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# THE PRESENCE OF AN ARYLPHORIN-TYPE STORAGE PROTEIN AT DIFFERENT LIFE STAGES OF *OSTRINIA NUBILALIS (LEPIDOPTERA: PYRALIDAE)*

ABSTRACT: Specific hemolymph proteins, termed storage proteins, are considered to play an important role in amino acid reserves in insects. Arylphorin-type storage proteins exist widely in insects and they appear as major proteins in the larval hemolymph. These proteins are rich in aryl groups and are thought to act as source of aromatic amino acids for protein synthesis during organ differentiation and adult development. In this study, we isolated an arylphorin-type storage protein from the larval hemolymph of the European corn borer, *Ostrinia nubilalis* Hübner (*Lepidoptera: Pyralidae*), and named it ostrinin. Using polyclonal antibodies against ostrinin, raised in a mouse and a rabbit, we analyzed the presence of this protein through different stages of the life cycle of *O. nubilalis*. Our results revealed that ostrinin was present in all life stages of the European corn borer (diapausing and nondiapausing larvae, pupae and adults), except in the eggs.

KEY WORDS: arylphorin, metamorphosis, *Ostrinia nubilalis*, ostrinin, storage protein

## INTRODUCTION

Holometabolous insects are thought to have developed the ability to store amino acids for anticipated metabolic needs, especially for metamorphosis. Pupae cannot take in any food, and thus the biosynthetic precursors common for adult structures must be accumulated prior to pupation. During the final larval stadium, large amounts of storage proteins are synthesized in larval fat body tissue and released into the hemolymph (Telfer and Kunkel, 1991) where they become the major constituent of the hemolymph. Once the insect ceases feeding and prepares to pupate, these proteins are partially or wholly sequestered by the fat body and stored in dense protein granules (Telfer and Kunkel, 1991; Tojo et al., 1978). Later, granules and their proteins are

hydrolyzed to produce amino acid needed for the synthesis of adult proteins. In the sweet potato hornworm (*Agrius convolvuli*), the amount of nitrogen, which mainly reflects the total amount of amino acids, is efficiently conserved during larval-adult development: 78—81% of the absorbed nitrogen was maintained in the pupal body and 40—55% still remained in the adult body (Shimoda and Saito, 1997).

In *Lepidoptera*, there are at least two kinds of storage proteins: arylphorins, which have a very high aromatic amino acid content (exceeding 15%), and methionine-rich storage proteins (Burmester, 1999; Haunerland, 1996). Both have molecular sizes of nearly 500 kDa and are composed of six identical or similar subunits in 70—90 kDa size range (Telfer and Kunkel, 1991). Arylphorin-type storage proteins exist widely in insects, accounting for up to 80% of the total protein content in the hemolymph of last instar larvae (Sekeris and Scheller, 1977). These proteins are rich in aryl groups and are thought to act as source of aromatic amino acids for protein synthesis during metamorphosis and development (Levenbook and Bauer, 1984).

In this study, we isolated an arylphorin-type storage protein from the larval hemolymph of the European corn borer, *Ostrinia nubilalis* Hübner (*Lepidoptera: Pyralidae*) and named it ostrinin. Using polyclonal antibodies against this protein, we analyzed the presence of ostrinin in the eggs, hemolymph and fat body of diapausing and nondiapausing larvae, as well as in pupae and adults, in order to shed more light to developmental pattern of this storage protein in *O. nubilalis*.

## MATERIAL AND METHODS

### *Chemicals*

All of the chemicals used in this study were purchased from Sigma Chemical Company (St. Louis, MO, USA), unless indicated otherwise.

### *Sample preparation*

Larvae (diapausing and nondiapausing) and pupae of the European corn borer, *Ostrinia nubilalis* Hübner (*Lepidoptera: Pyralidae*) were collected from maize plants in the fields of the Vojvodina Province, Serbia. Eggs and adults were supplied by Dr. F. Bača, Maize Institute, Zemun polje, Serbia. Larvae were carefully brushed to remove potentially contaminating particles. Prolegs of larvae were cut with scissors and hemolymph was squeezed out into 1.5-ml Eppendorf tubes containing a few crystals of phenylthiourea (PTU). After removal of hemocytes and cell debris by centrifugation at 12000 g for 5 min at 4°C, the supernatant was diluted two-fold with phosphate-buffered saline (PBS: 50 m*M* potassium phosphate, 150 m*M* NaCl, pH 7.4) containing 10 m*M* EDTA, 0.1 m*M* phenylmethyl sulfonyl fluoride (PMSF) and a few cry-

stals of PTU. Samples were divided into aliquots and stored at –20°C for later analysis. Larval fat bodies were dissected in ice-cold PBS, rinsed several times in the same buffer, divided into aliquots and then stored at –20°C until use. Prior to analysis, fat bodies were homogenized in 5 vol. of PBS containing 10 m*M* EDTA, 0.1 m*M* PMSF and a few crystals of PTU, and centrifuged at 12000 g for 5 min at 4°C. Eggs, pupae and adults were homogenized in 1.5-ml Eppendorf tubes, using the same buffer as mentioned above. Samples were than centrifuged at 12000 g for 5 min at 4°C. Resulting supernatant was divided into aliquots and stored at –20°C for later analysis.

### *Electrophoresis and elution of proteins*

Native polyacrylamide electrophoresis (native-PAGE) was carried out in a Bio-Rad Mini-PROTEAN II unit (Richmond, CA, USA) according to the method described by L a e m m l i (1970). Electrophoresis was performed on 10% separating gel, overlaid with 4% stacking gel, at a constant voltage of 25 V/cm. After native-PAGE, gel areas containing proteins of interest were sliced away from the rest of the gel, cut into a few millimeters thick sections and then incubated overnight at 4°C in PBS containing 10 m*M* EDTA, 0.1 m*M* PMSF and a few crystals of PTU. Afterwards, incubated gel pieces were centrifuged at 12000 g for 5 min at 4°C, using Ultrafree-MC filter unit (pore size 0.45 μm). Purity of eluted proteins was tested using native-PAGE. After electrophoresis, separated proteins were stained with 0.1% Coomassie Brilliant Blue R250 dissolved in water:methanol:acetic acid (50:40:10) solution. Protein content was determined by the method of L o w r y et al. (1951), with bovine serum albumin (Boehringer Mannheim, Germany) as a protein standard.

