dried separately in a hot-air oven at 85°C for 2 days prior to determination of dry weight and nitrogen content.

### *Nitrogen content*

Nitrogen content of dry matter was determined using Kjeldahl method (Bremner and Mulvaney, 1982). Data for plants from one pot were pooled and the presented data are the means of measurements made of five replicated samples.

### *Statistical analysis*

Experimental data were subjected to the analysis of variance (LSD-test).

## RESULTS

### *Cyanobacterial contribution to the growth and nitrogen content of plants grown in sand culture*

In the experiment carried out in sand, no specific associations between plant roots and cyanobacteria have been observed with the exception of the rice cultivar where a tight association and penetration into the root cells of the 2S9B strain were recorded. Microscope analyses of rice root demonstrated that single cells, cell groups or complete filaments of 2S9B strain covered some parts of roots. The strain 2S9B also penetrated the root epidermis and cortex and was present inside the root cells of rice.

Figures 1, 2 and 3 present the results of plant measurements made after a period of plant co-cultivation with the cyanobacterial strains. Significant effect of cyanobacterial strain on root dry weight and length compared with the control without nitrogen, was observed only in the rice cultivar. The strains 2S9B and LC2 decreased the length of root of rice, and increase in root length was observed in the case of Č5 strain. Distinctly stimulative influence on root growth was recorded in the case of the 2S9B strain, which significantly increased the root dry weight of the rice cultivar (Figure 1).

The root lengths and dry weights of sugar beet and wheat have not been significantly influenced by all the cyanobacteria. Still, some changes in root length were recorded in the presence of Č5 as stimulation and in the presence of LC2 as inhibition (Figures 2 and 3).

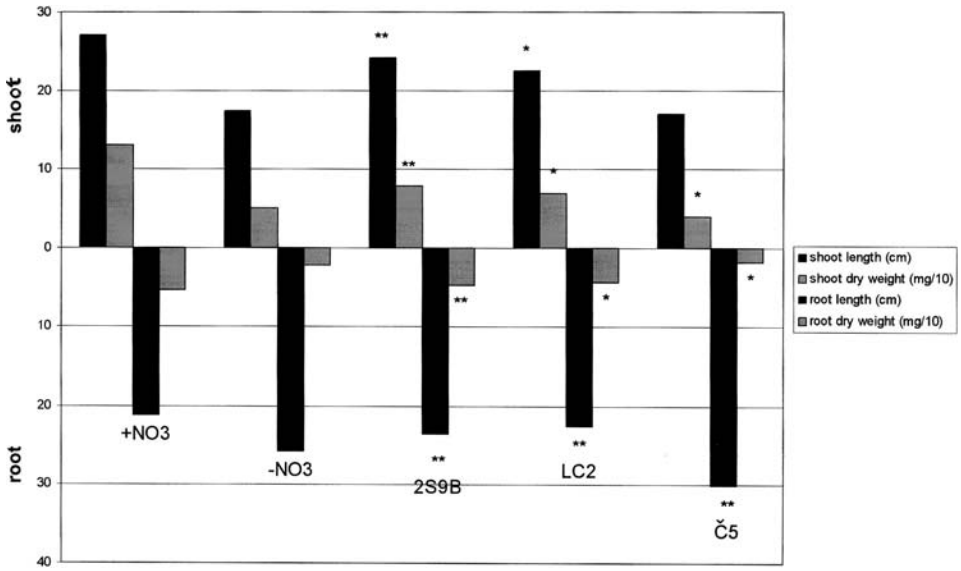



Fig. 1. — The effect of cyanobacterial strains on the length and dry weight of rice cultivar

* statistical significance of tested parameters compared with the control $-NO_3$ at the confidence level of 0.05
** statistical significance of tested parameters compared with the control $-NO_3$ at the confidence level of 0.01

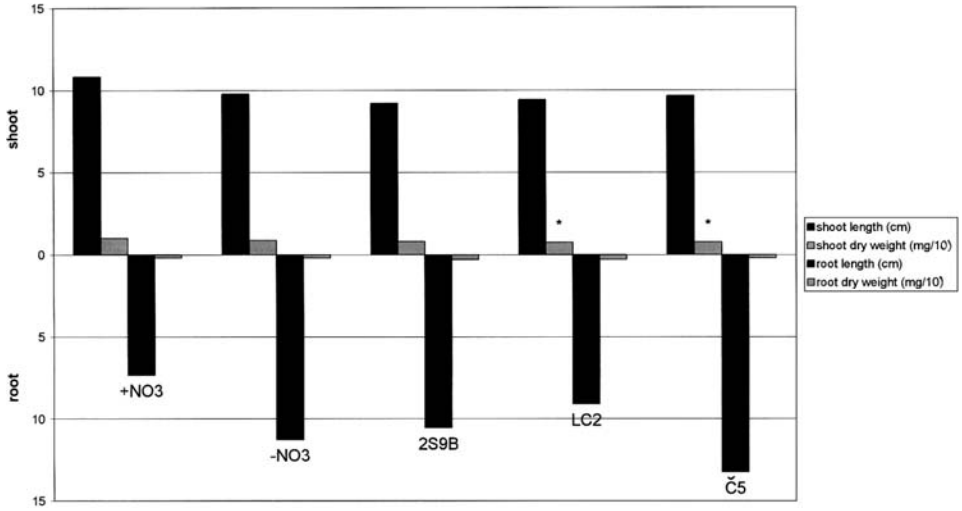



Fig. 2. — The effect of cyanobacterial strains on the length and dry weight of sugar beet

* statistical significance of tested parameters compared with the control $-NO_3$ at the confidence level of 0.05

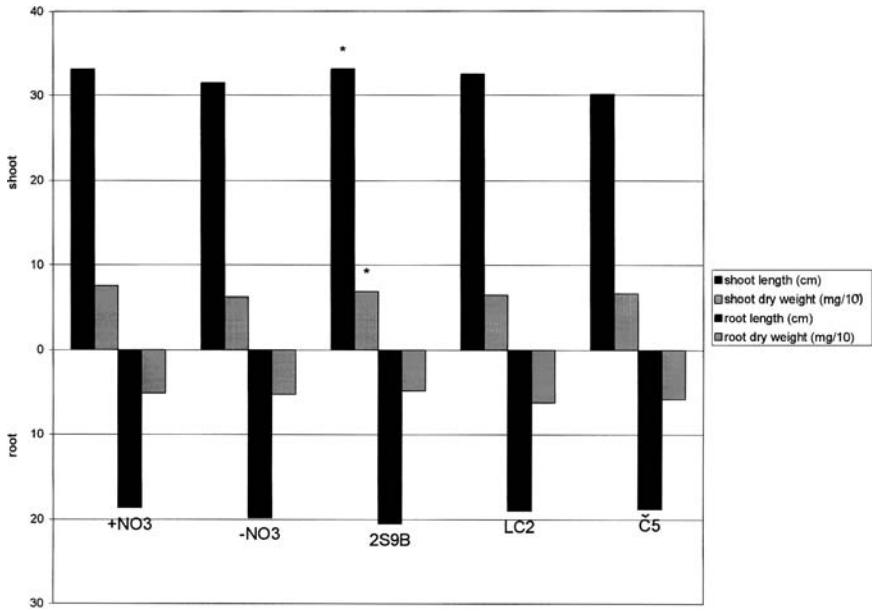



Fig. 3. — The effect of cyanobacterial strains on the length and dry weight of wheat

* statistical significance of tested parameters compared with the control $-NO_3$ at the confidence level of 0.05

The cyanobacteria exhibited similar effects on the tested parameters of shoots. The length and dry weight of rice shoots, compared with the control without nitrogen, have been statistically significantly increased by all cyanobacterial strains (Figure 1). In the case of wheat, only strain 2S9B increased the length and dry weight of shoots (Figure 3). At the same time the parameters of sugar beet have not been influenced by the cyanobacteria (Figure 2).

The nitrogen contents in roots and shoots of the examined plants are shown in Table 1. Significant increases in nitrogen content of both shoots and roots of the rice cultivar occurred with 2S9B and LC2 strains, while strain Č5 did not change the nitrogen content of rice. The same effect of the cyanobacteria on the nitrogen contents in wheat roots and shoots has been recorded. In the case of sugar beet, only strain 2S9B increased the nitrogen concentration of both shoots and roots. Strain LC2 decreased only the nitrogen content in shoots, while strain Č5 did not influence the nitrogen content in roots, but the content in shoots has been changed (Table 1).

Tab. 1. — Nitrogen contents in shoots and roots after co-cultivation in sand

| Plant species | Treatm. contr/strain | Shoot | Root |
|---|---|---|---|
| | | N (%) | N (%) |
| Rice (*Oryza sativa*) | $+NO_3$ | 145 | 166 |
| | $-NO_3$ | 100 | 100 |
| | 2S9B | 135** | 133** |
| | LC2 | 133** | 116* |
| | Č5 | 105 | 100 |
| Sugar beet (*Beta vulgaris*) | $+NO_3$ | 133 | 382 |
| | $-NO_3$ | 100 | 100 |
| | 2S9B | 119** | 221** |
| | LC2 | 76** | 117 |
| | Č5 | 92** | 104 |
| Wheat (*Triticum vulgare*) | $+NO_3$ | 130 | 137 |
| | $-NO_3$ | 100 | 100 |
| | 2S9B | 123** | 130* |
| | LC2 | 115* | 111 |
| | Č5 | 102 | 98 |

* statistical significance of tested parameters compared with the control $-NO_3$ at the confidence level of 0.05
** statistical significance of tested parameters compared with the control $-NO_3$ at the confidence level of 0.01
The results of nitrogen content measurements are presented in relative values with reference to control $-NO_3$

## DISCUSSION

During the co-cultivation in sand of cyanobacterial strains and some agronomicaly important plant cultivars, significant influence of cyanobacterial strains on the plants was observed. Cultivation of rice with cyanobacteria requires a high humidity of the medium. When we take this into consideration, these results and the results obtained during the co-cultivation in liquid culture (Svirčev et al., 1997) are similar. In the view of potential application of co-cultivation, these results confirm that the selection of appropriate plant species, characterized by high water demands, is necessary to enable favorable conditions for the growth of $N_2$-fixing cyanobacteria and also to enable the release of nitrogen compounds into the environment. The great importance of cyanobacteria in rice fields was discussed by Roger and Kulsooriya (1981) and Venkataraman (1981).

Among the tested strains, the greatest beneficial effect on the participating plant was determined in the case of 2S9B (*Nostoc*). This strain significantly increased the nitrogen content of roots and shoots of all tested plants grown in sand. Similar results were presented previously by Svirčev et al., (1997).

It is known that the cultivation of plants in nitrogen-free media leads to the elongation of the roots. In the light of this statement, the evident decrease in roots length observed during the co-cultivation of the 2S9B strain and rice should not be accepted as an inhibition but as an indicator of liberation of nitrogen compounds by cyanobacteria into the environment. According to these results and the results of our previous investigation (Obreht et al., 1993; Gantar et al., 1995), this strain should be underlined as a strain with great chances for potential application as biofertilizer. In addition, this strain was determined to be able to penetrate root cells of the rice cultivar, which could be significant for creating an artificial symbiosis. The way in which cyanobacteria penetrate the root epidermis and cortex of rice root is similar to the associations formed when *Rhizobia* were co-cultured with plant callus (Holsten et al., 1971) and when cyanobacteria were co-cultured with tobacco callus (Gusev et al., 1986). The strain Č5 showed a specific influence on the plant species. A negative effect on the rice cultivar grown in sand was recorded, leading to the decrease in plant biomass, followed by elongation of the root. During the co-cultivation of the cyanobacteria and plants in nitrogen-free media this kind of root elongation is quite rare, indicating the production and presence of biologically active substances to stimulate the growth of plants. The cyanobacteria have been previously shown to produce compounds that stimulate the growth of plants. The following cyanobacteria have been reported to stimulate plant growth: *Cylindrospermum muscicola* (Venkataraman and Neelakantan, 1967); *Anabaena* sp., *Nostoc* sp., *Oscillatoria* sp., *Plectonema* sp. and *Nodularia* sp. (Rodgers et al., 1979).

According to the presented results, the rice cultivar showed to be the most suitable plant species for co-cultivation with cyanobacteria. The influence of the cyanobacteria on the tested higher plants grown in sand culture seems to be highly specific and the kind of relations depends on both the strain and plant species taking part in the association.

## REFERENCES


Bremner, J. M., Mulvaney, C. S. (1982): *Nitrogen-Total*. In: *Methods of Soil Analysis (part 2) — Chemical and Microbiological Properties* (A. L. Page, R. H. Miller, D. R. Kenney, eds) pp. 194—248, Wisconsin, USA.

Gantar, M., Obreht, Z., Fojkar, O. (1991): *Occurence and characterization of nitrogen-fixing cyanobacteria in different temperate soils*, Microbiologia, 28: 33—44.

Gantar, M., Kerby, N. W., Rowell, P., Obreht, Z., Scrimgeour, C. (1995): *Colonization of wheat (Triticum vulgare) by $N_2$-fixing cyanobacteria: IV. Dark nitrogenase activity and effects of Cyanobacteria on natural $N^{15}$ abundance in plants*, New Phytol., 129: 337—343.

Gusev, M. V., Korzheneuskaya, G., Pyvovarova, D. U., Baulina, O. I., Butenko, R. G. (1986): *Introduction of a $N_2$-fixing cyanobacteria into tobacco shoot regenerates*, Planta, 167: 1—8.

Holsten, R. D., Burns, R. C., Hardy, R. E., Heberg, R. R. (1971): *Establishment of symbioses between Rhizobia and plant cells in vitro*, Nature (London), 232: 173—176.

Obreht, Z., Kerby, N. W., Gantar, M., Rowell, P. (1993): *Effect of root-associated $N_2$-fixing Cyanobacteria on the growth and nitrogen content of wheat (Triticum vulgare) seedlings*, Biol. Fert. Soils, 15: 68—72.

Rai, A. N. (1990): *Cyanobacteria in symbioses*. In: Rai A. N. (ed.) *CRC Handbook of Symbiotic Cyanobacteria*, CRC Press, Inc. Boca Raton, pp. 1—2.

Rippka, R., Deruelles, J., Waterbury, J. B., Herdman, M., Stanier, R. Y. (1979): *Generic assignments, strain histories and properties of pure cultures of cyanobacteria*, J. Gen. Microbiol, 111: 1—61.

Roger, P. A., Kulasooriya, S. A. (1981): *Nitrogen-fixing blue-green algae and rice*, Los Banos, Philippines: International Research Institute, 1—112.

Rodgers, G. A., Bergman, B., Henriksson, E., Urdis, M. (1979): *Utilization of blue-green algae as biofertilizers*, Plant Soil 62: 99—107.

Spiller, H., Gunasekaran, M. (1990): *Ammonia-excreting mutant strain of the cyanobacterium Anabaena variabilis supports growth of wheat*, Appl. Microbiol. Biotechnol. 33: 477—480.

Svirčev, Z., Gajin, S., Tamaš, I., Nenin, P., Drobac, A. (1997): *Co-cultivation of $N_2$-fixing cyanobacteria and some agriculturally important plants in liquid and sand cultures*, Proceedings of 1st Conference on „Progress in Plant Sciences from Plant Breeding to Growth Regulation", Mosonmogyarovar, Hungary, 207—212.

Venkataraman, G. S., Neelakantan, S. (1967): *Effect of the cellular constituents of the nitrogen-fixing blue-green alga Cylindrospermum muscicola on the root growth of rice plants*, J. Gen. Appl. Microbiol., 13: 53—61.

Venkataraman, G. S. (1972): *Algal biofertilizers and rice cultivation*, Today and Tomorrow's Printers, Faridabad, India.

Venkataraman, G. S. (1981): *Blue-green algae for rice production. A manual for its promotion*, FAO Soil Bull N° 46.

## УТИЦАЈ АЗОТОФИКСИРАЈУЋИХ ЦИЈАНОБАКТЕРИЈА НА ВИШЕ БИЉКЕ ГАЈЕНЕ У ПЕШЧАНОЈ КУЛТУРИ

Јелица Б. Симеуновић, Зорица Б. Свирчев, Славка К. Гајин
Институт за биологију, Трг Доситеја Обрадовића 2,
ПМФ Нови Сад, 21000 Нови Сад, Југославија

Резиме

У овом раду испитиван је утицај 3 различита азотофиксирајућа цијанобактеријска соја (2S9B, LC2 и Č5) на раст неких пољопривредно значајних култура: шећерне репе, пиринча и пшенице. Констатовано је да само сој 2S9B остварује чвршћу везу и да продире у ћелије корена пиринча. У случају пиринча уочен је значајан утицај испитиваних сојева на суву масу и дужину надземног дела и корена. Нису примећени статистички значајни утицаји цијанобактерија на суву масу и дужину корена пшенице и шећерне репе. Сој 2S9B (*Nostoc*) утицао је на повећање дужине и суве масе надземног дела код пшенице. Два соја 2S9B и LC2

(*Nostoc* и *Anabaena*) утицали су на повећање испитиваних параметара код пиринча. Установљено је да је сој 2S9B утицао на повећање концентрације укупног азота у корену и надземном делу током ко-култивације са шећерном репом и пшеницом, док у случају пиринча осим соја 2S9B и сој LC2 има позитиван ефекат. Током истраживања запажен је и негативан ефекат сојева LC2 и Č5 (*Anabaena*) на суву масу, као и на садржај укупног азота надземног дела код шећерне репе.