### *Antibody preparation*

Polyclonal antibodies against ostrinin were raised in a mouse and a rabbit. About 1 μg of the purified ostrinin was emulsified with Freund's complete adjuvant (FCA) and injected intraperitonealy into a mouse. After three weeks, 2 μg of protein were emulsified with Freund's incomplete adjuvant (FIA) and injected intraperitonealy. After three weeks, second and third booster injections were repeated with 5 μg and 125 μg of protein, respectively. Both were injected subcutaneously at three-week intervals. Ascites fluid was collected three weeks after the last injection, centrifuged, and the remaining fluid was divided into aliquots and than stored at –20°C until use.

About 10 μg of the purified ostrinin was emulsified with equal volume of FCA and injected into several positions on the back of a rabbit, followed by two booster injections at three week intervals with 15 μg and 20 μg of protein in FIA, respectively. The rabbit was bled two weeks after the last injection and the obtained serum was divided into aliquots and stored at –20°C until use.

*Immunoelectrophoresis*

Immunoelectrophoresis was performed on microscope slides coated with 1% agarose prepared in Michaelis buffer, pH 8.2. Electrophoresis was performed on CAMAG apparatus (Muttenz, Switzerland) within 60 min, at a constant voltage of 220 V. After electrophoresis, antiserum or ascites fluid was placed in a channel and slides were incubated overnight at 4°C to allow the formation of the precipitation arcs. Afterwards, gels were incubated in 1% NaCl for 12 hours, then stained with 0.5% Amido Black dissolved in methanol:acetic acid (9:1) solution and destained in the same solvent for maximum contrast (J o h n s t o n e and T h o r p e, 1982).

## RESULTS

After several injections of the pure ostrinin into a mouse, specific polyclonal antibodies against this storage protein were detected in the ascites fluid. This was confirmed by immunoelectrophoresis, where only one precipitation arc was formed (Figure 1).

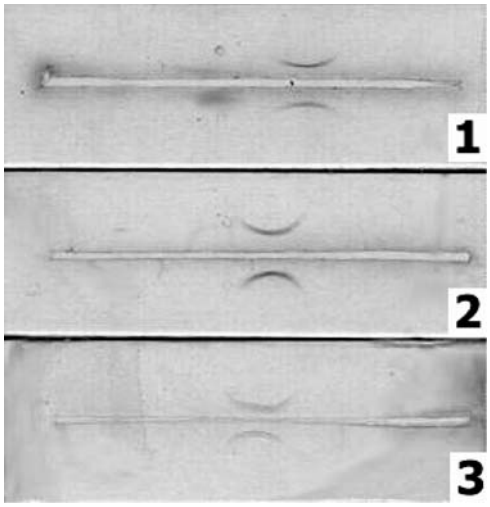


Figure 1. — Immunoelectrophoresis of the pure ostrinin to the mouse ascites fluid containing antibodies against ostrinin. (1) dilution of ascites fluid 1:1; (2) dilution of ascites fluid 1:10; (3) dilution of ascites fluid 1:100

The same immunological reaction was observed upon immunoelectrophoresis of the whole larval hemolymph, indicating the specific affinity of these antibodies against ostrinin (Figure 2).

Antiserum against ostrinin was obtained from a rabbit, and the presence of polyclonal antibodies was analyzed by immunoelectrophoresis. As shown in Figure 3, only one precipitation arc was observed.

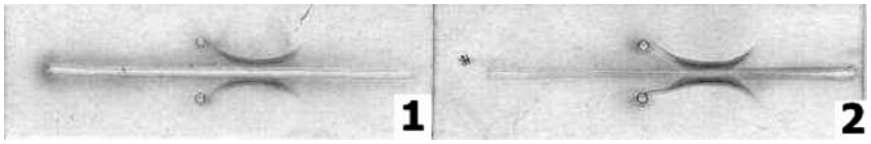


Figure 2. — Immunoelectrophoresis of the larval hemolymph of *O. nubilalis* to the mouse ascites fluid containing antibodies against ostrinin. (1) dilution of ascites fluid 1:1; (2) dilution of ascites fluid 1:10

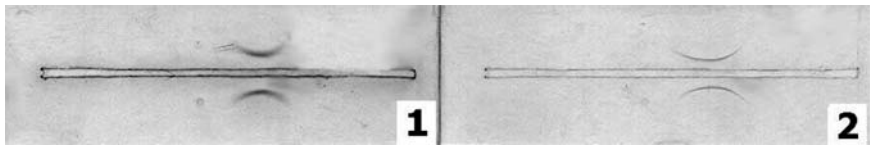


Figure 3. — Immunoelectrophoresis of the pure ostrinin to the rabbit antiserum against ostrinin. (1) dilution of antiserum 1:1; (2) dilution of antiserum 1:10

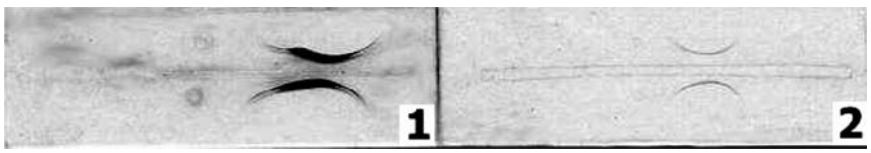


Figure 4. — Immunoelectrophoresis of the larval hemolymph of *O. nubilalis* to the rabbit antiserum against ostrinin. (1) dilution of antiserum 1:1; (2) dilution of antiserum 1:10

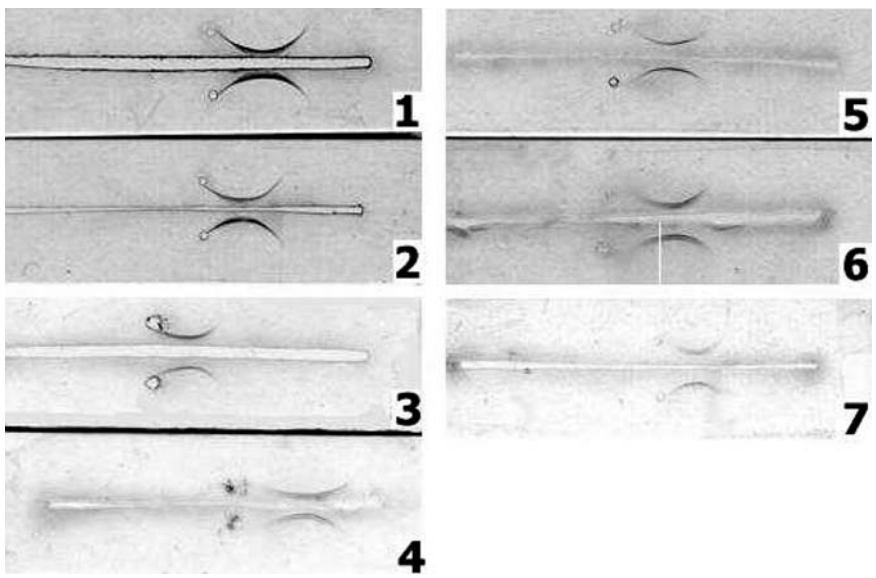


Figure 5. — Immunoelectrophoresis of *O. nubilalis* samples to the mouse ascites fluid containing antibodies against ostrinin. (1) hemolymph DL; (2) hemolymph NDL; (3) fat body DL; (4) fat body NDL; (5) pupae derived from NDL; (6) pupae derived from DL; (7) adults. DL = diapausing larvae; NDL = nondiapausing larvae

Only one precipitation arc was also formed upon immunological reaction of the whole larval hemolymph with antiserum against ostrinin, confirming the specific affinity of this antiserum towards ostrinin (Figure 4).

Using polyclonal antibodies against ostrinin, we analyzed the presence of this protein in different stages of the life cycle of *O. nubilalis*. As shown in Figure 5, ostrinin was present in all life stages of *O. nubilalis* (diapausing and nondiapausing larvae, pupae and adults), except in the eggs.

## DISCUSSION

During the last larval instar, many insects synthesize in the fat body and secrete into the hemolymph large amounts of proteins with high molecular weight (approximately 500 kDa). It is thought that these proteins primarily act as a source of amino acids and therefore are categorized as storage proteins. Previously, we have identified four major, high molecular weight proteins (named P1, P2, P3 and P4) in the hemolymph of the fifth-instar diapausing larvae of *Ostrinia nubilalis* (unpublished data). Based on their physico-chemical and immunological properties we concluded that P2 and P3 were methionine-rich, while P1 did not belong to the group of storage proteins. Based on its mobility in native- and SDS-PAGE and its affinity to antibodies against storage proteins of *Spodoptera litura* (gift from prof. Tojo), we identified P4 as arylphorin-type protein and named it ostrinin, in accordance with the suggestion of Thomson (1975) that storage proteins should be named after the species in which they were found.

In the present study, we analyzed the presence of ostrinin in the eggs, hemolymph and fat body of diapausing and nondiapausing larvae, in pupae and adults of *O. nubilalis*. Polyclonal antibodies against ostrinin, raised in a mouse and a rabbit, were used for immunological analysis. The presence and specific affinity of antibodies from the ascites fluid were tested with the pure ostrinin (Figure 1) and with the whole hemolymph (Figure 2). Only one precipitation arc was formed in each case, confirming good immunological properties of the obtained polyclonal antibodies. Clearly visible precipitation arc was detected even with 100-fold dilution of ascites fluid (Figure 1), which indicates a good titer of antibodies. Antiserum with polyclonal antibodies against ostrinin, obtained from a rabbit, was also tested with the pure ostrinin (Figure 3), as well as with the whole larval hemolymph (Figure 4), and only one precipitation arc was formed upon immunological reaction, confirming the specific affinity of this antiserum towards ostrinin. As shown in Figure 3, clearly visible precipitation arc was formed with 10-fold dilution of rabbit antiserum, indicating the lower titer of antibodies when compared with the mouse ascites fluid. Therefore, we have decided to use the ascites fluid for further analyses. We analyzed the presence of ostrinin at different stages of the life cycle of *O. nubilalis*. Our results revealed that ostrinin was present in all life stages, except in the eggs (Figure 5). These results are in accordance with the observations of other authors that storage proteins usually appear first in different

larval stages. In *Hyphantria cunea*, two storage proteins were observed to appear for the first time in hemolymph and fat body in the last instar larvae (Song et al., 1997). Furthermore, only very low levels of two methionine-rich storage proteins were present in the second instar larvae of *Plodia interpunctella*, but both mRNAs dramatically increased during the third instar, and peaked in the fourth/last instar (Zhu et al., 2002). In *Drosophila melanogaster* (Roberts et al., 1991) and *Calliphora vicina* (Levenbook and Bauer, 1984), concentration of the larval hemolymph protein increased just before pupation and than decreases in the pupal stage, whereas it was maintained at a low level during the adult stage. Moreover, similar developmental changes were observed for two storage proteins of *Bombyx mori* (Tojo et al., 1980). Until recently, the role of amino acid storage in adult forms of *Lepidoptera* has been largely overlooked. The recent results of Telang et al. (2002) show that both male and female pharate adults of *Heliothis virescens* retained a portion of total storage protein levels, although females retained greater levels overall. In females, post-eclosion protein reserves are likely used toward egg manufacturing, while the role of protein reserves in males remains speculative. Storage protein retention has also been observed in *Plutella xylostella* adults (Wheeler et al., 2000), while arylphorin was found to be effective in providing amino acids for vitellogenesis and chorion formation in pharate adult *Actias luna* moths (Pan and Telfer, 1996).

In conclusion, this study revealed a continuous presence of ostrinin, an arylphorin-type storage protein, during the life cycle and metamorphosis of *O. nubilalis*. Future work should reveal whether ostrinin possesses a specific role in regulation of complex metabolic processes involved in metamorphosis and egg production.

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## ПРИСУСТВО РЕЗЕРВНОГ ПРОТЕИНА ТИПА АРИЛФОРИНА У РАЗЛИЧИТИМ СТАДИЈУМИМА РАЗВИЋА *OSTRINIA NUBILALIS (LEPIDOPTERA: PYRALIDAE)*

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Резиме

Сматра се да код инсеката важну улогу у погледу обезбеђивања резерви аминокиселина имају специфични протеини из хемолимфе, који се називају резервни протеини. Резервни протеини типа арилфорина су широко распрострањени код инсеката и представљају главне протеине у ларвалној хемолимфи. Ови

протеини су богати арил-групама, те представљају извор ароматичних аминокиселина за синтезу протеина током диференцијације органа и развића адулта. У овом раду је изолован резервни протеин типа арилфорина из хемолимфе ларви кукурузног пламенца, *Ostrinia nubilalis (Lepidoptera: Pyralidae)*, и назван је остринин. Присуство овог протеина кроз различите стадијуме животног циклуса *O. nubilalis* је анализирано употребом поликлонских антитела на остринин добијених имунизацијом миша и зеца. Резултати истраживања су показали да је остринин присутан у свим стадијумима развића кукурузног пламенца (дијапаузирајуће и недијапаузирајуће гусенице, лутке и адулти), изузев у јајима.