На основу добијених резултата може се рећи да је утицај азотофиксирајућих цијанобактерија на раст биљака у циљу њихове примене као биофертилизатора најизразитији при ко-култивацији са пиринчем. Ефекти здруженог гајења су врло специфични за одређену комбинацију цијанобактеријског соја и биљне врсте, те се због тога не препоручује селекција универзалног соја као биофертилизатора, него одабирање једног или више сојева у односу на конкретан генотип биљне врсте.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 55—62, 2000

UDC 572.512.087.2(497.113 Srem)

*Tatjana M. Pavlica, Verica S. Božić-Krstić, Rada S. Rakić, Marija R. Savić*

Institute of Biology, Faculty of Natural Sciences
University of Novi Sad, Trg D. Obradovića 2
21000 Novi Sad, Yugoslavia

# ANTHROPOLOGICAL CHARACTERISTICS OF THE POPULATION OF SREM ACCORDING TO NATIVE ORIGIN

ABSTRACT: The paper presents the results of a biometrics analysis of morpho-physiological characteristics (body height, leg length, arm length, body mass, chest circumference and the vital capacity of lungs) of the adult population of Srem. The population structure, which combines the indigenous element and immigrants, was established in the Vojvodina Province after World Wars I and II. The investigation included 1826 individuals, 965 males and 859 females, all of them from 20 to 50 years of age. The data were processed by the variation statistical method, for males and females separately. Higher heterogeneity was observed for the male population, where the immigrants had higher values for all morphological characteristics. The females were much more homogeneous since no statistical difference could be found for any of the investigated characteristics.

KEY WORDS: arm length, body height, body mass, chest circumference, immigrants, indigenous, leg length, native origin, Srem, vital capacity of lungs

## INTRODUCTION

It is well-known that the forming of anthropological characteristics is influenced by both genetic and environmental factors. Their joint influence determines the development of an organism and its anthropological characteristics. These factors make up the structure of a certain human population. Because of the variability of environmental factors, it is necessary to provide data about anthropological characteristics for every region, even for individual towns and villages.

Historically, the region of Srem has been a scene of dramatic events that led to the disappearance of old populations and the appearance of completely new ones. The region had been originally inhabited by the Agatirs tribe, the earliest known inhabitants of the Vojvodina Province, then by the Illyric tribes of Amantins and Breuts, Dacians, Thracians and Celts, which were either subjugated or expelled by the Romans. During the great migration of peoples, a

large number of peoples either passed through or temporarily settled and ruled the area, to either leave or disappear altogether. However, it is certain that traces of these peoples have been preserved „in the toponymy and blood of the peoples in the countries where they used to live". In the 6th century Slavs migrated to this part of Pannonia, and in the 10th century Hungarians settled in the areas north of Srem (Popović, 1990).

The people that populated the region differed in geographic origin, ethnic structure and bioanthropological aspects. The Turkish invasion caused massive migrations. The Serbs intensively populated the territory of Srem and it is through these migrations that Srem acquired its permanent Serbian ethnic character. For several decades they lived in temporary dwellings. In mid 18th century they settled in villages which, for the most part, have survived until today. „This migration created the demographic basis which has remained until today" (Ćurčić, 1980).

Until the beginning of the 20th century the state and landowners moved in settlers belonging to several ethnic groups, which created ethnic variety in the area. Croats, together with Shoktsi and Bunievci as subgroups, had populated Srem during the Turkish rule. Hungarians moved to the area later, in the 18th century, after the Turks withdrew from Srem. From mid 18th century onwards, together with Hungarians, Slovakians first settled in the regions of Banat and Bačka, but were later resettled in Srem for the reasons of commerce. Ruthenians that inhabited Bačka since the 18th century moved later on to other areas, primarily to Srem.

The following events caused marked demographic changes in the 20th century: the World War I, the land reform and organized resettlement between the two wars, the World War II, the land reform and organized resettlement after the World War II. Apart from the migrations caused by warfare and resettlement programs, people spontaneously migrated to this region throughout this period. Large economic changes and urbanization were also important demographic factors (Ćurčić, 1980).

Popović (1990) interestingly notes that Srem, due to its special geographic position, has always been in the way of every migration of Serbs and that thus its population represents in miniature almost all segments of the Serbian people. He points out that the inhabitants of the areas along the Sava River (Posavina) and on the Fruška Gora Mountain originate mostly from Serbia, whereas the population along the left bank of the Danube River originally comes from western and northern parts of the former Yugoslavia.

Literature provides data on the morphological and other anthropological characteristics of the population that formerly lived in Srem. However, those were more descriptive observations of the physical appearance of the people, without conclusions made on the basis of exact measurements. Popović (1990) says that Proto-Slavs were „tall, red-haired and very strong" and that they differed among themselves. He also quotes the physician Marikovski from 1767, who described the inhabitants of Srem as tall, dark-eyed, black-haired, temperamental, and mostly of sanguine-choleric disposition. Another physician, doctor Kitl, wrote in 1795 that the people of Srem were of a rather strong and tall build, dark complexion and usually black hair, and that fat people were quite uncommon.

Vlahović (1994) points out that in the 12th century Hungarians had possessed the biophysical and anthropological characteristics typical of their Mongolian origin, but they lost them in the course of centuries, through intermarriage with other peoples, especially Slavs, acquiring their present appearance from Slavs.

Research into the bioanthropological characteristics of the adult population of the Vojvodina Province until 1976 was done on the basis of native and ethnic origin and mostly in the male population (Gavrilović 1960, 1961, 1962a,b, 1963, 1964). Božić (1976) studied the anthropological characteristics of adult inhabitants of the Vojvodina Province, above 20 years of age, of both sexes, both immigrant and indigenous. It was found that the immigrants of both sexes were on average taller than the natives. Gavrilović (1978), studying the adult population of the region of the Fruška Gora Mountain, of both sexes, aged 18—90, found that they were mostly of tall stature and that there was a larger proportion of very tall people among the immigrants than among the natives. The mean values of the Rohrer index were higher in the indigenous population of all age groups, with slightly smaller differences in the females. The research into 40 morpho-physiological and biotypological indicators in adult and elderly persons gave an insight into the change of anthropological characteristics during the process of ageing (Gavrilović and Božić-Krstić, 1981).

The object of this paper was to determine the basic anthropological characteristics of the population of Srem, natives and immigrants from various parts of Yugoslavia who settled in the Vojvodina Province after the World Wars I and II.

## MATERIAL AND METHOD

In the summer of 1995 an anthropological research was carried out on the population of Srem aged 20—50. The research was done on the basis of IBP, in 21 settlements situated evenly over the entire territory of Srem. The investigation included 1824 persons; 965 males and 859 females. The data were processed by the variation statistical method for both the immigrant and indigenous populations, separately for each sex. The basic statistical indicators calculated were: the mean value (X), minimum (MIN) and maximum (MAX) values, standard deviation (STD), variance (VAR), variability coefficient (VC) and random error (RE). Differences between the examined characteristics were determined by the univariate analysis of variance. The degree of differentiation of the population was compared by the intraclass correlation coefficient ($r_I$) (Sokal and Rohlf, 1969). It is necessary to point out that the research was carried out during the civil war in the former Yugoslavia that resulted in an intensive influx of refugees. The refugee population, however, was not included in the research. The results give an accurate picture of the bioanthropological characteristics of the autochthonous population that had lived in Srem before the beginning of the latest war.

## RESULTS

The results of the statistical analysis of the immigrant and indigenous populations of both sexes are given in Table 1.

Tab. 1. — The results of the statistical analysis of the immigrant and indigenous population of both sexes

| Distribution of anthropometric characteristics according to native origin | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| *Males — indigenous* | | | | | | | | |
| Value | N | $\bar{x}$ | Min. | Max. | STD | VAR | VC (%) | RE |
| Age | | 36 years | | | | | | |
| Stature | 675 | 172.85 | 149.2 | 194.0 | 6.72 | 45.12 | 3.89 | 0.26 |
| Leg length | 675 | 99.13 | 74.4 | 115.8 | 5.52 | 30.51 | 5.57 | 0.21 |
| Arm length | 675 | 75.25 | 64.7 | 97.6 | 3.77 | 14.22 | 5.01 | 0.15 |
| Body mass | 675 | 80.40 | 50.0 | 149.0 | 14.27 | 203.58 | 17.75 | 0.55 |
| Chest circumference | 675 | 101.89 | 79.0 | 150.0 | 9.04 | 81.66 | 8.87 | 0.35 |
| Vital capacity | 674 | 3489.91 | 1100.0 | 6600.0 | 763.26 | 582569 | 21.87 | 29.4 |
| *Males — immigrants* | | | | | | | | |
| Value | N | $\bar{x}$ | Min. | Max. | STD | VAR | VC (%) | RE |
| Age | | 36 years | | | | | | |
| Stature | 290 | 175.31 | 157.2 | 197.0 | 7.11 | 50.5 | 4.05 | 0.42 |
| Leg length | 290 | 100.91 | 86.0 | 114.0 | 5.35 | 28.60 | 5.30 | 0.31 |
| Arm length | 290 | 76.39 | 65.8 | 87.5 | 3.62 | 13.11 | 4.74 | 0.21 |
| Body mass | 290 | 82.37 | 56.0 | 135.0 | 14.14 | 199.88 | 17.16 | 0.83 |
| Chest circumference | 290 | 102.47 | 85.0 | 133.0 | 8.76 | 76.8 | 8.55 | 0.51 |
| Vital capacity | 290 | 3583.79 | 1500.0 | 6300.0 | 806.2 | 649979 | 22.50 | 47.34 |
| *Females — indigenous* | | | | | | | | |
| Value | N | $\bar{x}$ | Min. | Max. | STD | VAR | VC (%) | RE |
| Age | | 34 years | | | | | | |
| Stature | 545 | 160.26 | 142.0 | 179.0 | 5.99 | 35.95 | 3.74 | 0.26 |
| Leg length | 545 | 95.23 | 72.7 | 110.0 | 4.72 | 22.23 | 4.95 | 0.20 |
| Arm length | 545 | 68.94 | 59.0 | 86.0 | 3.36 | 11.32 | 4.88 | 0.14 |
| Body mass | 545 | 66.32 | 42.0 | 135.0 | 13.16 | 173.3 | 19.85 | 0.56 |
| Chest circumference | 545 | 92.59 | 70.0 | 127.0 | 9.376 | 87.9 | 10.13 | 0.40 |
| Vital capacity | 544 | 2304.78 | 1000.0 | 4000.0 | 544.74 | 296742 | 23.64 | 23.36 |
| *Females — immigrants* | | | | | | | | |
| Value | N | $\bar{x}$ | Min. | Max. | STD | VAR | VC (%) | RE |
| Age | | 35 years | | | | | | |
| Stature | 314 | 160.87 | 139.0 | 184.8 | 6.18 | 38.2 | 3.84 | 0.35 |
| Leg length | 314 | 95.67 | 80.0 | 110.5 | 4.61 | 21.27 | 4.82 | 0.26 |
| Arm length | 314 | 69.02 | 60.6 | 83.0 | 3.43 | 11.796 | 4.98 | 0.19 |
| Body mass | 314 | 66.18 | 44.0 | 107.0 | 12.09 | 146.07 | 18.26 | 0.68 |
| Chest circumference | 314 | 92.74 | 74.0 | 128.0 | 9.22 | 85.09 | 9.95 | 0.52 |
| Vital capacity | 314 | 2292.99 | 1000.0 | 4000.0 | 559.1 | 312626 | 24.38 | 31.55 |

The table shows that both groups of specimens are nearly of the same age. The immigrants have taller average stature (175.31 cm) than the natives (172.85 cm). The minimum value of stature was recorded in the indigenous population (149.2 cm) and the maximum in the immigrants (197.0 cm). The latter group is characterized by higher values of all other characteristics and the differences are statistically significant for the stature and leg and arm lengths. Body mass, chest circumference and vital capacity are also greater in the immigrants but the differences are not statistically significant. With females there is no difference between the average stature of the indigenous population (160.26 cm) and the immigrants (160.87 cm).

Tab. 2. — Analysis of variance according to native origin

| Males | | | | | | |
|---|---|---|---|---|---|---|
| | Stature | Leg length | Arm length | Body mass | Chest circumference | Vital capacity |
| $F_0$ | 23.83 | 19.44 | 16.65 | 3.167 | 0.762 | 2.436 |
| $r_I$ | 0.0557 | 0.0455 | 0.039 | 0.0056 | –0.0006 | 0.0037 |
| P | 1.2E-6 | 1.2E-5 | 4.9E-5 | 0.075 | 0.383 | 0.119 |
| | | P = 0.01 | $F$ = 6.663 | | P = 0.05 | $F$ = 3.852 |
| Females | | | | | | |
| $F_0$ | 2.0402 | 1.898 | 0.073 | 0.0714 | 0.0024 | 0.273 |
| $r_I$ | 0.0077 | 0.0067 | –0.007 | –0.007 | –0.0075 | –0.0055 |
| P | 0.1536 | 0.1687 | 0.787 | 0.789 | 0.961 | 0.601 |
| | | P = 0.01 | $F$ = 6.665 | | P = 0.05 | $F$ = 3.853 |

The examined female groups are uniform for the observed characteristics. Slightly higher values of all longitudinal characteristics are observed in the immigrants and higher values of body mass, chest circumference and vital capacity in the indigenous population.

The average values of the intraclass correlation coefficient are higher with the males (0.0248) than with the females (—0.0021), which is an indication of considerably higher heterogeneity of the examined characteristics in the males.

## DISCUSSION

The analysis of the results showed that the immigrants of both sexes are of taller stature than the natives. The difference is quite significant with the males (2.46 cm), whereas it is negligible with the females (0.61 cm). These findings are in accordance with earlier studies of the population of the Vojvodina Province (Božić, 1976, Gavrilović, 1978). It was observed that those inhabitants of the Vojvodina Province who were resettled in the area have taller stature than the indigenous population. This can be explained by the fact that the majority of them came to Srem from the mountainous areas of Bosnia, Herzegovina and Lika, regions where the population is characterized

by tall stature. When these results were compared with the results of an earlier research (Božić, 1976), acceleration in the stature values was observed. In the immigrant category it was 2.72 cm for the males and 1.8 cm for the females, and in the indigenous category it was 4.46 cm and 4.35 cm for the males and females, respectively. It is interesting to note that the natives of both sexes have a somewhat greater acceleration of the stature values than the immigrants, which confirms the opinion of some anthropologists that acceleration is larger in the groups of smaller stature.

As with stature, the immigrants of both sexes had greater leg length than the indigenous specimens. The difference is statistically significant only in the males whereas with females it is minor and statistically insignificant. The immigrants of both sexes had longer arms than the natives. This was to be expected considering the fact that the arm length is a longitudinal characteristic, which is highly correlated with the stature and leg length, for which it has already been said that it is greater within the immigrant category. With the males these differences are somewhat larger and statistically significant, whereas with the females the differences are insignificant. Gavrilović (1978) also found larger differences in the arm length between these two groups.

The male immigrants had larger body mass than the natives, and the female natives had larger body mass than the female immigrants, but these differences are not statistically significant. Gavrilović (1978) too points out a great discrepancy in body mass between immigrants and natives. In the present population of Srem there are no relevant differences in body mass between these two groups, which indicates that the immigrants have become adapted to the new environment.

Chest circumference of both sexes is somewhat greater in the immigrants but the variance analysis showed no relevant differences between the two groups.

As to vital capacity, higher values were observed in the male immigrants (3583.79 ml) and in the female natives (2304.78 ml). Statistically significant differences were not observed, which is consistent with an earlier research (Gavrilović, 1978).

The mean values of the intraclass correlation coefficient (males $r_I$ = 0.0248; females $r_I$ = 0.0021) suggest greater heterogeneity in the males, which has been noted in earlier studies (Pavlica et al., 1998, 1999).

## CONCLUSION

On the basis of the data presented above, it can be concluded that the male immigrants exhibited higher values of all characteristics and the differences were especially significant with the longitudinal characteristics. The two groups of females were not considerably different in any of the characteristics, which suggests their greater harmoniousness and eco-stability.

The research showed that both groups of the population of Srem were of tall stature and strong build. Acceleration is evident and it is interesting that it is greater with the indigenous population than with the immigrants. In compar-

ison with the research by doctor Marikovski (1767) and Kitl (1795), the present population has similar characteristics, with acceleration, the phenomenon present in the whole world.