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# ALLOZYME PATTERN FOR NEW RECORD OF *CRASPEDACUSTA SOWERBII* IN SERBIA

ABSTRACT: Cosmopolitan freshwater jellyfish *Craspedacusta sowerbii* Lankester, 1880 (*Cnidaria, Hydrozoa*) was recorded for the first time in the lake Velika peskara near Zrenjanin (Serbia and Montenegro) in summer 1998. A natural population of *C. sowerbii* from the lake Velika peskara was analyzed for genetic variability at 9 enzyme loci (*Gpi, Hk, Idh-1, Idh-2, Me, Mdh-1, Mdh-2, Pgm* and *Sod*) by polyacrylamide gel electrophoresis. A zymogram indicated that population was monomorphic at all analyzed loci.

KEY WORDS: allozymes, *Craspedacusta sowerbii*, freshwater jellyfish, polyacrylamide gel electrophoresis

## INTRODUCTION

Several species of inland water medusae have been described from locations all over the world. Freshwater species are widespread in most tropical-subtropical areas. In temperate areas they are found only in summer (Dumont, 1994). All freshwater medusa species (except *Halmomises*) belong to the family *Olindiidae (Hydroidomedusa, Limnomedusae). Craspedacusta sowerbii* Lankester, 1880 is the only species from the genus *Craspedacusta* described as cosmopolitan while the others are restricted to East Asia. The life cycle of *C. sowerbii* includes both a polyp stage and a free-swimming, sexually reproducing medusa stage. The polyp stage is solitary, or it forms small reptant colonies of 2—4, rarely 7 members (Bouillon and Boero, 2000). Like most *Cnidaria*, *C. sowerbii* is an opportunistic predator. Both polyps and medusae feed on various zooplankton taxa (DeVries, 1992; Dumont, 1994).

The objective of this study was to analyze the genetic variability of *C. sowerbii* population from the lake Velika peskara using allozyme electrophoresis.

## MATERIAL AND METHODS

A natural population (26 specimens) of *Craspedacusta sowerbii* was collected in mid-August 1998 from the lake Velika peskara near Zrenjanin, Serbia and Montenegro (Figure 1). Genetic variation was studied by standard 5%

Figure 1. The lake Velika peskara near Zrenjanin (Serbia and Montenegro)

polyacrylamide gel electrophoresis (M u n s t e r m a n n, 1979) with slight modifications (M i l a n k o v, 2001). Tris-Boric-EDTA (pH 8.9) buffer was used to assay glucosephosphate isomerase (5.3.1.9; GPI), hexokinase (2.7.1.1; HK), malic enzyme (1.1.1.40; ME), phosphoglucomutase (2.7.5.1; PGM) and superoxide dismutase (1.15.1.1; SOD). Tris-Citric (pH 7.1) buffer was used to assay isocitrate dehydrogenase (1.1.1.42; IDH, two loci: *Idh-1, Idh-2*) and malate dehydrogenase (1.1.1.37; MDH, two loci: *Mdh-1, Mdh-2*). Loci were numbered and alleles marked alphabetically with respect to increasing anodal migration.

## RESULTS AND DISCUSSION

Allozyme electrophoresis has been used to delineate morphologically similar marine taxa within the superclass *Hydrozoa*. A population-genetic analysis of 14 polymorphic loci in populations of *Aurelia* species from four distinct sites revealed the presence of fixed alleles at seven loci (*Idh-1, Idh-2, Pnp, Sod-1, Sod-2, Est-1* and *Est-2*). Further supported by significant fre-

quency differences at the *Cat, Estd, Peplgg, Gtdh* and *Peplp* loci, this has been compelling evidence that each *Aurelia* group is a distinct species (G r e e n b e r g et al., 1996). Analysis of gene-enzyme variability of the *Lap, Est* and *Acp* enzyme systems, and the application of electrophoretic data helped to elucidate the status of and relationships among 7 hydroid species from the family *Campanulariidae* (O s t m a n, 1982).

The analysis of allozyme variability at the *Gpi, Hk, Idh-1, Idh-2, Me, Mdh-1, Mdh-2, Pgm* and *Sod* loci in the natural population of *C. sowerbii* (Figure 2) from Velika peskara revealed the presence of only one allele at each

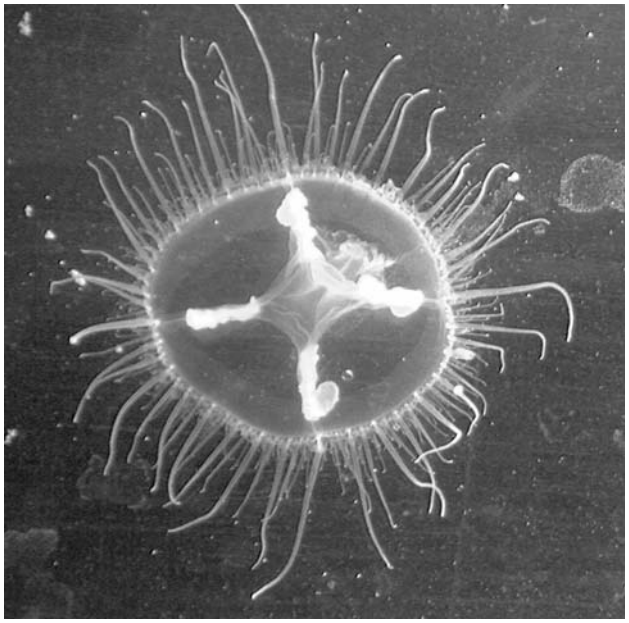

Figure 2. An adult specimen of *Craspedacusta sowerbii* L a n k e s t e r, 1880 from the lake Velika peskara near Zrenjanin

locus. Monomorphic loci *Idh-1, Idh-2, Mdh-1, Mdh-2*, and *Sod* were also registered in all analyzed populations of *Aurelia* sp., which corresponds to the results in this study. Absence of heterozygotes was observed at all enzyme loci analyzed for *C. sowerbii* that were included in the gene-enzyme variability analysis of the aquarium-cultured population of *Aurelia* sp. from Japan. This population of *Aurelia* sp. was heterozygous only at *Pnp* and *Estd* loci that were not assayed in *C. sowerbii*. Because of inconsistent activity, *Hk*, *Pgm*, and *Mdph-2* enzyme loci were not included in the analyses of *Aurelia* sp. ( G r e e n b e r g et al., 1996), while distinct banding patterns were recorded for *C. sowerbii*.