## REFERENCES


Божић-Крстић, В. (1976): *Неке физичке особине становништва САП Војводине*, магистарски рад, Универзитет у Новом Саду, ПМФ, Институт за Биологију, Нови Сад.

Ђурчић, С. (1980): *Становништво фрушкогорске области*, Матица српска, Нови Сад.

Гавриловић, Ж. (1960): *Прилог познавању утицаја послератне миграције на телесно стање Црногораца насељених у Војводини*, посебно издање МС, Нови Сад.

Гавриловић, Ж. (1961): *Прилог проучавању телесног стања насељеника из Лике после Првог светског рата у Војводини*, посебно издање МС, Нови Сад.

Гавриловић, Ж. (1962а): *Прилог биологији Херцеговаца насељених у Војводини после Првог и Другог светског рата*, Зборник природних наука МС, Нови Сад, стр. 22—43.

Гавриловић, Ж. (1962б): *Заједничке црте у антрополошким особинама становништва у Војводини*, Етнолошки преглед, 4, Београд, стр. 32—35.

Гавриловић, Ж. (1963): *Антрополошке карактеристике аутохтоних Војвођана*, Зборник за природне науке МС, св. 24, Нови Сад, стр. 145—148.

Гавриловић, Ж. (1964): *Прилог познавању утицаја послератне миграције на телесни развој насељеника из Босне у Војводини*, Зборник за природне науке, Матица српска, св. 27, Нови Сад, стр. 112—129.

Гавриловић, Ж. (1978): *Антрополошке карактеристике становништва Фрушке горе*, Матица српска, Одељење за природне науке, Нови Сад.

Гавриловић, Ж., Божић-Крстић В. (1981): *Морфофизиолошке и биотиполошке карактеристике одраслих и старих особа*, Гласник АДЈ, Свеска 18, pp. 33—80, Београд.

Павлица, Т., Божић-Крстић, В., Савић М., Ракић Р. (1998): *Bioanthropological characteristics of population of some villages in Srem and their biological relationship*, Зборник Матице српске за природне науке, N° 94, 99—107, Нови Сад.

Павлица, Т., Божић-Крстић, В., Ракић Р., Савић М. (1999): *Неке биоантрополошке карактеристике становништва појединих етничких група у Срему*, Гласник АДЈ, 34, 47—54, Београд.

Поповић, Д. (1990): *Срби у Војводини*, Матица српска, Нови Сад.

Sokal, R. R., and Rohlf, F. J. (1969): *Biometry*, San Francisco: WH. Freeman.

Влаховић, П. (1994): *Етно-антрополошке одлике Војводине*, Гласник АДЈ, 30, Београд, 9—17.

# АНТРОПОЛОШКЕ КАРАКТЕРИСТИКЕ СТАНОВНИКА СРЕМА ПРЕМА ЗАВИЧАЈНОМ ПОРЕКЛУ

Татјана М. Павлица, Верица С. Божић-Крстић,
Рада С. Ракић, Марија Р. Савић
Природно-математички факултет, Институт за биологију
Трг Доситеја Обрадовића 2, 21000 Нови Сад, Југославија

Резиме

У раду је изнета биометријска анализа морфолошко-физиолошких особина (висине тела, дужине ноге, дужине руке, масе тела, обима грудног коша и виталног капацитета плућа) одраслог становништва у Срему, староседелаца и досељеника настањених у Војводини после I и II светског рата из других крајева Југославије. Истраживањем је обухваћено 1824 особе, и то 965 мушкараца и 859 жена, старости од 20 до 50 година. Подаци су обрађени варијационо-статистичком методом, посебно за сваки пол. Утврђена је већа хетерогеност код мушког пола, где су досељеници имали веће вредности за све морфолошке карактеристике. Статистички значајна разлика испитиваних карактеристика код жена није уочена, што указује на њихову хомогеност.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 63—67, 2000

UDC 616.31-089:621.318.38

*Ђорђе М. Тешић[1], Milanko Đ. Đurić[1], Neda M. Pekarić-Nađ[2]*

[1] Faculty of Medicine, Clinic for Stomatology
Veljka Petrovića, 21000 Novi Sad
Yugoslavia
[2] Faculty of Engineering, Department of Electrical Engineering and Computers
Sq. of Dositey Obradović 6
21000 Novi Sad, Yugoslavia

# THE INFLUENCE OF THE PULSATING ELECTROMAGNETIC FIELD (PEMF) ON POSTOPERATIVE BLEEDING AND PAIN AFTER THE MWFO

ABSTRACT: Postoperative pain and postoperative bleeding are expected side effects after modified Widmann flap operation (MWFO). Such operation is performed as a radical solution for Parodonthopatia, which includes surgical implantation of hydroxylapatit in infrabony periodontal defects. Two groups of patients were monitored, the experimental (E) and the control (C) group. The objective of this study was to compare the pain intensity and postoperative bleeding in patients who were and who were not treated by the pulsating electromagnetic field (PEMF). The treatment was performed immediately after the operation and lasted for 30 minutes. Next day after the operation, the patients were requested to fill in the questionnaire about the pain intensity and postoperative bleeding. As reported by the patients from the E group, the PEMF treatment had an analgesic effect. The pain was reported to be weaker and more tolerable than in the C group. No postoperative bleeding was reported either by the patients from the E or C group.

KEY WORDS: Parodonthopatia, PEMF, pain, bleeding

## INTRODUCTION

Pain (dolor) is a subjective feeling which is manifested as physical disturbance of different intensity and character. Pain receptors are represented by nerve ends, particularly the ones located in clusters in mouth cavity. The pain in mouth cavity is most often the result of injuries, dental diseases, soft tissue diseases, disturbed functions, but also the consequence of stomatological interventions, oral surgery and periodontal operations. As explained in (1), in the case of the described operation, no particular irritations are present. More likely the pain was provoked by the release of the tissue hormones, which, in turn, affected the nerve ends.

Surgical treatment of periodontal disease, modified Widmann flap operation (MWFO), is followed by postoperative pain and bleeding of different intensity.

Beneficial effects of the PEMF in human medicine were reported before (2, 3, 4). Pain reduction by the PEMF was reported earlier by the authors from different medical domains. For instance, Jacobson (5) treated his patients with 8Hz PEMF and found significant pain decrease in patients suffering from knee and elbow osteoartrit. At the end of the treatment, 80% of his patients reported a complete lack of pain.

Analgesic effect of the PEMF treatment is not completely explained. This non-invasive treatment is believed to activate enzyme systems and enzyme synthesis. A direct anti-inflammatory effect is also possible. As described by Patinjo (8) similar anti-inflammatory effects were achieved by the PEMF and indomethacin (non-steroid antirheumatic with anti-inflammatory and analgesic activity). The results of the therapies were measured objectively, as the levels of Prostaglandin E2 were monitored. Patinjo concluded that the PEMF therapy seems more beneficial for the patients, as the side effects of indomethacin (gastrointestinal problems, leukopenia, agranulocitosis, etc.) were avoided.

The PEMF effect on DNK synthesis was proved by Liboff et al. (1984). Improved protein synthesis was shown by many authors: Cossarizza et al. (1993), Goodman et al. (1983, 1987), Goodman and Henderson (1988), Murraz and Farndale (1984). A PEMF increased level of inozitol-triphosphate was reported by Korzh-Sleptosova et al. (1995). Intracellular calcium oscillations provoked by PEMF were reported by Lindstrom et al. (1993, 1995).

The effect of an increased osteogenesys was reported in many papers (6, 9, 10).

This study was performed having on mind all possible beneficial effects of the PEMF therapy. The objective was to increase the cells metabolism and sanation of the wounds caused by the MWFO. Possible complications of this therapy were expected in a form of increased bleeding, due to increased blood circulation and lower blood viscosity. Because of that, special attention was paid to postoperative bleeding.

## MATERIALS AND METHODS

The research was performed on Cathedra for Oral Diseases and periodontology, in Department of Stomatology. The explored patients were suffering of fully developed periodontal disease, with deep infabony defects. Two groups were formed of healthy volunteers, aged 25 to 60. Each group consisted of 10 patients who were chosen randomly. All the patients were prepared for the research, which included: positive motivation, plaque identification and removal, good oral hygiene education, correction of nonadequate fillings, correction of occlusion and articulation. At the beginning of the operation hard and soft tissue deformities were corrected and rinsed with physiological solution. During

the MWFO hydroxylapatit (Osprovit) was inserted into infabony defects. After the intervention, the wound was closed with stitches and covered by sterile compress to prevent bleeding. This was necessary to provide certain tooth immobilization and prevent plaque formation.

Immediately after the MWFO, the patients from the E group were exposed to the PEMF, and the patients from the C group were sham exposed. The exposure lasted for 30 minutes. The PEMF was generated by microprocessor controlled device. The pulses were 70 s wide. Peak intensity was 5 mT. The repetition rate was 30 per second. The applicator was made in a shape of saddle-like coil and was placed externally, on the top of the affected region.

Bleeding and the pain intensity were reported subjectively by the patients. The patients were asked to report on peak pain intensity, which, in most cases occurred 6 hours after the MWFO. The patients were asked to comment on bleeding and mark the intensity of their pain on an analog scale, illustrated in Figure 1.

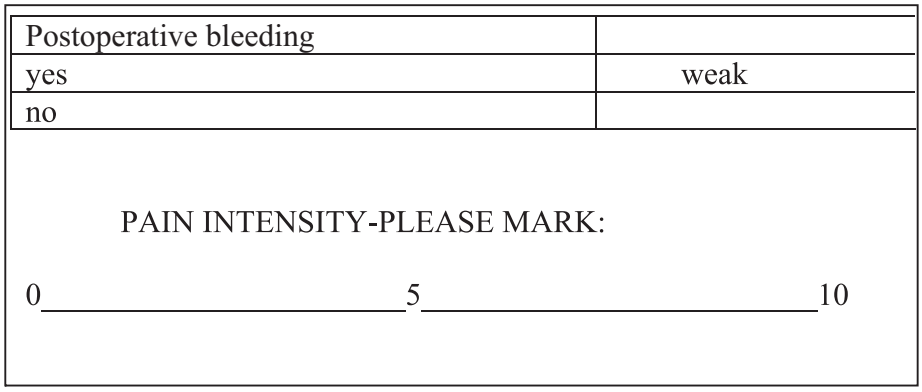



Fig. 1. — Part of the questionnaire

## RESULTS

All patients, from both groups, filled the questionnaire. No patient reported any bleeding. The results of their estimation of postoperative pain are illustrated in Table 1 and Graph 1.

Tab. 1. — Postoperative pain 6 hours after the MWFO

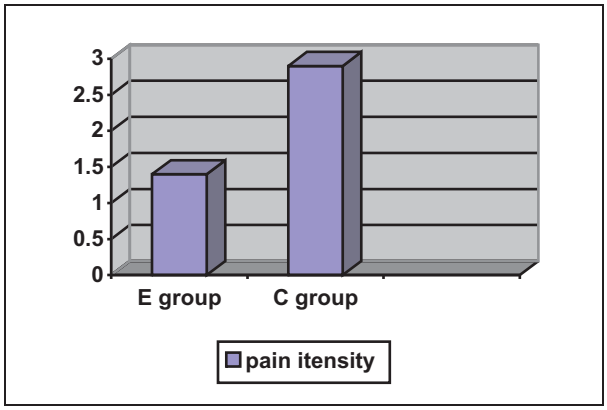
| The experimental group E | Control group C |
|---|---|
| Average pain reported 1.4 ± 0.2 | Average pain reported 2.9 ± 0.9 |

The results of this study showed that:

— Patients from the E group, who were exposed to PEMF immediately after the MWFO, had weaker and more tolerable pain than the patients from the C group.

— Vasodilatation and lower viscosity of blood, provoked by the PEMF treatment, did not cause bleeding. Bleeding was not reported either in the E or C group.

— More investigations are needed and greater number of patients is needed in order to make any final conclusion about the PEMF treatment after the MWFO. Initial results seem promising, particularly for the patients who do not tolerate chemical analgetics.




Graph. 1. — Average postoperative pain intensity reported by the patients

REFERENCES

(1) Анђић, Ј. (1981): *Основи оралне физиологије и биохемије*, Научна књига, Београд.

(2) Basset, A.: *Fundamental and practical aspects of therapeutic uses of pulsed electromagnetic fields*.

(3) Cheng, K., Goldman, R. J. (1998): *Electric fields and proliferation in dermal wound medel; Cell cycle kinetics*, Biolelectromagnetics 19: 68—74.

(4) Hillier Kolarov, V., Pekarić-Nađ, N., Stojšin I. (1996): *Pulsating electromagnetic field (PEMF) in therapy of oral pathologies*, Zbornik Matice srpske za prirodne nauke, Novi Sad, N° 91, 51—59.

(5) Jakobson, J. I. (1998): *Range finding clinical studies stilizing extremely low intensity, low frequency magnetic fields in the treatment of chronic pain*, Abstract book, 20th BEMS Ann. Meeting, Florida, June.

(6) Kotani, H., Ueno, S., Kawaguci, H., Kurokawa, T. (1998): *The effects of repetitive pulsed magnetic stimulation on osteoblast like cells*, Abstract book, 20th BEMS Ann. Meeting, Florida, June.

(7) Лажетић, Б., Козарчић, Т., Станков, К. (1997): *Утицај нискофреквентних електромагнетских поља на неуроендокрини систем*, Медицински преглед, Л 9—10, 357—362.

(8) Patino, O., Grana, D. R., Mino, J. (1998): *PEMF effects on an experimental model of inflamation*, Abstract book, 20th BEMS Ann. Meeting, Florida, June.

(9) Сеп, Д., Крајчиновић, Ј. (1989): *Електробиолошка индукција остеогенезе*, зборник радова, I едукациони семинар са међународним учешћем, Нови Сад, 6—7. октобра.
(10) Tabrah, F. L., Ross, P., Hoffmeier, M., Gilbert, F. (1998): *Clinical report on long term bone density after short term EMF application*, Bioelectromagnetics 19: 68—74.

## УТИЦАЈ ПУЛСИРАЈУЋЕГ ЕЛЕКТРОМАГНЕТСКОГ ПОЉА (ПЕМП) НА ПОСТОПЕРАТИВНО КРВАРЕЊЕ И БОЛ НАКОН MWRO

Ђорђе М. Тешић[1], Миланко М. Ђурић[1], Неда М. Пекарић-Нађ[2]
[1] Медицински факултет, Клиника за стоматологију,
Вељка Петровића, 21000 Нови Сад, Југославија
[2] Факултет техничких наука, Одсек за електротехнику и рачунарство,
Трг Доситеја Обрадовића 6, 21000 Нови Сад, Југославија

Резиме

У раду су праћени постоперативни бол и постоперативно крварење до којег може доћи након оперативног третмана пародонтопатије, познатог као модификована Widmann режањ операција (MWRO). У испитивању су поређени постојање и интензитет бола и постоперативног крварења код пацијената који су били изложени дејству пулсирајућег електромагнетског поља (ПЕМП) непосредно након операције у трајању од 30 минута у односу на групу пацијената која није била излагана овом третману. Сви пацијенти су на контролном прегледу анкетирани о овим тегобама. На основу добијених одговора можемо закључити да је испољен аналгетски ефекат ПЕМП-а: излагани пацијенти су субјективно имали слабији постоперативни бол од неизлаганих. Постоперативно крварење се није јавило ни код једног пацијента из обеју група.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 69—79, 2000

UDC 595.7(497.11-15)

*Bela F. Blesić*

Faculty of Natural Sciences
University of Kragujevac
34000 Kragujevac, Yugoslavia

# INVESTIGATION OF *DIPLURA* AND *PROTURA* IN WESTERN SERBIA

ABSTRACT: We have collected 380 individuals from terrestrial material, 150 from the order *Diplura* and 230 from the order *Protura* (*Insecta*). The individuals of *Diplura* belonged to thirteen (13) species, of *Protura* to ten (10) species. *Podocampa serbica* was the most common *Diplura* species, *Eosentomon transitorium* the most common *Protura* species. Among the *Diplura* species, *Japyx solifugus* was found for the first time in the fauna of Yugoslavia (Serbia and Montenegro). Three new *Protura* species were also found, *Gracilentulus gracilis, Gracilentulus meridionalis* and *Hesperentomon carpaticum*. Soil samples were taken from meadows but mostly from the litter of deciduous forests.

KEY WORDS: *Insecta, Diplura, Protura*, distribution, Serbia

## INTRODUCTION

The fauna of *Diplura* and *Protura*, groups of primitive wingless insects, have been investigated in Western Serbia. It was the first investigation of this kind on the territory of Serbia. The insects were typically found in moist soils, where they searched for food. *Diplura* (*Campodaidae*) mostly fed on detritus, but some of them preyed for food. The species from the family *Japygidae* were exclusively predators. The *Diplura* may thus be considered as omnivorous. The *Protura* were smaller (about 2 mm) than the *Diplura* (about 5—10 mm, in some cases longer).

## MATERIAL AND METHODS

Soil samples were taken from the 20 km perimeter around the town of ≥ Čačak, located about 150 km southwest of Belgrade. They were taken in 20 locations, mostly from the soil litter of mixed deciduous forests and from meadows. The soil samples, taken from the depth of 0—10 cm, had the dimensions 15 x 15 x 10 cm.

Three hundred eighty individuals were separated from the samples in the conventional way. The *Diplura* individuals belonged to thirteen (13) species, the *Protura* individuals to ten (10).

Toto-microslides were used for the determination of insects. Classification was done according to Condé (1956), Ionescu (1955), Nosek (1973), Paclt (1957) and Páges (1953).

The materials mounted on slides are kept at the Faculty of Natural Sciences, University of Kragujevac.

## RESULTS AND DISCUSSION

The article supplies data for twenty-one (21) species. These information are important for the distribution and zoogeographic studies on the Balkans as well as the entire Europe.

Order: *DIPLURA*
Suborder: *RHABDURA* Cook, 1896
emend Pages, 1959
Family: *Campodeidae* Lubbock, 1873
Genus: *Campodea* Westwood, 1842
Subgenus: *Campodea* Westwood, 1842
emend Silvestri, 1932

1. *Campodea (Campodea) colladoi* (Silvestri, 1932)
Syn.: *Campodea ilixonis* Denis, 1932
In the litter of mixed deciduous forest: June 24, 1990 (Riđage, about 4 km W of Čačak) 1 male.
Body length of about 2.8 mm, the number of segments of the antenna 21—22.
Distribution — Spain, France, Germany, Romania, Yugoslavia (Serbia — Karaman and Blesić, 1984)

2. *Campodea (Campodea) posterior* (Silvestri, 1932)
Syn.: *Campodea (Campodea) posterior* Wygodzinsky, 1944
In the soil of hilly meadow: May 20, 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 1 male, 1 female and 1 larva.
Body length about 2.7 mm, the number of segments of the antenna 18—20.
Distribution — Spain

3. *Campodea (Campodea) sillvestrii* Bagnall, 1918
In the soil of:
— valley meadow: April 25, 1990 (Pridvorica, about 6 km W of Čačak) 3 males and 5 females; May 20, 1990 (Međuvršje, about 10 km NW of Čačak) 5 males and 6 females.

— hilly meadow: May 20, 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 6 males and 2 females; June 10, 1992 (Konjevići, about 3 km E of Čačak) 1 larva; October 6, 1992 (Šebeći, about 3 km NE of Čačak) 1 male; March 1993 (Milićevci, about 10 km NW of Čačak) 1 female.

Body length about 3.5 mm, the number of segments of the antenna 14—25.

Distribution — Europe

4. *Campodea (Campodea) wallacei* Bagnall, 1918

In the soil of:

— valley meadow: July 1993 (Milićevci, about 10 km NW of Čačak) 1 male and 1 larva.

— hilly meadow: April 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S of Čačak) 2 females; June 1993 (Milićevci, about 10 km NW of Čačak) 1 larva; June 1993 (Drenova, about 9 km NW of Čačak) 1 male and 1 larva.

Body length about 5.5 mm, the number of segments of the antenna 20—29.

Distribution — Great Britain, Sweden, France, Romania, Yugoslavia (Serbia and Montenegro), Republic Macedonia (Blesić, 1997; 1998)

Subgenus: *Dicampa* Silvestri 1932

5. *Campodea (Dicampa) campestre* Ionescu, 1955

In the litter of:

— mixed deciduous forest: April 15, 1990 (Markovica, about 9 km SW of Čačak) 1 male and 1 female; June 24, 1990 (Riđage, about 4 km W of Čačak) 2 males; October 6, 1992 (Ljubić, about 1 km N of Čačak) 1 female and 1 larva; October 1992 (Rakova, about 10 km N of Čačak) 1 larva and (Sokolići, about 10 km NW of Čačak) 1 female.

— hornbeam trees: May 6, 1990 (Vidova, about 6 km NW of Čačak) 1 male.

— oak forest: April 25, 1990 (Čačak) 1 male; May 20, 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 1 female; June 24, 1990 (Riđage, about 4 km W of Čačak) 4 males, 5 females and 1 larva; June 1992 (Ljubić, about 1 km NW of Čačak) 1 larva; July 25, 1992 (Sokolići, about 10 km NW of Čačak) 1 larva; May 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S of Čačak) 2 females; May 1993 (Rožanj, 20 km N of Čačak) 1 larva; June 1993 (Drenova, about 9 km NW of Čačak) 2 females.

— oak and fir trees: June 1993 (Klik, about 10 km NW of Čačak) 1 female.

— conifer trees: June 1993 (Milićevci, about 10 km NW of Čačak) 1 larva.

In the soil of:

— valley meadow: April 25, 1990 (Čačak) 1 male; June 1993 (Klik, about 10 km NW of Čačak) 2 males, 2 females and 1 larva.

— hilly meadow: May 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S of Čačak) 1 female; June, 1993 (Milićevci, about 10 km NW from Čačak) 1 female; August 1993 (Drenova, about 9 km NW of Čačak) 1 female.

Body length about 3.6 mm, the number of segments of the antenna 23—25, the number of segments of the cercus 11.

Distribution: Europe

6. *Campodea (Dicampa) frenata* Silvestri, 1931

In the litter of:

— elm trees: June 24, 1990 (Riđage, about 4 km W of Čačak) 1 male and 1 female; July 25, 1992 (Brđani, about 15 km NE from Čačak) 1 larva; June 1993 (Drenova, about 9 km NW of Čačak) 1 female.

— mixed deciduous forest: October 2, 1992 (Ljubić, about 1 km NW of Čačak) 1 male.

In the soil of:

— valley meadow: April 25, 1990 (Čačak) 2 males; June 1993 (Klik, about 10 km NW of Čačak) 3 larvae.

— hilly meadow: May 6, 1990 (Vidova, about 6 km NW of Čačak) 1 female.

Body length about 4.5 mm, the number of segments of the antenna 18—26.

Distribution: Bulgaria, Romania, Czech Republic, Slovakia, Yugoslavia, Republic Macedonia (Blesić, 1993, 1997).

7. *Capodea (Dicampa) malpighii* Silvestri, 1912

In the soil of:

— hilly meadow: May 6, 1990 (Vidova, about 6 km NW of Čačak) 2 males, 1 female and 5 larvae; June 24, 1990 (Riđage, about 4 km W of Čačak) 1 male and 1 female.

Body length about 2.7—3 mm, the number of segments of the antenna 16—26, the number of segments of the cercus 9—15.

Distribution: Spain, Italy, Romania, Poland, Czech Republic, Yugoslavia (Serbia — Blesić, 1993)

8. *Podocampa serbica* Karaman and Blesić, 1983

In the litter of:

— oak forest: May 6, 1990 (Vidova, about 6 km NW of Čačak) 1 male; May 1992 (Ljubić, about 1 km NW of Čačak) 1 larva; July 1992 (Sokolići, about 10 km NW of Čačak) 1 female; April 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S from Čačak) 1 female; June 1993 (Drenova, about 9 km NW of Čačak) 2 females; September 7, 1993 (Milićevci, about 10 km NW of Čačak) 1 female and 2 larvae.

— mixed deciduous forest: May 1992 (Ljubić, about 1 km NW of Čačak) 1 male and 1 female; July 1992 (Preljina, about 5 km NW of Čačak) 1 larva.

— conifer trees: June 1993 (Milićevci, about 10 km NW of Čačak) 1 female; June 1993 (Klik, about 10 km NW of Čačak) 2 males, 6 females and 1 ?.

— garden: April 22, 1990 (Čačak) 2 females; May 13, 1990 (Čačak) 6 males and 2 females.

In the soil of:

— valley meadow: June 1993 (Klik, about 10 km NW of Čačak) 3 males, 5 females and 7 larvae; June and July 1993 (Drenova, about 9 km NW of Čačak) 2 females and 6 larvae; July 1993 (Milićevci, about 10 km NW of Čačak) 1 female.

— hilly meadow: June 1993 (Milićevci, about 10 km NW of Čačak) 2 females and 3 larvae;

June 1993 (Klik, about 10 km NW of Čačak) 4 males, 4 females and 4 larvae; August 1993 (Drenova, about 9 km NW of Čačak) 1 female.

Body length about 3.8 mm, the number of segments of the antenna 20—27, the number of segments of the cercus 5—12.

Distribution: Yugoslavia (Serbia — B l e s i ć , 1993).

Family: *Japygidae* L u b b o c k , 1873

9. *Japyx* sp.

In the litter of:

— hornbeam trees: April 15, 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 1 female; May 6, 1990 (Vidova, about 6 km NW of Čačak) 1 larva; May 20, 1990 (Preobraženje Monastery, about 12 km W of Čačak) 2 larvae.

— oak forest: April 15, 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 1 larva; April 25, 1990 (Čačak) 1 female; June 24, 1990 (Riđage, about 4 km W of Čačak) 1 female and 1 larva; May 1992 (Ljubić, about 1 km NW of Čačak) 1 female; June 1992 (Ljubić, about 1 km NW of Čačak) 1 male and 1 female.

— beech forest: July 1992 (Brđani, about 15 km NE of Čačak) 1 female; July 1992 (Preljina, about 5 km NW of Čačak) 1 female.

— mixed deciduous forest: April 15, 1990 (Markovica, about 9 km SW of Čačak) 1 male, 3 females and 1 larva; June 24, 1990 (Riđage, about 4 km W of Čačak) 1 female and 3 larvae; October 1992 (Ljubić, about 1 km NW of Čačak) 1 larva.

— garden: April 22 and May 13, 1990 (Čačak) 2 and 4 females, respectively.

In the soil of:

— valley meadow: August 1992 (Trbušani, about 3 km NW of Čačak) 1 larva.

— hilly meadow: June 24, 1990 (Riđage, about 4 km W of Čačak) 3 females; May 1992 (Ljubić, about 1 km NW of Čačak) 1 female; September 1992 (Šebeći, about 3 km NE of Čačak) 1 female.

10. *Catajapyx conffusus* (S i l v e s t r i , 1929)

Syn.: *Japyx conffusus* S i l v e s t r i , 1929

In the litter of:

— oak forest: April 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S of Čačak) 1 female; July and September 1993 (Milićevci, about 10 km NW of Čačak) 1 female, 2 males and 5 larvae; June 1993 (Drenova, about 9 km NW of Čačak) 4 females, 2 males and 1 larva.

— conifer trees: June 1993 (Milićevci, about 10 km NW of Čačak) 1 female and 4 larvae; June 1993 (Klik, about 10 km NW of Čačak) 1 male and 1 female.

In the soil of hilly meadow: May 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S of Čačak) 1 larva; August 1993 (Drenova, about 9 km NW of Čačak) 1 female and 8 larvae.

Body length between 6—8.5 mm, the number of segments of the antenna 28.

Distribution: Austria, Romania, Czech Republic, Slovakia, Germany.

11. *Metajapyx serratus* (S t a c h , 1929)

Syn.: *Japyx serratus* S t a c h , 1929; *Japyx braueri* V e r h o e f , 1904; *Japyx sinuosus* T u x e n , 1930

In the litter of:

— oak forest: October 1993 (Klik, about 10 km NW of Čačak) 1 male.

— mixed conifer trees: August 1993 (Drenova, about 9 km NW of Čačak) 1 female and 2 larvae.

Body length between 4 and 5.6 mm; the number of segments of the antenna 28.

Distribution: Belgium, Germany, Switzerland, France, Yugoslavia (Montenegro) (B l e s i ć , 1998)

12. *Japyx solifugus* (S i l v e s t r i , 1903)

Syn.: *Dicellura solifuga* H o l i d a y , 1865

In the litter of:

— oak forest: June 1993 (Drenova, about 9 km NW of Čačak) 1 male.

— mixed conifer trees: August 1993 (Drenova, about 9 km NW of Čačak) 1 larva.

Body length between 8 and 10 mm, the number of segments of the antenna 28.

Distribution: Italy, Austria, France, Germany.

13. *Protojapyx maior* S i l v e s t r i , 1948

In the litter of conifer trees: June 1993 (Klik, about 10 km NW of Čačak) 1 larva.

In the soil of hilly meadow: April 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S of Čačak) 1 female and 1 larva.

Body length about 14 mm, the number of segments of the antenna from 33 to 36.

Distribution: Mediterranean region, Germany, Yugoslavia (Montenegro — B l e s i ć , 1998).

Order *PROTURA*
Family *Acerentomidae* S i l v e s t r i , 1907

14. *Acerentulus traegardhii* I o n e s c u , 1937
In the litter of:
— elm trees: May 6, 1990 (Vidova, about 6 km NW of Čačak) 1 male; May 20, 1990 (Preobraženje Monastery, about 12 km W of Čačak) 1 female.
— oak forest: June 20, 1990 (Riđage, about 4 km W of Čačak) 1 male and 1 larva.
— mixed deciduous trees: May 20, 1990 (Preobraženje Monastery, about 12 km W of Čačak) 2 males, 3 females and 1 larva; June 24, 1990 (Riđage, about 4 km W of Čačak) 3 males and 5 females.
Body length about 0.95 mm.
Distribution: Sweden, Ireland, Denmark, Germany, Belgium, Poland, Czech Republic, Slovakia, Hungary, Romania, Spain, France, Italy, Greece, Yugoslavia and Republic Macedonia (B l e s i ć , 1997).

15. *Acerella* sp.
In the litter of oak forest: June 24, 1990 (Riđage, about 4 km W of Čačak) 1 male and 1 female.

16. *Acerentomon* sp.
In the litter of:
— hornbeam trees: May 6, 1990 (Vidova, about 6 km NW of Čačak) 3 females; May 20, 1990 (Preobraženje Monastery, about 12 km W of Čačak) 1 male and 5 females; June 20, 1990 (Riđage, about 4 km W of Čačak) 1 male, 2 females and 1 larva.
— oak forest: June 20, 1990 (Riđage about 4 km W of Čačak) 2 females and 2 larvae.
In the soil:
— valley meadow: June 1990 (Riđage, about 4 km W of Čačak) 1 female.
— hilly meadow: May 1993 (on the Jelica Mountain, at the altitude between 400 and 500m, S of Čačak) 1 larva.

17. *Acerentomon balcanicum* I o n e s c u , 1993
In the litter of:
— hornbeam trees: May 1990 (Vidova, about 6 km NW of Čačak) 3 males and 2 females; April 1990 (Čačak) 3 females.
— oak forest: May 1990 (Vidova, about 6 km NW of Čačak) 1 female; May 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 5 females and 1 male; April 1990 (Čačak) 2 females and 2 males; June 1993 (Drenova, about 9 km NW of Čačak) 1 male and 1 female.
— mixed deciduous trees: May 20, 1990 (Preobraženje Monastery, about 12 km W of Čačak) 1 male.
In the soil of:
— valley meadow: May 1990 (Međuvršje, about 10 km NW of Čačak) 3 females and 2 males.

— hilly meadow: May 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 1 larva.

Body length about 1.5 mm.

Distribution: Yugoslavia and Greece (B l e s i ć , 1993).

18. *Gracilentulus gracilis* (B e r l e s e , 1908)

Syn.: *Gracilentulus gracilis* T u x e n , 1964

In the litter of:

— oak forest: April 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S of Čačak) 2 females and 1 male; May 1993 (Rožanj, about 20 km N of Čačak) 1 female; July 1993 (Milićevci, about 10 km NW of Čačak) 1 female and 1 male; October 1993 (Klik, about 10 km NW of Čačak) 1 female.

— conifer trees: June 1993 (Milićevci, about 10 km NW of Čačak) 5 females and 3 males.

Body length about 1.5 mm.

Distribution: cosmopolitan (N o s e k , 1973).

19. *Gracilentulus meridianus* C o n d é , 1945

Syn.: *Gracilentulus meridianus* T u x e n , 1964

In the litter of oak forest: July 1993 (Milićevci, about 10 km NW of Čačak) 1 female; August 1993 (Drenova, about 9 km NW of Čačak) 2 males, 4 females and 1 larva.

Body length between 1.1 and 1.3 mm.

Distribution: France and Spain (N o s e k , 1973).

Family *Eosentomidae* B e r l e s e , 1909

20. *Eosentomon transitorium* B e r l e s e , 1908

In the litter of:

— hornbeam trees: May 1990 (Vidova, about 6 km NW of Čačak) 2 males, 1 female and 1 larva; June 1990 (Riđage, about 4 km W of Čačak) 7 females, 2 males and 6 larvae.