Possible reasons for the absence of genetic variability in the analyzed population are probably correlated with the pattern of its migration and reproduction. More than 120 years ago *C. sowerbii* spread from South America to all parts of the world except Antarctic, by passive dispersal (aquatic plants, fish,

human activity) (Dumont, 1994; Boothroyd et al., 2002). The species *C. sowerbii* has been registered only in a few locations in Serbia: in a pond by the Morava River near Ćuprija (Grozdanović and Manojlović, 1958), in the Danube river near Novi Sad, in the lake Sava in Belgrade (Kalafatić et al., 1999), and in the lakes Velika peskara and Mala peskara near Zrenjanin (Radišić, personal communication). Formed at the site of an old sand pit, the lake Velika peskara is a recent (less than 20 years old) artificial water basin. It is probable that *C. sowerbii* was introduced to this site, since it has no natural connections with other locations. Also, predominance of a single sex was regular in the populations of the analyzed species (Pannek, 1956; according to Kalafatić et al., 1999). This unisexuality is believed to result from the small size (possible one resting stage) of the introduced propagules (Dumont, 1994). In addition, since the life cycle of *C. sowerbii*, like most cnidarians, includes asexual reproduction, a possibility of sampling clones within the populations must be considerable. Specimens of the Japanese population of *Aurelia* from aquarium culture were identical at all loci, indicating that they were clonemates (Greenberg et al., 1996). Finally, based on the above data, it can be assumed that genetically identical medusae of the analyzed population of *C. sowerbii* are the first generation, originating by budding of polyps recently introduced to the lake Velika peskara.

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## АЛОЗИМСКА АНАЛИЗА НОВОГ НАЛАЗА ВРСТЕ *CRASPEDACUSTA SOWERBII* У СРБИЈИ

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Резиме

Космополитска слатководна медуза *Craspedacusta sowerbii* L a n k e s t e r, 1880 (*Cnidaria*, *Hydrozoa*) регистрована је први пут у вештачком језеру Велика пескара код Зрењанина (Србија и Црна Гора) у лето 1998. Природна популација врсте *C. sowerbii* из језера Велика пескара проучена је методом полиакриламид гел електрофорезе и притом је анализирана генетичка варијабилност 9 ензимских локуса (*Gpi, Hk, Idh-1, Idh-2, Me, Mdh-1, Mdh-2, Pgm* и *Sod*). Тумачењем зимограма утврђена је мономорфност свих анализираних локуса популације. Одсуство генетичке варијабилности у анализираној популацији вероватно је резултат пасивне дисперзије и смене полног и бесполног размножавања врсте *C. sowerbii*, те је могуће да су генетички идентичне јединке прва генерација медуза насталих пупљењем полипа који су недавно интродуковани у језеро Велика пескара.

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# BIOENERGETIC ASPECTS OF COMPETITION BETWEEN NITROGEN METABOLISM AND CARBOHYDRATE SYNTHESIS IN SMALL GRAINS

ABSTRACT: Yields of small grains have been significantly increased in recent decades, primarily by breeding. No corresponding progress has been made regarding protein content in the grain. That was mostly due to a negative correlation existing between yield level and protein content. Protein synthesis requires a much larger amount of energy that that required for carbohydrate synthesis. A hypothesis explains this negative correlation by competition for energy between nitrogen metabolism and carbohydrate synthesis. The proponents of this hypothesis surmise that nitrate reduction unfolds mostly at the expense of the energy released upon oxidation of carbohydrates and organic acids (heterotrophic), and not at the expense of light energy (autotrophic). In small grains, nitrate reduction takes place mostly in leaves, i. e., it is autotrophic. Under conditions of optimum nitrogen supply, protein content and yield per unit area increase but grain yield does not decrease although it could be expected if there existed a competition for energy between nitrogen metabolism and carbohydrate synthesis. Protein content reduction in consequence to yield increase could be explained by the dilution effect, as evidenced by similar reductions of other substances in the grain, for example mineral elements. When considering the competition for energy between nitrogen metabolism and carbohydrate synthesis, it should not be overlooked that nitrogen stimulates the energy transport in plants indirectly, by stimulating the photosynthetic activity and thus the utilization of light energy by plants.

KEY WORDS: bioenergy, protein content, yield

## INTRODUCTION

In the course of seed filling, reserve substances needed for the heterotrophic stage in plant life, i. e., for germination, are accumulated. Carbohydrates, proteins and oils are most frequent reserve substances in seed. Depending on the prevalence of one of these three groups of organic substances, plants can be divided into those predominantly accumulating carbohydrates (corn, wheat, rice), proteins (soybean, bean) and oil (sunflower, flax). There are many transitional plants among these three main groups (K a s t o r i, 1984). The different reserve substances in seed require different amounts of energy to synthesize.

The energy released by the oxidation of 1 g of glucose may synthesize 0.8 g of starch but only 0.4 g of proteins. In other words, protein synthesis requires much more energy than carbohydrate synthesis (Penning de Vries et al., 1974). Competition for energy required for carbohydrate and protein synthesis could be a limiting factor for protein synthesis and a reason for negative correlation between yield level and protein content (Bhatia and Rabson, 1976).

Numerous studies have reported the existence of negative correlation between grain yield and protein content in grain in different varieties of small grains (Fossati et al., 1993; Peltonen-Sainio and Peltonen, 1994; Stoddard and Marshall, 1990; Mächler et al., 1988; Feil, 1997) and, in most cases, the existence of positive correlation between grain yield and protein yield (Simmonds, 1995). Old varieties of small grains typically had a higher protein content than the new, higher yielding ones. This reduction in protein content was due to the dilution effect or genetic, ecological or physiological reasons. Unfavorable weather conditions may reduce 1000-seed mass. This reduction is a consequence of lower starch accumulation. Simultaneously, protein content increases but this protein yield is lower than that obtained under optimum conditions of grain forming and filling (Bálint, 1977). In this connection, the question is raised to which extent is the competition between nitrogen assimilation for protein content and carbohydrate content based on the competition for energy.

### *Bioenergetic aspects of nitrate assimilation*

Plants take up nitrogen predominantly in the form of nitrate and ammonium ions. In the soil with favorable chemical and physical properties, nitrification is quite intensive. This is why plants take the largest part of nitrogen in the nitrate form. The physiological values of nitrate and ammonium ions are more or less equal in most plants; however, they need different amounts of energy to take part in plant metabolism. While ammonium ions join directly the biosynthesis of amino acids, i. e., proteins, nitrate ions must first be reduced, which requires energy. The extent to which nitrate reduction burdens the plant energy balance depends on the plant part in which the reduction takes place. In chlorophyll-containing tissues, reduction equivalents are provided by the light phase of photosynthesis, $FS_I$, i. e., by reduced ferredoxin (nitrite reduction)

$$NO_3^- + 2e^- + 2H^+ \xrightarrow{\text{nitrate-reductase}} NO_2^- + H_2O,$$

$$NO_2^- + 6e^- + 6H^+ \xrightarrow{\text{nitrite-reductase}} NH_3 + H_2O + OH^-$$

This autotrophic system of nitrate reduction uses small amounts of energy, because, among other things, there is no energy loss on photorespiration (Schrader and Thomas, 1981). If a plant receives sufficient light and carbon dioxide assimilation is moderate, there occurs an excess photochemical

energy. The expenditure of this energy, which fuels a reaction taking place in chloroplasts, i. e., the nitrite reduction to glutamate, is not too exacting for the plant. Opinions have been voiced that, under such conditions, even the nitrate reduction taking place in the cytoplasm does not overtax the plant energy balance (Miflin, 1980).

Nitrate reduction also takes place in tissues that do not contain chlorophyll, for example in those that make the root. The required energy, i. e., the reduction equivalents needed for this heterotrophic nitrate reduction provides the oxidation of organic acids and carbohydrates. The energy needed for heterotrophic nitrate reduction is approximately double that needed for autotrophic reduction. It follows from this that, from the point of energy consumption, it matters in which plant part nitrate reduction takes place. Nitrate-reductase distribution differs among plant species (Kastori and Petrović, 2003). In most plants, nitrate reduction occurs in the root and the aboveground plant part. Only a limited number of plant species has this process occurring exclusively in the root or in the aboveground part. According to Andrews (1986), in small grains grown in a moderate climate, as much as 90% of the absorbed nitrates are reduced in the aboveground part, i. e., in the leaves that receive maximum light. The results of Petrović and Kastori (1991) showed that in wheat the intensity of nitrate reduction was higher in the aboveground part than in the root. With increased access to light, nitrate-reductase activity increases more in the aboveground part than in the root. Effect of light on nitrate-reductase activity depends on plant provision with nitrates and genotype. The effect of light is more pronounced when the plant is abundantly supplied with nitrates. This strategy regarding the site of nitrate reduction allows the plant to efficiently utilize light energy for nitrate reduction, which improves the energy balance in the plant. Under the conditions of intermediate nitrate supply, the intensity of nitrate reduction is much higher in the aboveground part than in the root. High or low nitrate concentrations in the nutritive medium lower the difference in intensity of nitrate reduction between the aboveground part and the root. In the same plant species, the rate of nitrate reduction in the root increases together with the increase in ambient temperature. These and other research results show that numerous ecological factors affect the site of nitrate reduction. They also highlight the importance of nitrate-reductase in nitrate assimilation (Simmonis and Moss, 1978; Kastori and Petrović, 2003).

Generally speaking, under the conditions of inadequate energy supply, competition for energy needed for carbon dioxide fixation and nitrate reduction is more favorable in the root. The time of most intensive reduction of nitrates and assimilation of carbon dioxide in the course of the day and the site of these processes in the plant tend to overlap. Adequate light supply during midday probably supplies sufficient energy for both, nitrate reduction and carbon dioxide assimilation, mitigating the competition for energy required for the synthesis of carbohydrates and nitrogen metabolism.

*Relationships among protein content, carbohydrate content and yield level*

Numerous studies have indicated that the metabolisms of carbohydrates and nitrogen are mutually related (Izmajlov and Smirnov, 1985). Opinions are contrasted regarding the competition for energy between nitrogen metabolism and carbohydrate synthesis (Feil, 1998). According to Bhatia and Rabson (1976), the negative correlation between grain yield and protein concentration in grain proves that such competition does exist. The authors who are in favor of the hypothesis on the competition between nitrogen metabolism and carbohydrate synthesis start from the assumption that nitrate reduction unfolds primarily at the expense of the energy obtained from the decomposition of carbohydrates and organic acids. This is not the case with cereals, since they reduce nitrates predominantly in leaves, mostly at the expense of light energy.

Adequate plant supply with nitrates not only increases energy consumption but also, indirectly, increases energy production. Nitrogen increases chlorophyll content, leaf area and duration and intensity of photosynthesis, which in turn increase the synthesis of carbohydrates (Bálint, 1977; Sztanev, 1981). Additionally, a significant portion of the acquired nitrogen accumulates in RuBP-carboxylase and PEP-carboxylase; the contents of these enzymes increase simultaneously with the increase in plant provision with nitrogen. The chloroplasts of the $C_3$ plants accumulate significant amount of nitrogen in the form of rubisco (Millard, 1988). Rubisco is considered the major protein in chloroplasts' stromata. In most plant species, its concentration in chloroplasts exceeds 50% of the total protein content in leaves (Edwards and Walker, 1983). In that way, under the conditions of adequate nitrogen supply, the increased energy requirement needed for nitrogen metabolism is compensated for a greater part, or even an excess energy is produced which may be used for the synthesis of other compounds.

Numerous research results have indicated that only high nitrogen doses affect protein content; low doses primarily affect yield level (Kastori, 1967). Protein content in grain of plants insufficiently provided with nitrogen is sometimes higher than that in plants that received optimum nitrogen nutrition. This apparent contradiction ensues from the fact that, under optimum nitrogen nutrition, photosynthetic activity in plants is increased, which in turn intensifies the production of photosynthates used for grain growth. Under such conditions, although plants take up a larger amount of nitrogen, the relative protein content may be lower because of increased organic matter production, i. e., because of larger dilution. This explains why low and medium doses of nitrogen fertilizer have no effect on protein content. Protein content will increase only if the dose of nitrogen fertilizer is so high that nitrogen compounds accumulate in plant tissues in spite of increased production of organic matter.