— oak forest: May 1990 (Vidova, about 6 km NW of Čačak) 1 female and 1 larva; May 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 1 female and 5 males; June 1990 (Riđage, about 4 km W of Čačak) 6 females, 8 males and 1 larva; October 1992 (Konjević, about 3 km E of Čačak) 1 larva: April 1993 (on the Jelica Mountain, at the altitude between 400 and 500 m, S of Čačak) 1 female; May 1993 (Rožanj, about 20 km N of Čačak) 1 larva; September 1993 (Milićevci, about 10 km NW of Čačak) 1 female, 1 male and 1 larva; August — September 1993 (Drenova, about 9 km NW of Čačak) 2 males, 3 females and 1 larva; October 1993 (Klik, about 10 km NW of Čačak) 1 female.

— mixed deciduous trees: May 20, 1990 (Preobraženje Monastery, about 12 km W of Čačak) 1 female and 3 larvae; June 1990 (Riđage, about 4 km W of Čačak) 11 females, 15 males and 9 larvae.

— conifer trees: June 1993 (Milićevci, about 10 km NW of Čačak) 1 male.

In the soil of:

— valley meadow: May 1990 (Međuvršje, about 10 km NW of Čačak) 1 larva; May 1990 (Vidova, about 6 km NW of Čačak) 1 female; June 1993 (Milićevci, about 10 km NW of Čačak) 1 female.

— hilly meadow: July 1993 (Milićevci, about 10 km NW of Čačak) 1 female, 5 males and 1 larva; May 1990 (Vidova, about 6 km NW of Čačak) 1 female, 3 males and 1 larva; June 1993 (Milićevci, about 10 km NW of Čačak) 1 female; September (ibid) 3 females and 1 larva; August 1993 (Drenova, about 9 km NW of Čačak) 3 males and 1 female.

Body length between 1.3 and 1.5 mm.

Distribution: cosmopolitan (B l e s i ć , 1993, 1997 and 1998).

21. *Eosentomon coiffaiti* C o n d é , 1961

In the litter of:

— oak forest: June 1993 (Klik, about 10 km NW of Čačak) 1 male; July—August 1993 (Drenova, about 9 km NW of Čačak) 4 males and 8 females.

— conifer trees: June 1993 (Milićevci, about 10 km NW of Čačak) 1 female; June 1993 (Klik, about 10 km NW of Čačak) 1 female.

Body length about 1.3 mm.

Distribution: Maiorca (N o s e k , 1973).

Family *Protentomidae* E w i n g , 1936

22. *Hesperntomon* sp.

In the litter of:

— conifer trees: May 20, 1990 (Preobraženje Monastery, about 12 km W of Čačak) 1 female.

— mixed deciduous trees: May 20, 1990 (Preobraženje Monastery, about 12 km W of Čačak) 1 female.

— valley meadow: July 1993 (Vidova, about 6 km NW of Čačak) 1 female.

— hilly meadow: May 1990 (Vavedenje Monastery, about 6 km W of Čačak, along the river W. Morava) 1 female; July 1993 (Milićevci, about 10 km NW of Čačak) 1 larva.

23. *Hesperntomon carpaticum* (I o n e s c u , 1930)

Syn.: *Hesperntomon carpaticum* T u x e n , 1964

In the litter of oak forest: July 1993 (Drenova, about 9 km NW of Čačak) 1 male.

Body length about 1.75 mm.

Distribution: Romania, Greece, Poland, Croatia (Dalmatia), Bosnia (C v i j o v i ć , 1970)

## CONCLUSION

Twenty-three (23) species belonging to the orders *Diplura* (13) and *Protura* (10) were found in twenty (20) localities in the surroundings of Čačak.

The order *Diplura* was represented by two (2) families (*Campodeidae* and *Japygidae*) and six (6) genera. The species *Japyx solifugus* was found for the first time in the fauna of Yugoslavia (Serbia and Montenegro). *Podocampa serbica* (with 73 individuals) was the most common and the most numerous while the other species were fewer.

The order *Protura* was represented in the surroundings of Čačak by 3 families (*Acerentomidae, Eosentomidae* and *Protentomidae*) and 6 genera (*Acerentulus, Aceentomon, Acerella, Gracilentulus, Eosentomon* and *Hesperentomon*). Six (6) species belonged to the family *Acerentomidae*, two (2) to the family *Eosentomidae* and also two (2) to the family *Protentomidae*. Three species were found for the first time in the fauna of Yugoslavia (Serbia and Montenegro), *Gracilentulus gracilis, Gracilentulus meridionalis* and *Hesperentomon carpaticum. Eosentomon transitorium* (with 117 individuals) was the most common and the most numerous, the other species were less numerous.

This investigation provides data for further zoogeographical and ecological studies and it broadens our knowledge of the diversity of this group of primitive wingless insects.

## REFERENCES


Blesić, B. (1993): *Fauna of Protura and Diplura (Apterygota, Insecta) of Kotlenik*, Collection of scientific papers of the Faculty of Science, Kragujevac, 14: 81—84.

Blesić, B. (1997): *Protura and Diplura of Republic Macedonia*, Frag. Balc. Mus. Sci. Nat., Skopje, in press.

Blesić, B. (1998): *Knowledge of Protura and Diplura of Montenegro*, The Montenegrin. Academy of Sciences and Arts Glasnik of the section of Natural Sciences, Podgorica, 12: 63—70.

Condé, B. (1956): *Materiaux pour une monographie des Diloures*, Campodeides. Mem. Mus. Hist. Nat. Paris, A Zoologie, 12: 1—202.

Цвијовић, М. (1970): *Прилог познавања фауне Acerentomidea (Protura) на планинама Маглић, Волујак и Зеленгора*, Гласник Зем. муз. Сарајево, Н. С., 9: 31—36.

Ionescu, M. A. (1955): *Diplura*, in: *Fauna Republicii Populare Romine, Insecta*, 7(2): 1—50.

Karaman, M. and Blesić, B. (1984): *Fauna Diplura of SR Serbia*, Collection of scientific papers of the Faculty of Science, Kragujevac, 5: 91—96.

Nosek, J. (1973): *The Europian Protura*, Mus. Hist. Natur., Geneva, 346.

Paclt, J. (1957): *Diplura*. In: P. Wytsman, *Genera Insectorum*, 212: 1—123, Cranheim.

Páges, J. (1953): *Japyginae (Japygidae, Insecta, Diplura) de la Yugoslavie et des regions limitrophes*, Bull. Mus. Hist. Nat. pays Serbe, Belgrade, sB 5—6: 235—264.

# ИСТРАЖИВАЊА *DIPLURA* И *PROTURA* У ЗАПАДНОЈ СРБИЈИ

Бела Ф. Блесић
Природно-математички факултет, Универзитет у Крагујевцу,
34000 Крагујевац, Југославија

Резиме

Истраживања у Западној Србији на око 20 локалитета (околина Чачка) током неколико година (у шумским и ливадским екосистемима) резултирала су са 150 јединки *Diplura* и 230 јединки *Protura*. Јединке *Diplura* спадају у 13 врста, а *Protura* у 10 врста.

Најмногобројнија врста *Diplura* је била *Podocampa serbica* (са 73 јединке) а од *Protura* врста *Eosentoman transitorium* (са 117).

По први пут је у фауни Југославије (Србија и Црна Гора) од *Diplura* установљена врста *Japyx solifugus*, а од *Protura* врсте: *Gracilentulus gracilis, meridionalis* и *Hesperentomon carpaticum*.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 81—86, 2000

UDC 599.325:575.17(497.113)

*Ljiljana B. Vapa*[1], *Vukoman R. Šelmić*[2], *Tamara S. Popović*[1], *Milan M. Vapa*[3]

[1] Faculty of Sciences, Trg Dositeja Obradovića 2
21000 Novi Sad
[2] Faculty of Forestry, Kneza Višeslava 1
11000 Belgrade
[3] Faculty of Agriculture, Trg Dositeja Obradovića 8
21000 Novi Sad

# GENETIC IDENTITY OF BROWN HARE POPULATIONS IN VOJVODINA

ABSTRACT: Low genetic variability is connected to inbreeding depression and the loss of heterozygosity, which weakens most of the components of adaptive value of a population, including metabolic efficiency, reproductive efficiency, disease resistance, etc. Therefore, the main objectives of this paper are to establish genetic identity of hare (*Lepus europaeus* P a l l a s ) populations in Vojvodina on the basis of allozyme polymorphism. Polyacrylamide gel (PAAG) electrophoresis was used to study variability of 12 allozyme loci. Loci for *Est-1, Est-2, Est-3, Est-4, Est-5, Est-6, Me-1* and *Pgm-2* were polymorphic, possessing from two to seven alleles. Genetic identity (I) of hares from Turija and Novo Miloševo was I = 0.95, while lower values were obtained with hares from Donji Petrovac and Turija (I = 0.91), as well as those from Donji Petrovac and Novo Miloševo (I = 0.87).

KEY WORDS: allozyme, brown hare (*Lepus europaeus* P a l l a s ), genetic identity

## INTRODUCTION

Genetic variability within population has enormous significance for the living world. If this variability is high, possibilities exist for combining genetic materials and developing new properties. Therefore, the basic objective of conservational biology is preservation of genetic variability within population ( H a r t l , 1991).

Causes of lowering the genetic variability in wild animals, in comparison with domestic ones, are even more frequent, as the result of mating within small populations (inbreeding depression), selective hunting for trophies, reintroduction, random loss of certain alleles (random genetic drift). These populations are exposed to high inbreeding level, loss of genetic biodiversity and random fixations of non-adaptive alleles, which could bring unfavorable phenotype properties into population (R a n d i , 1993). In such populations, dur-

ing one generation, certain genes could be extinguished, and other fixed (Emery, 1986).

Genetic variability may be estimated in several ways. One of them is based on genetic markers showing polymorphism, i.e., appearing in several molecular forms. Since enzymes are direct gene products, they can be used as genetic markers in the estimation of population variability, identity and distance within and between population, genetic exhaustion etc.

Isozymes are different molecular forms of enzymes with the same substrate specification, of which every form catalyzes the same type of biochemical reaction. Each isozyme has a specific and unique role in cell physiology. Isozymes coded by different alleles of one gene are called allozymes, which enable rapid adaptation to environment changes. These changes are relatively quick and they directly influence the selection.

Role of isozymes (allozymes) was emphasized by the discovery of genetic polymorphism (Harris, 1966). On the basis of single parameter on the molecular level, being direct gene product, it is possible to obtain precise quantitative data on genetic variability. Isozymes are markers for genetic activity enabling identification of biochemical phenotypes by electrophoresis method.

For all game species, especially for the hare as our autochthonous species, whose numbers are rapidly declining over the years (Šelmić, 1997; Vapa and Šelmić, 1997), it is necessary to conduct genetic and population studies in order to conserve specific genetic properties, i.e., biodiversity preservation, since they are of permanent biological interest and an important part of hunting economy.

Investigations of genetic diversity in wild animals, especially in hares, are relatively rare (Hartl et al., 1987, 1990, 1992; Vapa et al., 1994, 1995, 1997, 1999; Suchentrunk et al., 1999).

Therefore, the main objective of this paper is to establish the genetic identity of the hare population in Vojvodina, on the basis of the allozyme polymorphism detected by the method of PAAG electrophoresis.

## MATERIAL AND METHODS

Brown hares were collected at three locations in Vojvodina: Novo Miloševo, Donji Petrovac and Turija. All animals were obtained during hunts in winter 1999, and were sexed and aged. Liver was frozen in liquid nitrogen immediately after death of the animals, and stored at —20°C until electrophoresis. Liver was macerated at 4°C in Tris Citrate stock buffer pH 7.1 (Tris 0.8M, citric acid 0.24M, sucrose 10%, TritonX-100 0.1%, Bromophenol Blue 0.1%), and the ratio was 1: 10 (w/v). After centrifugation for 3 minutes at 4°C and 16 000 rpm, 1.5 to 10 μl (depending of enzyme) of clear supernatant were applied into gel slots.

The following enzyme systems were screened:

1. Esterase (EST, E.C. 3.1.1.1)
2. Malic enzyme (ME, E.C. 1.1.1.40)
3. Phosphoglucomutase (PGM, E.C. 2.7.5.1)

4. Octanol dehydrogenase (ODH, E.C. 1.1.1.73)
5. Superoxide dismutase (SOD, E.C. 1.15.1.1)

Vertical polyacrylamide gel (PAAG) electrophoresis was performed in LKB system at 4°C, in Tris-Borate-EDTA buffer system pH 8.9 (0.08 TRIS, 0.0015M EDTA, 0.02M boric acid). Electrophoresis was performed in electrical field at constant 300 V for 4 hours, gels were stained and fixed in 7% acetic acid (V a p a et al., 1999).

Genetic identity (I), genetic distance (D), and UPGMA dendogram were calculated according to N e i (1972).

## RESULTS AND DISCUSSION

Screening for the five enzyme systems in the brown hare populations, polymorphism was determined only in three enzymes: esterase, malic enzyme and phosphoglucomutase. Loci for *Odh-1, Odh-2, Sod-1, Pgm-1* were monomorphic, while loci for *Est-1, Est-2, Est-3, Est-4, Est-5, Est-6, Me-1* and *Pgm-2* were polymorphic showing two to seven different alleles in different sequences (Table 1).

Tab. 1. — Allele frequencies in the hare populations

| Locus | Allele | Locality and number of hares | | | Mean value |
|---|---|---|---|---|---|
| | | Novo Miloševo 6 | Donji Petrovac 4 | Turija 3 | |
| *Est-1* | a | 0.83 | 0.75 | 1.0 | 0.86 |
| | b | 0.17 | 0.25 | 0.0 | 0.14 |
| *Est-2* | a | 0.58 | 0.5 | 0.0 | 0.36 |
| | b | 0.08 | 0.0 | 0.0 | 0.03 |
| | c | 0.08 | 0.0 | 0.17 | 0.08 |
| | d | 0.17 | 0.125 | 0.33 | 0.21 |
| | e | 0.08 | 0.25 | 0.17 | 0.17 |
| | f | 0.0 | 0.125 | 0.17 | 0.098 |
| | g | 0.0 | 0.0 | 0.17 | 0.06 |
| *Est-3* | a | 0.08 | 0.0 | 0.0 | 0.027 |
| | b | 0.0 | 0.0 | 0.17 | 0.057 |
| | c | 0.0 | 0.125 | 0.0 | 0.042 |
| | d | 0.92 | 0.875 | 0.83 | 0.875 |
| *Est-4* | a | 0.5 | 0.75 | 0.5 | 0.58 |
| | b | 0.5 | 0.25 | 0.5 | 0.42 |
| *Est-5* | a | 0.0 | 1.0 | 0.33 | 0.44 |
| | b | 1.0 | 0.0 | 0.67 | 0.56 |
| *Est-6* | a | 0.25 | 0.375 | 0.17 | 0.265 |
| | b | 0.33 | 0.25 | 0.33 | 0.303 |
| | c | 0.17 | 0.25 | 0.33 | 0.25 |
| | d | 0.25 | 0.125 | 0.17 | 0.182 |
| *Me-1* | a | 0.83 | 0.75 | 0.67 | 0.75 |
| | b | 0.17 | 0.25 | 0.33 | 0.25 |

| | | | | | |
|---|---|---|---|---|---|
| *Odh-1* | a | 1.0 | 1.0 | 1.0 | 1.0 |
| *Odh-2* | a | 1.0 | 1.0 | 1.0 | 1.0 |
| *Pgm-1* | a | 1.0 | 1.0 | 1.0 | 1.0 |
| *Pgm-2* | a | 0.8 | 1.0 | 1.0 | 0.93 |
| | b | 0.2 | 0.0 | 0.0 | 0.07 |
| *Sod-1* | a | 1.0 | 1.0 | 1.0 | 1.0 |

Esterases are monomers or dimers, showing high levels of polymorphism in all six loci. Octanol dehydrogenase is a dimer controlled by two monomorphic loci *Odh-1* and *Odh-2*, represented with one band on each zymogram. The locus for malic enzyme, *Me-1*, showed one or two bands with different electrophoretic mobility. Phosphoglucomutase is a monomer controlled by the loci *Pgm-1* and *Pgm-2*. In *Pgm-1* one band was observed, in *Pgm-2* one or two bands with different migration distances. The locus for superoxide dismutase was monomorphic, represented with only one band in the zymogram.

Investigating the variability of liver isozymes in *Lepus europaeus* from Central Europe, Hartl et al. (1990, 1992) found loci of the enzymes PGM and SOD to be monomorphic. Examining the allozyme variability of natural populations of the brown hare in Vojvodina, Vapa et al. (1999) showed that the enzymes SOD (locus *Sod-1*) and PGM (locus *Pgm-1*) were monomorphic. Polymorphism of loci for the enzyme systems EST-I and EST-D were found by Hartl et al. (1990, 1992) as well as Suchentrunk et al. (1999) while investigating genetic identity of hare populations in Bulgaria.

Analyzing isozyme variability in various tissues of purebred and crossbred strains of the domestic rabbit (*Oryctolagus cuniculus* L.), Hartl and Hoger (1986) found polymorphism for both loci, *Me-1* and *Me-2*. Locus *Me-1* in these investigations was polymorphic, while Hartl (1987) and Vapa et al. (1999) found that the isozyme ME (for locus *Me-1*) is monomorphic. Hartl et al. (1990, 1992), Vapa et al. (1994, 1999) and Suchentrunk et al. (1999) described the polymorphism in *Pgm-2* in the same way as it was treated in this paper.

Based on the allele frequencies at the eight polymorphic loci Nei-s values of genetic identity (I) and distance were calculated among the hare populations (Table 2). The value of genetic identity was higher between the hares from Turija and Novo Miloševo 0.95, while lower values were obtained with the hares from Donji Petrovac and Turija, I = 0.91, as well as with those from Donji Petrovac and Novo Miloševo, I = 0.87 (Figure 1). The highest value for genetic identity in hare populations in Vojvodina was obtained between the populations from Despotovo and Pačir (I = 0.98) by Vapa et al. (1999).

Tab. 2. — Genetic identities (above diagonal) and genetic distance (below diagonal) in the hare populations

| Population | Novo Miloševo | Donji Petrovac | Turija |
|---|---|---|---|
| Novo Miloševo | — | 0.87 | 0.95 |
| Donji Petrovac | 0.14 | — | 0.91 |
| Turija | 0.05 | 0.09 | — |

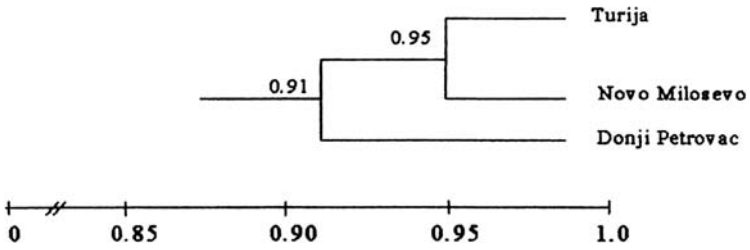



Fig. 1. — UPGMA dendogram summarizing the genetic identity among the hare populations

## CONCLUSION

In this paper, genetic identity was investigated in *Lepus europaeus* on the basis of the polymorphism of liver tissue allozymes. A total of 13 individuals was analyzed, originating from three locations: Turija, Novo Miloševo and Donji Petrovac. Variability of 5 enzyme systems (EST, ME, ODH, PGM, SOD) was investigated, being coded with 12 loci. The loci for *Est-1, Est-2, Est-3, Est-4, Est-5, Est-6, Me-1* and *Pgm-2* were polymorphic, possessing from two to seven different alleles. The highest genetic identity was found between the individuals from Turija and Novo Miloševo, I = 0.95, somewhat lower between Turija and Donji Petrovac, I = 0.91, and the lowest between the individuals from Novo Miloševo and Donji Petrovac, I = 0.87.

## REFERENCES

Emery, A. E. H. (1986): *Основи медицинске ґенетшке*, Поглавље 9: „Популациона генетика и природна селекција", 185—215, Савремена администрација, Београд.

Harris, H. (1987): *Enzyme polymorphism in man*, Proc. Roy. Soc., Ser. B, 164: 298—310.

Hartl, G. B., Hoger, H. (1986): *Biochemical variation in purebred and crossbred strains of domestic rabbits (Oryctolagus cuniculus L.)*, Genet. Res. Camb., 48: 27—34.

Hartl, G. B. (1987): *Biochemical differentiation between the Wild rabbit (Oryctolagus cuniculus L.), the Domestic rabbit and the Brown hare (Lepus europaeus Pallas)*, Sonderdruck aus Z. fur Zool. Sys. und Evolutionsforsch, 25 (4): 309— 316.

Hartl, G. B., Markowski, J., Kovacs, G., Grillitsch, M., Willing, R. (1990): *Biochemical variation and differentiation in the brown hare (Lepus europaeus) of Central Europe*, Z. Saugetierkunde, 55: 186—193.

Hartl, G. B. (1991): *The influence of game management on allelic variation in large mammals of Central Europe*. In: E. Randi and M. Spagnesi (Eds.) *Atti del convegno genetica e conservazione della fauna*, Vol XVIII. Istituto nazionale di biologia della selvaggina „Alessandro Ghigi", Bologna, pp. 77—94.

Hartl, G. B., Markowski, J., Swiatecki, A., Janiszewski, T., Willing, R. (1992): *Genetic diversity in the Polish brown hare Lepus*

*europaeus Pallas, 1778: Implications for conservation and management*, Acta Theriologica, 37: 15—25.

Nei, M. (1972): *Genetic distance between populations*, Am. Nat., 106: 283—292.

Randi, E. (1993): *Genetica delle piccole popolazioni*, Suppl. Ric. Biol. Selvaggina XXI: 151—166.

Suchentrunk, F., Michailov, C., Markov, G., Haiden, A. (1999): *Population genetics of brown hares Lepus europaeus: allozymic diversity at zoogeographical crossroads*, Acta Theriologica (in press).

Шелмић, В. (1997): *Зец у савременим аграоекосистемима*, зборник радова „Зец и јаребица у савременим агроекосистемима". У: В. Шелмић (Ed.) *Симпозијум из ловства*, Нови Сад, pp. 1—19.

Вапа, Љ., Радовић, Д., Вапа, М. (1994): *Isozyme polymorphism in the hare (Lepus europaeus Pallas)*, Proceedings for Natural Sciences, Матица српска 86: 13—19.

Вапа, М., Вапа, Љ., Радовић, Д. (1995): *Polymorphism of tissue isozymes in hare (Lepus europaeus Pallas)*, Proc. XXV Congress of World Veterinary Association, Yokohama, Japan.

Вапа, М., Вапа, Љ., Борота, Ј., Радовић, Д. (1997): Генетичка разноврсност у популацији зеца (*Lepus europaeus Pallas) у Војводини*, зборник радова „Зец и јаребица у савременим агроекосистемима". У: В. Шелмић (Ed.) *Симпозијум из ловства*, Нови Сад, pp. 172—177.

Вапа, М., Шелмић, В. (1997): *Садашње стање и будућност европског зеца*, зборник радова „Зец и јаребица у савременим агроекосистемима". У: В. Шелмић (Ed.) *Симпозијум из ловства*, Нови Сад, pp. 33—45.

Вапа, М., Шелмић, В., Обрехт, Д., Вапа, Љ. (1999): *Allozyme variability of natural population of hares (Lepus europaeus Pallas)*, Proceedings for Natural Sciences, Матица српска, 97: (in press).

## ГЕНЕТИЧКА БЛИСКОСТ У ПОПУЛАЦИЈИ ЗЕЦА ВОЈВОДИНЕ

Љиљана Б. Вапа[1], Вукоман Р. Шелмић[2],
Тамара С. Поповић[1], Милан М. Вапа[3]
[1] Природно-математички факултет, Трг Доситеја Обрадовића 2, 21000 Нови Сад
[2] Шумарски факултет, Кнеза Вишеслава 1, 11000 Београд
[3] Пољопривредни факултет, Трг Доситеја Обрадовића 8, 21000 Нови Сад

Резиме

У раду је испитивана генетичка блискост популација врсте *Lepus europaeus* из ткива јетре. Анализирано је 13 јединки које потичу са три локалитета: Турија, Ново Милошево и Доњи Петровац. Испитивана је варијабилност 5 ензимских система (EST, ME, ODH, PGM, SOD) који су кодирани са 12 локуса. Полиморфни су били локуси *Est-1, Est-2, Est-3, Est-4, Est-5, Est-6, Me-1* и *Pgm-2* са два до седам алела. Највећа генетичка блискост установљена је између јединки са локалитета Турија и Ново Милошево I = 0.95, нешто нижа између Турије и Доњег Петровца I = 0.91, а најнижа између јединки из Новог Милошева и Доњег Петровца I = 0.87.

Зборник Матице српске за природне науке / Proceedings for Natural Sciences, Matica Srpska Novi Sad, № 99, 87—96, 2000

UDC 636.293.01(497.1)

*Timotej M. Čobić*

Faculty of Agriculture
Trg D. Obradovića 8
21000 Novi Sad, Yugoslavia

# ORIGIN, DOMESTICATION AND EXPANSION OF DOMESTIC BUFFALO (*BUBALUS BUBALIS*) IN YUGOSLAVIA

ABSTRACT: The origin and zoological characteristics of buffalo, its domestication and expansion in the world and in Yugoslavia are presented in the paper. Buffaloes arrived to our country from two directions, southern and northern. They were primarily used as draft animals, then for milk production, while the meat production was secondary. It is considered that as draft animals they are stronger than oxen, and their modesty in nutrition and capability of using poor quality feeds is specially appreciated. Under conditions of intensive management they may yield 1800 kg of milk with high butterfat content. As draft animals they were gradually replaced by horses, and then by tractors, which caused a decrease of their importance in agriculture. However, elsewhere in the world, the buffalo is still indispensable, specially in the regions of rice growing.

KEY WORDS: domestic buffalo, origin, characteristics, production traits

## ORIGIN AND ZOOLOGICAL CHARACTERISTICS

Buffalo belongs to relatives of cattle, which means that in the zoological order it is close to cattle in the narrowest sense, but the cattle do not originate from it. According to Mason (1974) buffaloes belong to the order *Artiodactyla*, suborder *Ruminantia*, family *Bovidae* and subfamily *Bovinae*. Within that subfamily Bohlken (1958) differentiates three groups, *Bovina* (cattle), *Bubalina* (Asian buffalo) and *Syncerina* (African buffalo).

According to general appearance, the two groups of buffaloes are similar and they clearly differentiate from cattle according to horns, which are angular at the cross section, while those in cattle are round. However there are fundamental anatomical differences between the African and Asian buffaloes which justify their separation, not into different genera, but into different groups.

The African buffalo resembles the Asian according to the straight back line, massive form and spare hairs. However in *Bubalus* the hairs of the median back line between thurls and the neck are directed cranially, while in *Syncerus*

they are directed normally, i.e., caudally. The Asian buffalo has relatively smaller ears and a long narrow skull, while the African buffalo has large ears, with a wider and shorter skull. The most important difference is in the bones of the head. In *Bubalus*, the ploughshare bone (vomer) and parietal bones are conected, and the rear opening of the nostrils is completely divided by the ploughshare bone. In the *Syncerus* the ploughshare and the palatine are separated and the opening of the posterior nares is not divided by the vomer. Differences exist also in the bones of the temple. In the African buffalo, the frontal and temporal bones touch each other, whereas the parietal bone and the ala temporalis of the sphenoidal bone do not touch. That latter apophysis of the Asian buffalo is so much elongated that it touches the parietal bone and thus prevents the meeting of the frontal and temporal bones.

The horns of the Asiatic buffalo have a simple form, they are shaped like a sicle. They are thinner than those of the African buffalo and they are not thickened at the base, which is characteristic for the African buffalo.

## DOMESTICATION AND EXPANSION OF THE ASIAN BUFFALO

The utilization of the domestic buffalo in the south and southeastern Asia is connected with rice growing, and it may be assumed that these regions were the first places of domestication, but there is no archeological evidence for that. The first mentions of buffaloes are found in the archeological findings of Harappa culture in the locality of Mohenjo-Daro in Western Pakistan. A buffalo by a through was presented on the steatite seals, which could point to its domestication in the third millenium B.C.

The first certain date about the existence of buffalo in the River Jordan valley was registered in 723 A.D. It is obvious that they were brought by Arabs from Mesopotamia and then to Egypt, where they arrived in the seventh century B.C. In the post Hittite layers from 12th to 7th century B.C., remains of buffalo have been found. In the 2nd century buffaloes were often mentioned in the Arabic poetry. The buffalo found its way to Persia in the 4th century, and from there probably came to South Russia where its presence was confirmed in the 5th century A.D. (B ö k ö n y i , 1974). There is no mention of the buffalo in the ancient Greece and Rome.

It is not definitely concluded wherefrom the buffalo came to Europe, nor is it known who brought it first. The movement of the buffalo toward the Balkan Peninsula was from several directions: eastern, through Byzantium, Bulgaria and Greece, northern, by the invasion of Huns and Avars, and southern, through Italy. There are no information for Slavs, who crossed the Danube River and settled on the Balkan Peninsula, that they bred the buffalo as domestic animal.

The Avars took the buffaloes along from South Russia and brought them to the Carpathian Basin in 560 A.D. and then to Italy in 596 A.D. The Lombard King Agilulf has received buffaloes as a gift from the Khan of Avars in Hungary. St Willibald, travelling to Palestine through Italy and Sicily in 723 A.D., was astonished to see buffaloes there. The Avars introduced the first

buffaloes to Germany, where in 874 A.D. there was a mention of a place called Buffileba (B ö k ö n y i , 1974). The Bulgarians brought the buffalo to the Balkan Peninsula in 679 A.D. Historical documents from the time of Khan Omortag show that in 814 A.D. he demanded two buffaloes for each Byzantine prisoner of war (Z a h a r i e v , 1963). That shows that Omortag knew the economic value of buffaloes and that in that time their breeding was developed in Byzantium. The next information of the presence of buffaloes in Bulgaria was found in the annals of a French historian Villehardouin, who accompanied the Emperor Baldwin and his brother Henry in the crusade to Jerusalem. In 1206 A.D. when the crusaders seized the town of Veroi, they won many cows, oxen and buffaloes (I s h i r k o v , 1931).

With the penetration of Turks into Europe, the buffaloes started to be bred in all areas under their power, as they are bred nowadays by the Moslems who follow the rules of their religion refering to diet. In the invasion of the West, the Turks utilized the buffaloes as draft animals for drawing of heavy weapons, equipment and food. For Turkish noblemen it was prestigious to be driven in a cart pulled by a large number of buffaloes. The most eminent of Crimean sultans, Selim Gerej, in 1702 A.D., was driven in a cart pulled by 50 buffaloes on his way to get treatment at the Kush Bunar spring. The road was narrow and steep, so that only buffaloes could pull the sultan's cart (T a b a k o v , 1911).

The Hungarians probably introduced many buffaloes into Europe, according to data presented by B ö k ö n y i (1974). S z e n t k i r á l y (1889) mentions buffaloes in Transilvania in the 11th century. In the King Bela's foundation deed of Dömös Abbey (1183) the buffaloes were mentioned also. At the end of the 12th century there was an estate in South Hungary named Biwol, and in the documents of later centuries in the Middle Ages too the names derived from buffalo occured. Endre II, King of Hungary, presented to Serbian King Stephen (1195—1224) horses, bisons and „Saracen buffaloes". Wladislas, King of Poland, visiting Sigismund King of Hungary and Emperor of the Holy Roman Empire in 1412, was taken ill from buffalo cheese.

B u f f o n (1958) considers that buffaloes arrived to Italy from Central Europe through the invasion of Avars at the end of the 6th century. He also cites monsignor Gaetani, a member of the family that had large herds of buffaloes in the Pontian marshes. D e v e r i u s attributed to Tunisian bey the introduction of buffaloes to Italy in the 7th century, during the Arab invasion.

## BUFFALOES IN YUGOSLAVIA AND NEIGHBORING COUNTRIES

From the previous presentation it was seen that the buffaloes came to Yugoslavia from one side through Bulgaria and Greece, and from the other side from Hungary. There are literature data about breeding of buffaloes in this country, although they are rather sparse. U l m a n s k i (1926) quotes that already about 1200 A.D. they were numerous in Macedonia. The monastery Dečani had its own „bivolare" (buffalomen) who were engaged in the breeding of buffaloes. In connection with buffaloes there are the toponyms of Bivoljak vil-

lage, as well as Bivol Grad, which were mentioned in a charter of Emperor Dušan issued in Hilandar in 1342 A.D. It seems that Duke Lazar was keeping many buffaloes, and the place where they were kept is still called Bivolje.

In the statute of town Kotor there are many data concerning the organization of the city slaughterhouse and collection of excise tax from the slaughter of animals, where, among other species of domestic animals, buffaloes are also mentioned. It was decided that every butcher must pay each evening certain tax per head of buffalo slaughtered. By the same document it was forbidden to keep livestock in the town, including buffaloes (K a t i ć , 1978). At the coast and in Konavli, according to J i r e č e k (1930), the buffalo (lat. *bubalus, bufalus*) was known, the same species that in the Middle Ages was mentioned among domestic animals in Thrace, Epirus and Bulgaria. The hides of buffaloes were exported to Venice. M i j a t o v i ć (1873) bear witness about it also, quoting that in the 15th and 16th century people of Dubrovnik imported considerable amounts of hides from Serbia, and one boat in 1574 A.D. bore the load of 14,597 cattle hides, 2,999 bufalo hides and 100 bales of wool. In collecting of material for agricultural history of Serbia, K o s t i ć (1921) cited several sources speaking about breeding of buffaloes. K o n t a r i n i (1550) on the part of Constantinople road that passes through Serbia, met a cart pulled by two buffaloes.