In support to this explanation of nitrogen effect on protein content goes the fact that in dry years, in which the nitrogen uptake and production of organic matter are considerably reduced because of insufficient water supply, the

protein content in grain is invariably higher than in humid years in which the production of organic matter is increased.

It has been established that excessive nitrogen nutrition reduces the content of carbohydrates. K a s t o r i (1964) found that corn fertilization with 200 kg N/ha reduced the contents of starch and oil in grain by 2.44% and 0.12%, respectively, while the yield of grain increased instead of going down. This was an indication that in the case of excessive nitrogen nutrition, the major part of the primary photosynthetic products is used for the synthesis of nitrogen compounds, i. e., amino acids, at the expense of the synthesis of carbohydrates. H e h l and M e n g e l (1972) found that in the English ryegrass the increase in ammonium nitrate dose resulted in increases of dry matter and nitrogen content and decreases of the contents of starch, sucrose and polyfructosane. These results too indicated that increased nitrogen supply caused the different metabolic processes to compete for photosynthates. Since a significant yield increase occurred subsequently instead of yield reduction, the reduction in carbohydrate content could not be attributed to the competition for energy between nitrogen metabolism and carbohydrate synthesis. If the competition for light energy between nitrogen metabolism and carbohydrate synthesis had been high, the yield would have been reduced in the case of excessive nitrogen nutrition and the related increase in protein synthesis. This, however, was not the case (B ä z i n g e r et al., 1994; Z e r u l l a and K n i t t e l, 1988). All this seems to indicate that the negative correlation between yield level and protein content does not have a bioenergetic background but that it is primarily due to the dilution effect.

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# БИОЕНЕРГЕТСКИ АСПЕКТИ КОНКУРЕНЦИЈЕ МЕТАБОЛИЗМА АЗОТА И СИНТЕЗЕ УГЉЕНИХ ХИДРАТА У ЖИТА

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Резиме

Најзначајније резервне материје у зрну жита су пре свега угљени хидрати (скроб) и протеини. Садржај протеина у великој мери одређује хранидбену и технолошку вредност зрна жита. Између висине приноса и концентрације протеина у зрну генотипова жита обично је негативна корелација. Повећање генетског потенцијала приноса у већини случајева довело је до смањења концентрације протеина у зрну и повећање приноса протеина по јединици површине. За асимилацију азота т. ј. синтезу протеина потребна је већа количина енергије него за синтезу угљених хидрата. С тим у вези поставља се питање да ли је разлог смањењу концентрације протеина при повећању приноса конкуренција за енергијом или је то само ефекат разблажења. Обилна исхрана азотом повећава концентрацију протеина, али истовремено утиче на морфолошке и физиолошке параметре биљака које омогућавају ефикасније искоришћавање светлосне енергије. Да је конкуренција за светлосном енергијом између метаболизма азота и синтезе угљених хидрата велика, принос би, у случају обилне исхране азотом и с тим у вези повећане синтезе протеина, морао да се смањи. То, међутим, није случај, што указује да негативна корелација између приноса и садржаја протеина нема биоенергетску основу, него је то пре свега ефекат разблажења. На то указује и чињеница да се са повећањем приноса смањује и садржај других материја у зрну, на пример концентрација минералних материја.

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# GENETIC VARIABILITY OF MAIZE BREEDING MATERIAL (*Zea mays* L.)

ABSTRACT: A study has been conducted in order to assess genetic variability in three maize populations, which are part of breeding material of Institute of Field and Vegetable Crops in Novi Sad. Variability has been analyzed for seven enzymes. PGM enzyme was monomorphic while enzymes ACP, GLU, IDH, MDH, PHI and PGD had from 2 to 5 alleles. In the total sample which consisted of 228 lines, 34 alleles were found. The greatest variability was found in *Pgd1* and *Pgd2*. The average heterozygosities of loci per population were from 0.203 to 0.274, and polymorphism varied between 0.727 and 0.818.

The genetic distances and heterozigosities among the populations marked them as suitable material for selecting parental pairs in development of maize hybrids.

KEY WORDS: allelic variability, genetic variability, isozymes, populations

## INTRODUCTION

Genetic diversity plays a key role for future progress in maize breeding. The development of molecular markers provides a tool for assessing the genetic diversity at the DNA level in plant science. Information on the genetic diversity and relationships of lines or populations, and the choice of heterotic groups are fundamental in hybrid breeding of maize (Hallauer et al., 1988; Melchinger and Gumber, 1998). Knowledge of the genetic relationships among breeding materials could help to avoid great risk for an increasing uniformity in the elite germplasm, and could ensure long-term selection gains (Messmer et al., 1993).

Until a decade ago, trends in the genetic diversity within and between populations were mostly evaluated with morphological markers or biometric analyses of quantitative variation. Genetic distance based on molecular markers has been suggested as a tool for grouping of similar germplasms as a first step in identifying promising heterotic patterns (Melchinger, 1999). Genetic distances between genotypes are highly correlated with the distance based on known pedigrees, and the distance based on isozymes and RFLP are also

highly correlated with coefficient of parentage and with the distance between inbred lines (Smith and Smith, 1992). Inter-population variability and geographic origin of populations are important in heterotic breeding (Zlokolica et al., 2002).

Molecular markers allow direct comparison of the similarity of genotypes at the DNA level. The technique of random amplified polymorphic DNA (RAPD) (Williams et al., 1990) and SSR markers provide a powerful tool for grouping maize germplasm (Reif et al., 2003), and offer considerable advantages in speed and technical simplicity compared with RFLP and other methods.

The aim of this investigation was to compare the genetic variability and diversity of two populations originated from US Cornbelt and a local population on the basis of their genetic markers.

## MATERIALS AND METHODS

A total of 228 maize inbred lines from three populations with different origin were analyzed: Population 1 (81 lines), developed by crossing inbred lines from American population Iowa Stiff Stalk Synthetic (BSSS); Population 2 (94 lines), derived from Lancaster Sure Crop and population 3 (53 lines) made from the local maize populations (Vukovar yellow dent, Šid yellow dent, Novi Sad yellow dent, Novi Sad gold dent, etc.). All three populations were developed from the F1 generation using pedigree method of selection.