On the road between Niš and Pirot, G e r l a c h (1573) saw buffaloes also. Turkish hired coachmen most often hitched buffaloes. In Turkish times, buffalo meat was considered as unclean, and it was not consumed. B o u é (1839) said about his entry to Skadar „we entered Skadar, having behind us and in front of us herds of buffaloes, oxen and cows", and H a n in the „Travel through Drina and Vardar valleys" writes: „From the fields in the surroundings of the town, every evening, large number of buffaloes, cows, goats, pigs and geese are driven to town". Karađorđe wrote to bishop Petar I on September 16, 1809, that in Resava he routed some Turks and captures 40 carts with buffaloes. Describing newly liberated towns K o s t i ć (1922) stated that in Mitrovica buffaloes were replacing oxen as well as that „travelers were crossing the River Ibar by carts or by riding horses or buffaloes". In his description of the Knjaževac district, M a č a j (1966) cites that some buffaloes are seen in the very district town.

Concerning the buffalo breeding in Transylvania, a detailed description was provided by V u n d e r l i c h (1888). In 1870 in Transylvanian districts there were 42,176 and in 1884 75,489 buffaloes, which was contrary to tendencies in the other parts of Hungary, where the Podolian breed of cattle predominated. That was explained by an exceptional modesty of buffalo nutrition and by the quality of buffalo milk, which was specially praised by the Sachsen. In their area every third milking animal was the buffalo cow. Describing the winter feeding, the author states that „buffalo during the winter must be satisfied with cornstalks, of which it does not consume the leaves only, but, due to hunger, the hard parts of the stem also. At that, it is housed in the dark, cold stable, but endures cold and hunger and at such feeding and housing, which is astonishing, gives although small amount, but quite good milk". Buffalo cow yields on average 925 liters of milk, with the following average com-

position: 80.64% water, 8.45% butterfat, 5.57% casein, 4.518% milk sugar, and 0.845% salt. Buffalo cows allow to be milked only by persons known to them. At the age of four they are fully developed. Average height at withers in buffalo cows is 138 cm, and in bulls 139 cm. Completely developed buffalo cows weigh 520 kg, bulls 540 and working oxen 590 kg. Gestation is several weeks longer than in domestic cows. They have difficulties to become pregnant, which could be attributed to careless management.

For the presentation of buffalo breeding in Serbia and Macedonia, interesting information were given by S a v i ć (1924) which will be presented as a whole.

„The buffaloes are kept in sheltered marshy places along rivers and swamps; they are not kept in hilly villages. In marshes and swampy places it is easier to feed them, since they graze the grass in the marsh and during the winter they eat chaff, mostly straw, cornstalks and sometimes hay and some grains. The feed is given in somewhat higher amounts than to cattle, so that it is easier to feed them. Male and female buffaloes achieve the mature size at the same time as cattle. Buffalo cow gives more milk, which contains more butterfat, and it is healthier than that of domestic cows. It is especially excellent for tuberculous persons. The sour buffalo milk is so firm that it may be cut with a knife. Buffalo milk gives an excellent, best sour milk. One liter of buffalo milk may give 100—200 grams of butter. Good milking buffalo animal is always better accepted than milking domestic cow. The data are very different because they depend on feeding. Well bred buffalo cow may yield 6—8 okas (1 oka = 1.28 kg) of milk. From 2.5 okas of milk, one oka of cheese and about 120 g of butter are obtained. From Bujanovac is reported that good buffalo cow may give 10—15 kg of milk a day, and from it 0.5 to 1 kg of butter and 2—5 kg of cheese are obtained. From buffalo milk, two times more butterfat are obtained than from the domestic cow's milk.

The buffaloes are much stronger for work. They are used as working animals for 10—20 years or more. Fattened buffalo or buffalo cow are bought not for slaughter but for work. The price of buffaloes is two times higher than that of domestic cattle. Nobody keeps buffaloes only. The price of buffalo meat is by 1/4 lower' than that of beef, but it is rarely seen in the butchers shop. The buffalo meat is less palatable although it is much fatter than beef, and people do not consume it as ready as they do beef.

The butter of buffalo cows commands the price which is by 1/4 higher than those of cattle and sheep butter. In towns buffalo milk is more expensive than cow milk, by l/8 of the price. The buffalo hide is much more expensive than that of domestic cattle. Adult buffaloes may yield 200—300—500 of lean meat and 10% of tallow. It depends on the breed, i.e., the breed kept in the northern parts of Kosovo yields smaller amounts of meat than the breed kept in the vicinity of Skoplje and Gevgelia. Buffalo yields 60—70% of lean meat. In the summer buffaloes must spend at least one hour in the mud or water, where they lie in order to cool themselves. In the winter they have to be kept in warm stables and during work they must be covered by a blanket over the rump.

In the spring, the buffaloes are shawed and all the last year's hairs from the back, neck and a half or more of the abdomen are removed. In such way the hide is cleaned from all impurities, because it must be well lathered with warm water and soap before shawing.

Buffaloes need to be combed often, otherwise they are invaded by lice. For ploughing, buffaloes are two times more endurable, and they can pull a three times heavier load than oxen. Buffaloes are also kept in the vicinity of towns, because they are specially suitable for waggoners, as well as because of milk, which is more expensive. They are calmer than oxen and capable of pulling heavier loads than horses. Even a child may handle buffaloes. In winter, they are fed on straw and during the summer they graze. Buffalo milk has 6% of fat (two times more than cow milk). With respect to butter production it does not fall behind the best Swiss cow. Buffalo hides are 50% more expensive than cattle hides.

Buffaloes are kept in the Nišavski district of Pirot area, in Barje, Čiflik of the Rasnica community. Along the addition to Točica river in the Dobrički district people keep buffaloes in cattle and they are very satisfied with them. In the Zvečan district, a small number of owners keep buffaloes, only those near the Ibar and Sitnica rivers. Buffaloes are prefered to domestic cattle in the Kosovo area, because they can live on straw. The buffalo cow yields 5—6 okas per milking, from which one oka of butter, or 1.5 okas of cheese are obtained (a report from Priština). A common buffalo gives about 200 okas of meat. Buffaloes are also raised in Bujanovac and Trnavac. In the Gračanica district, the following villages raise buffaloes: Bivoljak, Crkveno, Vodice, Kruševac, Dobrotin, Lepina, Rajevo, all of them near the Sitnica river. They are fed on wheat straw, pumpkins, leftovers after other livestock; otherwise, the feed is like that for cattle. The buffalo cow gives 7—8 okas of milk and from 5—4 okas of milk one oka of butter and one oka of skim milk cheese are obtained. There it is easier to feed buffaloes than cattle. In Prizren, the male buffalo yields about 180 okas of meat and the female from 120 to 150 okas. In Giljane and in Lower and Upper Morava, along the river Moravica, buffaloes are raised. The buffalo cow gives annually 1400—1800 okas of milk, or 70 okas of butter and 300 to 400 okas of cheese. In Djakovica, the buffalo cow yields 8 okas of milk a day, from which 3 okas of butterfat or 5 okas of cheese are obtained. In Metohija, specially the Moslems (because they own the best land) rear buffaloes near the water, in villages as well as in towns. There are 32.000 buffaloes in our country.

For the defense of the country it is of enormous importance to maintain buffalo breeding, because during the previous war our buffaloes pushed through the worst and muddiest places and roads, where oxen and horses failed".

Although the author probably collected these data by means of inquiry while some statement about meat production or the medicinal qulity of milk ought to be taken with reserve, this report provides ample information about buffalo raising in the southern parts of own country at the beginning of the 20th century.

In the Vojvodina Province, buffaloes were mainly raised at large estates. B o r o v s z k y (1921) reported that count Čekonić presented at an exibition

one bull, 12 females and 8 buffalo calves. Describing the estate of count Čekonić in Jimbolia, Popović (1924) states that buffaloes were used for the heavy work of hauling and transportation of steam engines. The animals were useful because they were giving good manure and were satisfied with low-quality feed. For the first time, 2 bulls and 50 female buffaloes were bought, which served for drawing and reproduction, while the surplus was sold. In 1910 there was a total of 320 animals. From that number 134 served for drawing only. In the journal „Težak" from 1921, 14 heifers and 16 buffalo oxen were offered for sale from the same estate. On the estate of count Kotek in Futog there were 18 buffaloes. Jovičić (1970) quoted that in 19th century in Bela Crkva in Banat the buffaloes were bred within the cattle „but to much smaller extent than the true cattle, and they were used for work only".

The buffaloes were present in other estates. According to knowledge of the author, in 1951 there were four buffaloes on the estate „Sokolac" in Novi Bečej, and even in 1946 eight buffaloes were transferred from Čoka to Novi Kneževac for agricultural estate „Spasoje Stejić". Last buffaloes have been seen on the agricultural estate „Zobnatica" in 1967, when 5 animals were sold (one of which was albinic), so that it may be considered as complete disappearance of that species in the Vojvodina Province.

However in recent years one farmer in Bačka Topola (Tružinski) bought one male and two female buffaloes in an effort to save the genetic resources of that species.

By liberation of southern parts of Serbia, 1876—1918 the number of buffaloes increased in the country. Denić (1898) reported that the people of the Leskovac area raise buffalo females for work. On the basis of Statistical Yearbook of 1904, Tartler (1918) registered that the number of buffaloes in Serbia was as follows: 1890 — 8,494, 1895 — 7,471, 1900 — 6,929, 1905 — 7,710. By presenting the economy in the Balkan countries, Nemeth (1913) concluded that in Sandžak and Old Serbia there were 52,000 buffaloes. It is interesting to compare that census with those in the neighboring countries. So in Macedonia (part of Vardarian and Aegean Macedonia) on the eve of war there were 101,000 buffaloes and in 1892 in Bulgaria 343,193, while in 1900 in the same country there were 431,497 buffaloes.

Tartler (1918) reported about changes in the number of buffaloes in Hungary.

| Year | Number according to census | Export of buffaloes |
|---|---|---|
| 1895. | 132,578 | |
| 1904. | 157,164 | |
| 1905. | 162,318 | |
| 1906. | 158,996 | 7,777 |
| 1907. | 166,410 | 4,470 |
| 1908. | 186,586 | 6,851 |
| 1909. | 182,138 | 17,118 |

In Italy, Maymone (1940) reported that in 1881 there were 11,000 buffaloes, in 1909 the number was 19,000, in 1930 it was 14,000 and in 1937

it declined to 12,000. However Salerno (1974) pointed to continuous increase of the number of buffaloes in that country, so that in 1970/71 it reached 55,000 animals owing to breeding for milk, introduction of modern technology and recording of productivity, so that milk production per head achieved about 1,800 kg.

After the establishment of Kingdom of Serbs, Croats and Slovenes, the number of buffaloes in the country, on the basis of the census of 1920, according to Frangeš (1926), was as follows:

| | |
|---|---|
| Northern Serbia | 6,806 |
| Southern Serbia | 42,045 |
| Montenegro | 41 |
| Voyvodina | 1,184 |
| Croatia and Slavonia | 268 |
| Dalmatia | 50 |
| Slovenia | 1 |
| Bosnia and Herzegovina | 400 |
| Total | 50,883 |

The trend of the next censuses of buffaloes in the Kingdom of Yugoslavia was presented by Belić (1995):

| | 1921 | 1931 | 1939 |
|---|---|---|---|
| Number of buffaloes | 51,470 | 40,563 | 38,004 |
| Per 1 km$^2$ | 0.21 | 0.16 | 0.25 |
| Index | 100 | 76 | 71 |
| Per 100 inhabitants | 0.43 | 0.29 | 0.24 |

About 82—84% of buffaloes were in Vardarska regional unit, and about 12.5% in Zetska regional unit, which included Kosovo and Metohia areas, as well as Sandžak.

By creation of new Yugoslavia, after 1944, the tendency of decrease of number of buffaloes was expressed again, as a consequence of mechanization of agricultural production, depopulation of villages and changes in eating habits of the population.

Trend of numbers of buffaloes was as follows:

| | 1949 | 1989 | 1990 | 1991 |
|---|---|---|---|---|
| Number of buffaloes | 78,000 | 25,000 | 21,000 | 37,000 |
| Index | 100 | 32 | 27 | 47 |
| Per 100 inhabitants | 0.48 | 0.11 | 0,09 | 0,16 |
| Per 1 km$^2$ | 0.31 | 0.10 | 0.08 | 0.14 |
| Index | 100 | 32 | 11 | 45 |

The republics of Serbia and Montenegro, which make the present Yugoslavia, in 1992 have had 19,000 buffaloes, in order that the number in 1997

amounted 16,000 animals only. The present population of buffaloes is found mainly in Kosovo and Metohia and in the Raška area.

## REFERENCES


Белић, Ј. (1993): *Сточарство у Југославији од њеног настанка до распада 1991*, САНУ, посебна издања, књига DCXXXIII, Одељење природно-математичких наука, књига 71, Београд.

Bohlken, H. (1958): *Vergleichende Untersuchungen an Wildrindern (Tribus bovini Simpson 1945)*, Zool. Jb., Abt. Allg. Zool., 68:113—202.

Borovszky, S. (1912): *Magyarország Vármegyéi és Városai*, Budapest.

Boué (1839): *Turquie*, III.

Bökönyi, S. (1988): *History of domestic mammals in Central and Eastern Europe*, Akadémiai kiadó, Budapest.

Buffon, G. L. L. (1938): *Oeuvres complètes*, Vol. IV, Paris.

Денић, Р. (1898): *Допис из Медвеђе, срез Јабланички*, Тежак, бр. 16 и 17, Београд.

Франгеш, О. (1926): *Аграрна статистика*, Народна енциклопедија СХС, Загреб.

Ishirkov, A. (1931): *Buffalo breeding in Bulgaria*, J. Vet. Collection, N° 8 and 9.

Јиречек, К. (1923): *Историја Срба*, друга свеска, Београд.

Јовичић, С. (1970): *Бела Црква у прошлости*, Бела Црква.

Катић, Р. (1978): *Сточарство Средњовековне Србије*, САНУ, посебно издање, књига DXIII, Београд.

Костић, К. (1921): *Грађа за историју пољопривреде код нас*, Министарство пољопривреде и вода, Београд.

Костић, К. (1922): *Наши нови градови на југу*, СКЗ, Београд.

Мачај, С. (1866): *Грађа за топографију округа Књажевачког*, Гласник Српског ученог друштва, XIX.

Mason, I. L. (1974): *Species, types and breeds*. In: „The Husbandry and Health of the Domestic Buffalo", FAO, Rome.

Maymone, B. (1940): *Талијанско сточарство*, Пољопривредни гласник, бр. 7/8, Нови Сад.

Мијатовић, Ч. (1873): *Студије за историју српске трговине XIII и XIV века*, Гласник Српског ученог друштва, књига XXXVII, Држ. штампарија, Београд.

Németh, J. (1913): *A balkáni államok mezögazdasági termelési és forgalmi viszonyai*, Budapest.

Поповић, Ч. (1924): *Имање грофа Чекоњића из Жомбоља — Банат*, Тежак, br. 3—7.

Salerno, A. (1974): *The buffaloes of Europe. Italy*. In: „The Husbandry and Health of the Domestic Buffalo", FAO, Rome.

Савић, М. (1924): *Где и зашто се гаје биволи код нас*, Наша индустрија, занати и трговина, IV део, Државна штампарија, Сарајево.

Szentkirály, A. (1889): *Über den Buffel*, Magyaróvár.

Tabakov, T. (1974): *Sliven district during the time of the Crimean Gereys at the end of the 17th and the beginning of the 18th century*, I, Sliven.

Tartler, G. (1918): *Der Siebenbürgische Buffel*, M. N. Verlagsbuchhandlung, Hannover.
Улмански, С. (1926): *Биво*, Народна енциклопедија Српско-хрватско-словеначка, Загреб.
Вундерлих, Л. (1886): *О држању бивола у Ердељу*, Тежак, св. 5, Београд.
Zahariev, I. (1963): *Buffalo breeding in Bulgaria*, J. Nature (6).

## ПОРЕКЛО, ДОМЕСТИКАЦИЈА И ШИРЕЊЕ ДОМАЋЕГ БИВОЛА (*Bubalus bubalis*) У ЈУГОСЛАВИЈИ

Тимотеј М. Чобић
Пољопривредни факултет, Трг. Д. Обрадовића 8, 21000 Нови Сад

Резиме

У раду су приказани порекло и зоолошке одлике домаћег бивола (*Bubalus bubalis*), његова доместикација и ширење у свету и код нас. Биволи су у нашу земљу дошли из два правца, јужног и северног. Првенствено су коришћени као теглеће животиње, затим за производњу млека, док је производња меса била секундарног карактера. Сматра се да су као вучна стока јачи од волова, а посебно се истиче њихова скромност у исхрани и могућности коришћења хране лошијег квалитета. У условима интензивног држања могу да постигну млечност од 1800 kg, са високим садржајем млечне масти. Као запрежне животиње биволи су замењени најпре коњима, а затим тракторима, што је довело до опадања њиховог значаја у пољопривреди Европе. Међутим, у свету је биво још увек незамењив, посебно у реонима гајења пиринча.

UDC 614.23(497.1):929 Milin J.

# IN MEMORIAM

## Проф. др ЈОСИФ МИЛИН
## (1943—2000)

Прераним одласком у вечни смирај проф. др Јосифа Милина (18. јула 2000), великог радника и знатижељника, немирног духа, у широкој научној јавности афирмисаног научника, на универзитетима у Југославији познатог и посебно уваженог наставника, и Матица српска је осиромашена за једног угледног Сталног члана сарадника.

Јосиф Милин је рођен 14. марта 1943. године у Бечеју, Основну школу и гимназију је завршио у Сарајеву, а Медицински факултет у Новом Саду 1967. године. Након дипломирања изабран је за асистента на Катедри за хистологију и ембриологију. Овим избором означен је почетак интензивног рада на стручној и научној надградњи, образовању других и комплетирању своје наставно-научне личности.

Одбраном магистарског рада 1974. и докторске дисертације 1976. године уврстио се у запажене хистофизиологе неуроендокриног система, што је, уз обиман научноистраживачки рад и педагошко искуство, резултирало избором у сва наставничка звања (доцент 1977, ванредни професор 1982. и редовни професор 1987. године).

Специјализацију из гинекологије и акушерства завршио је 1978. године.

Своје усавршавање, образовање и надградњу реализовао је стручно-научним боравцима и радом у Институту за патологију Медицинског факултета у Загребу, Институту за хистологију и ембриологију у Лиону, Институту за биологију Универзитета Jyvaskyli у Финској, Секцији за неуроендокринологију Вајсмановог центра Медисон у САД, Научном центру у Сан Антонију у САД...

Опсежним истраживачким радом и стваралаштвом научној јавности и критици ставио је на увид своја достигнућа исказана у седам монографија у југословенским издањима; осам монографија у иностраним издањима; у осам универзитетских уџбеника; у 23 рада у иностраним часописима, у 27 радова у домаћим часописима, у 48 саопштења на иностраним научним скуповима и 80 на домаћим, што представља завидну афирмацију његове и наше научне мисли.

Проф. др Јосиф Милин је, поред значајног доприноса образовним институцијама у којима је радио у научном стваралаштву из различитих области, запажену активност испољио у многобројним стручним и научним удружењима у земљи и иностранству.

Својим прераним и изненадним одласком проф. др Јосиф Милин ништа није однео са собом. Све најлепше и најбоље оставио је својој породици, научно-образовним институцијама медицинских факултета у Новом Саду, Крагујевцу, Подгорици, природноматематичким факултетима у Новом Саду и Подгорици, стручним и научним удружењима, као и Матици српској. Проф. др Јосиф Милин је оставио веома значајан научноистраживачки опус и допринео угледу и афирмацији поменутих институција, чиме је, поред сећања и захвалности, заслужио посебно поштовање и уважавање.

*Проф. др Драгиша Поповић*

# INSTRUCTIONS FOR AUTHORS

1. General

1.1. *Proceedings for Natural Sciences* accepts original works, review papers, and short communications on all subjects implied by the journal's title. Review papers are published only at the invitation of the board of Editors. Papers already published or under consideration elsewhere cannot be accepted.

1.2. Manuscripts are accepted in English. They must be correct in terms of grammar and style. Authors are requested to submit manuscripts in triplicate (original plus two copies). Authors whose native tongue is not English should also submit a copy of the paper in the original language.

1.3. Upon receipt of a manuscript, the author will be sent the file number of the paper. This number should be quoted in all correspondence. The Editors will try to inform the author about the status of the paper within three months. Each paper will be reviewed by at least two reviewers. If a paper is not accepted, the manuscript will not be returned to the author.

1.4. Manuscripts submitted for publication should be sent to the Editorial Office of *Proccedings for Natural Sciences*, 21000 Novi Sad, Ul. Matice srpske 1, Yugoslavia.

2. Preparation of manuscript

2.1. The maunscript must be typed double space throughout (including references, table, etc.), on A4 paper, and all margins should be kept wide (2.5 cm).

2.2. Manuscripts should be divided into sections, viz. Abstract, Key words, Introduction, Material and/or Methods, Results, Discussion, References, Abstract in Serbo-Croatian, Acknowledgements.

2.3. The title of the paper should contain as many relevant terms as possible, but should be limited to about 10 words.

2.4. The key words should indicate the scope of the paper. They should be given in alphabetical order and separated by commas. Key words should not exceed 100 characters.

2.5. The names, family names and middle names, of all authors with at least one first name should be spelled out for each author. The affiliation of the authors (without abbreviations) and where the contribution originated, including complete postal addresses, should be specified.

2.6. The abstract should be given in two languages, English and Serbo-Croatian. It should be as informative as possible, and it should summarize the contents of the paper. The former should not exceed 5% and the latter not 10% of the length of the entire manuscript. The abstract in Serbo-Croatian should include the title of the paper, the name(s) of the author(s), and his/their affiliation.

2.7. Acknowlegdements of financial support, advice, or other kinds of assistance should be made at the end of the paper, under the heading „Acknowledgements".

2.8. Papers should not exceed 12 typewritten pages, including references, tables, legends, and figures.

3. References

3.1. References should be limited to those that are absolutely necessary.

3.2. References to literature should be arranged alphabetically; cite exactly as follows:

a. Journal articles

Author CD, Author DC (1990) Title of article. *Plant and Soil* 135: 102—134.

b. Book articles

Author ED, Author SI, Author BB (1991) Title of article. In: A Blom, B. Lindau, Eds., *Title of book*, Ed 3, Vol 2, Publisher, City, pp 242—255.

c. Doctoral theses, M. A. Theses and Habilitations

Author VA (1989) *Title of thesis*. PhD Thesis. University, City.

d. Books

Author AE (1987) *Title of the book*. Publishers, City, pp 237.

e. No authors or editors

Title of booklet, pamphlet, etc. (1989) Publisher of company, City.

f. Unpublished works

Cite an article as „in press" only if accepted for publication; cite the journal in which it will appear.

3.3. Journal names should be abbreviated in conformity with the Bibliographic Guide for Authors and Editors (BIOSIS, Chemical Abstracts Service and Engineering Index, Inc., 1974).

3.4. References to literature in the text should be made by mentioning the last name of the author and the year of publication. In the case of two authors, both should be mentioned, but with three or more only the name of the first author plus „et al." should be given.

3.5. If an author is cited who published several papers in the same year, add a, b, c, etc, to the year of publication, both in the text and references.

4. Illustrations

4.1. Figures are of two kinds: black-and-white photographs and drawings. Photographs must have good contrast; line drawings must be neatly drawn,

boldly in black ink on good quality white tracing paper. In addition to the usual line-drawn graphs, also treat metabolic schemes, complicated formulas, and large or complex tables as figures.

4.2. All letters, numbers, and symbols must be large enough in the original to be at least 1.5 mm high after reduction. The lettering of figures should also be drawn in black ink.

4.3. Figures should be added separately, i.e., not inserted in the typed text. Legends should, if possible, be inserted in the figures.

4.4. The position of figures should be indicated at the left margine. Figures are to be numbered with Arabic numerals.

4.5. Every figure must be accompanied by a caption. The caption must explain the contents of the figure. Captions are not to be typed under the figures, but should be compiled on a separate page.

5. Tables

5.1. Tables are to be typed on extra pages (one page per table), at the end of the manuscript.

5.2. Tables are to be numbered with Arabic numerals.

5.3. Each table must begin with a caption. The caption must explain the contents of the table.

5.4. Footnotes to a table should be typed directly under the table.

5.5. The position of tables should be indicated at the left margin.

6. Units, names, formulas, and abbreviations.

6.1. Only SI quantities and units are to be used (SI = Systeme International d'Unit's); in exceptional cases, other officially accepted units may be used.

6.2. For molar concentration, an italicized M (underlined) should be used.

6.3. Biological names in Latin should be italicized (underlined).

6.4. Chemical structural formulas and equations should be drawn (not written or typed), ready for photographic reproduction.

6.5. Only standard abbreviations should be used. Where specialized abbreviations are used, the term should be given initially in full with the abbreviation indicated in parentheses.

6.6. Mathematical expressions should be written in such a way as to use the minimal number of lines, while retaining their clarity, for example: 2/3 instead of 2:3, exp (-ab) instead of $e^{-ab}$, etc.

7. Short Communications

7.1. Proceedings for Natural Sciences offers an opportunity to publish short communications on all aspects that are implied by the journal's title.

7.2. Short communications are limited to 4 typewritten pages including all illustrations.

7.3. The presentation and format of the short communications are similar to those of a normal paper, except for the list of references, in which the titles of the papers should be omitted.

8. Information of authors

8.1. When the manuscript has been accepted, the author will be informed of the approximate time of publication.

8.2. Corrections of the proofs should be restricted to printer's errors only. Other than these, substantial alterations will be charged to the author. Proofs should be handled promptly and returned to the Editorial Office.

8.3. Fifty offprints are supplied free of charge. Copies in addition to these may be ordered and paid for through the Editorial Office.

9. DISKETTES: After acceptance, the final revision should be submitted on disk. Include text, tables and figures on a double-density or high-density 3.5-inch diskette. An accompanying printout is needed to facilitate the incorporation of electronic tables and figures. Word for Windows (any version) is the preferred word-processing program. When copying the paper on disk, it is important to follow this procedure: *File>Save as>Options>Embed True Type fonts>ok>Save*.

# УПУТСТВО ЗА АУТОРЕ

1. Опште напомене

1.1. Зборник за природне науке прима оригиналне радове и прегледне чланке као и кратка саопштења из свих научних области које обухвата назив часописа. Прегледни радови се објављују само на позив редакције. Радови који су већ објављени или су послани за објављивање у другом часопису не могу бити прихваћени.

1.2. Прихватају се рукописи писани на енглеском језику. Језик мора бити исправан у погледу граматике и стила. Аутори треба да предају рукопис у три примерка (оригинал и две копије). Аутори чији матерњи језик није енглески такође треба да приложе и копију рада на изворном језику.

1.3. По примању рукописа, аутори ће добити редни број свога рада. Тај број треба наводити у даљој преписци. Редакција ће обавестити ауторе о приспећу рукописа и мишљењу рецензената у року од три месеца од пријема. Сваки рад рецензирају најмање два рецензента. Ако рад не буде прихваћен, рукопис се не враћа аутору.

1.4. Рукописе за објављивање треба слати на адресу редакције Зборника за природне науке, 21000 Нови Сад, Ул. Матице српске 1, Југославија.

2. Припрема рукописа

2.1. Рукописи се куцају са двоструким проредом у свим деловима текста (укључујући литературу, табеле итд.), на папиру формата А4. Све маргине треба да буду широке 2,5 сантиметра.

2.2. Рукопис треба поделити на: Сажетак, Кључне речи, Увод, Материјал и/или методе, Резултати испитивања, Расправа, Литература, Сажетак на српско-хрватском језику, Захвалност.

2.3. Назив рада треба да буде информативан, али не дужи од десет речи.

2.4. Кључне речи треба да указују на целокупну проблематику истраживања. Треба их навести абецедним редом и одвојити зарезима. Кључне речи не треба да пређу сто словних знакова.

2.5. Треба навести презимена, средње слово и имена аутора рада као и назив установе (без скраћеница) у којој је рад настао, заједно са пуном поштанском адресом.

2.6. Сажетак, на енглеском и српском, треба да буде информативан и да резимира садржај рада. Дужина енглеског сажетка треба да буде до 5%, а српског до 10% дужине укупног текста. Српски сажетак треба да садржи наслов рада, презимена и имена аутора и назив установе у којима су аутори запослени.

2.7. Податке о финансијској помоћи, саветима и другим врстама помоћи треба навести на крају рада, под насловом Захвалност.

2.8. Радови не смеју бити дужи од 12 куцаних страна, укључујући литературу, табеле, легенде и слике.

3. Литература

3.1. Литературу треба ограничити на неопходан број навода.

3.2. Литературне наводе треба сложити абецедним редом, на следећи начин:

а. Чланци из часописа Аутор CD, Аутор DC (1990) Назив рада. Име часописа 135: 102—134.

б. Чланци из књига

Аутор ED, Аутор SI, Аутор BB (1991) Назив цитираног дела књиге. У: A. Blom, B. Lindau, Eds., Назив књиге, Ed 3, Vol 2, Издавач, Град, 242—255.

в. Дисертације

Аутор VA (1989) Назив тезе. Докторска дисертација. Универзитет, Град.

г. Књиге

Аутор AE (1987) Назив књиге, Издавачи, Град, 237.

д. Публикације без аутора или уредника

Назив књиге, брошуре, итд. (1989) Издавач или установа, Град.

ђ. Необјављени радови

Навод „у штампи" треба да се односи само на прихваћене радове; навести име часописа у којем ће рад бити објављен.

3.3. Имена часописа треба скраћивати према „Bibliographic Guide for Authors and Editors" (BIOSIS, Chemical Abstracts Service and Engineerings Index, Inc., 1974).

3.4. Референце у тексту треба да укључе презиме аутора и годину издања. Ако има два аутора, треба навести обојицу, а у случају три или више аутора треба навести првог аутора и назначити „et al.".

3.5. Ако се наводе два или више радова истог аутора, објављених у истој години, потребно је у тексту и списку литературе ставити a, b, c итд. иза године објављивања.

4. Илустрације

4.1. За илустрације могу се користити црно беле фотографије и цртежи. Фотографије треба да имају добар контраст а цртежи треба да буду цртани тушем, на папиру доброг квалитета. Осим графикона, метабо-

личке шеме, компликоване формуле и велике или компликоване табеле такође треба третирати као слике.

4.2. Сва слова, бројке и симболи треба да буду довољно велики у оригиналу, тако да после смањивања не буду мањи од 1,5 mm. Текст на сликама и графиконима такође треба исписати тушем.

4.3. Илустрације треба приложити уз рад а не уметнуте у текст. По могућности, легенде треба назначити на илустрацијама.

4.4. Места илустрација треба означити на левој маргини, арапским бројевима.

4.5. Свака илустрација треба да има текст који објашњава садржај прилога. Текст за илустрације треба куцати на посебној страни.

5. Табеле

5.1. Табеле треба куцати на одвојеним странама (једна табела по страни) и приложити их на крају рада.

5.2. Табеле се означавају арапским бројевима.

5.3. Свака табела треба да почне насловом који објашњава њен садржај.

5.4. Напомене треба куцати одмах испод саме табеле.

5.5. Места табела у тексту треба означити на левој маргини.

6. Јединице, имена, формуле и скраћенице

6.1. Треба користити SI ознаке количина и јединица (SI Systeme International d'Unit's), изузетно се могу користити и друге званично прихваћене јединице.

6.2. Моларну концентрацију треба означити са M и подвући.

6.3. Биолошка имена на латинском треба подвући.

6.4. Хемијске структурне формуле и једначине треба нацртати (не исписивати или куцати) и припремити за фотографску репродукцију.

6.5. Прихватају се само стандардне скраћенице. При коришћењу специјалних скраћеница, пун термин треба навести приликом првог спомињања, а скраћеницу додати под наводним знацима.

6.6. Математички изрази треба да буду написани тако да се користи најмањи број редова, али да се сачува читљивост, нпр. 2/3 уместо 2:3, exp (-ab) уместо a-ab, итд.

7. Кратка саопштења

7.1. Зборник за природне науке нуди могућност објављивања кратких саопштења о свим научним областима обухваћеним називом часописа.

7.2. Величина кратког саопштења је ограничена на 4 куцане стране, укључујући све илустрације.

7.3. Кратко саопштење се пише по упутствима за припрему рада нормалне дужине, сем што у литератури треба изоставити наслове рада.

8. Обавештавање аутора

8.1. Када рукопис буде прихваћен, аутор ће о приближном времену објављивања бити обавештен у писаној форми.

8.3. Исправљање текста припремљеног за штампу треба ограничити на штампарске грешке. Значајне промене текста ће се наплаћивати. Кориговани текст треба вратити уредништву у најкраћем могућем року.

8.3. Аутори добијају 50 бесплатних примерака сепарата. Ако аутор жели већи број сепарата може их наручити код издавача уз надокнаду.

## 9. КОПИЈА РАДА НА ДИСКЕТИ

После прихватања рада потребно је доставити дискету са коначном верзијом рада. Дискета треба да садржи текст рада, табеле и слике (прилоге) на DD или HD дискети од 3,5 инча. Приложите и једну копију одштампаног рада ради лакше обраде табела и слика. Молимо да обраду текста вршите програмом Word for Windows (било која верзија). Приликом копирања рада на дискету, придржавајте се следеће процедуре: File> Save as>Options>Embed True Type fonts>ok>Save.