Genetic characters of the populations were assessed on the basis of isozymic genotypes belonging to seven enzymes (11 loci): acid phosphatase, β glucosidase, isocitrate dehydrogenase, malate dehydrogenase, phosphoglucomutase, 6-phosphogluconate dehydrogenase, phosphohexose isomerase. The analyses were done according to Stuber et al. (1988).

The tested materials were analysed for frequency of the detected alleles, polymorphism and heterozygosity of the loci and standard genetic distance (Nei, 1978). Genetic diversity between populations was determined by the cluster analysis according to Euclidean distances (Statistica software).

## RESULTS AND DISCUSSION

Populations with high genetic variability, besides other characteristics, are superior breeding material. Development of new maize lines depends on initial breeding material or, more precisely, on the variability present in the material (Mišević, 1986). Local populations as well as breeding material derived from them, have a great potential value in maize improvement as sources of desirable alleles. Sources of resistance to agents of major diseases and pests were found in Yugoslav populations of dents, semi dents and flints (Penčić et al., 1984). In total sample of 228 inbred lines 34 alleles were found, while the number of alleles found in individual populations ranged from 24 to 28. The number of detected allelic variants per locus ranged from 2 to 5. The ave-

rage heterozygosity per population ranged from 0.203 to 0.274, the average polymorphism was 76%. (Tables 1 and 2). Some of the tested enzimic loci were not polymorphic. The *Pgm 1* locus was monomorphic in all three tested populations, *Idh1* in populations 1 and 2 and *Pgd2* in populations 2 and 3. The greatest number of alleles was obtained for the locus for 6 phosphogluconate dehydrogenase (5).

Table 1. Frequencies of the alleles detected in maize populations

| Locus — allele | Frequency | | |
|---|---|---|---|
| | Population 1 | Population 2 | Population 3 |
| Acp1—2 | 0.39 | 0.70 | 0.55 |
| 3 | 0.29 | 0.09 | 0.03 |
| 4 | 0.32 | 0.20 | 0.42 |
| 6 | | 0.01 | |
| Glu1—2 | 0.15 | 0.04 | 0.07 |
| 7 | 0.85 | 0.94 | 0.90 |
| N | | 0.02 | 0.03 |
| Idh1—4 | 1.00 | 1.00 | 0.93 |
| N | | | 0.07 |
| Idh2—4 | 0.55 | 0.87 | 0.40 |
| 6 | 0.45 | 0.13 | 0.60 |
| Mdh1—1 | | 0.03 | 0.19 |
| 3 | | 0.01 | |
| 6 | 1.00 | 0.96 | 0.81 |
| Mdh2—3 | 0.53 | 0.04 | 0.30 |
| 3.5 | | 0.08 | 0.10 |
| 6 | 0.47 | 0.88 | 0.60 |
| Pgm1—9 | 1.00 | 1.00 | 1.00 |
| Pgm2—3 | 0.07 | | 0.15 |
| 4 | 0.93 | 0.86 | 0.85 |
| 8 | | 0.14 | |
| Pgd1—2 | 0.10 | 0.09 | 0.17 |
| 2.8 | 0.10 | | |
| 3.8 | 0.70 | 0.72 | 0.75 |
| N | 0.06 | 0.19 | 0.08 |
| 9 | 0.04 | | |
| Pgd2—2.8 | 0.03 | | |
| 5 | 0.84 | 1.00 | 1.00 |
| 6 | 0.09 | | |
| 8 | 0.03 | | |
| N | 0.01 | | |
| Phi1—2 | 0.01 | | |
| 4 | 0.90 | 0.70 | 0.92 |
| 5 | 0.09 | 0.30 | 0.08 |

Table 2. Heterozygosity of loci in maize populations

| Locus | Population 1 | Population 2 | Population 3 |
|---|---|---|---|
| *Acp1* | 0.661 | 0.462 | 0.520 |
| *Glu1* | 0.255 | 0.144 | 0.184 |
| *Idh1* | 0 | 0 | 0.130 |
| *Idh2* | 0.495 | 0.226 | 0.480 |
| *Mdh1* | 0 | 0.077 | 0.380 |
| *Mdh2* | 0.498 | 0.218 | 0.540 |
| *Pgm1* | 0 | 0 | 0 |
| *Pgm2* | 0.130 | 0.241 | 0.255 |
| *Pgd1* | 0.485 | 0.437 | 0.402 |
| *Pgd2* | 0.284 | 0 | 0 |
| *Phi1* | 0.182 | 0.420 | 0.147 |
| Average heterozygosity | 0.272 | 0.203 | 0.274 |

The average number of alleles per locus in different maize collections ranged from 2.3 in American hybrids, to 1.8 in Yugoslav, while in exotic germplasm it was much higher 4—4.5 (Doebley et al., 1983; Goodman and Stuber, 1983a, b). In tested populations number of alleles per locus ranged from 1 to 3.67, and average value calculated for all loci was 2.33. Heterozygosity per locus varied from 0.077 to 0.661.

The tested populations can be differentiated on the basis of presence or absence of certain alleles in loci, as well as calculated frequencies for individual alleles.

High frequency of alleles Pgm2—4, as well as approximated participation of alleles in locus *Acp1* are characteristic for population 1 derived from Lancaster population. Only one allele, Phi1—2, was found in locus Phi1. Alleles Pgd2—8 and Pgd1—9, which were not present in the other two populations, were found in the Pgd1 locus. Polymorphism of the Pgd2 locus in which 5 alleles were found was also characteristic for this population.

The results obtained by Smith et al. (1985) pointed out that Lancaster Sure Crop lines had fixed alleles: Pgd1—3.8, Glu1—7 and Idh2—4, while alleles Acp1—2, Acp1—3, Glu1—2 and Mdh2—3.5 were not present. The results obtained for population 2 originating from Lancaster Sure Crop lines showed that its genetic variability had been increased by breeding, and that new alleles occurred which could be sources of desirable genes for important agronomic traits.

Population 2 differed from the other two populations in high frequencies of alleles Idh2—4, as well as in allele Acp1—6 found in the Acp1 locus, which was not detected in the other two populations. A zero allele was found in the Idh1 locus only in population 3.

Analyzing maize populations, Gerić et al. (1989) found 5 alleles on the Pgd1 locus with high frequency of allele Pgd1—3.8, which indicated an increasing influence of northern flints on the Yugoslav commercial germplasm.

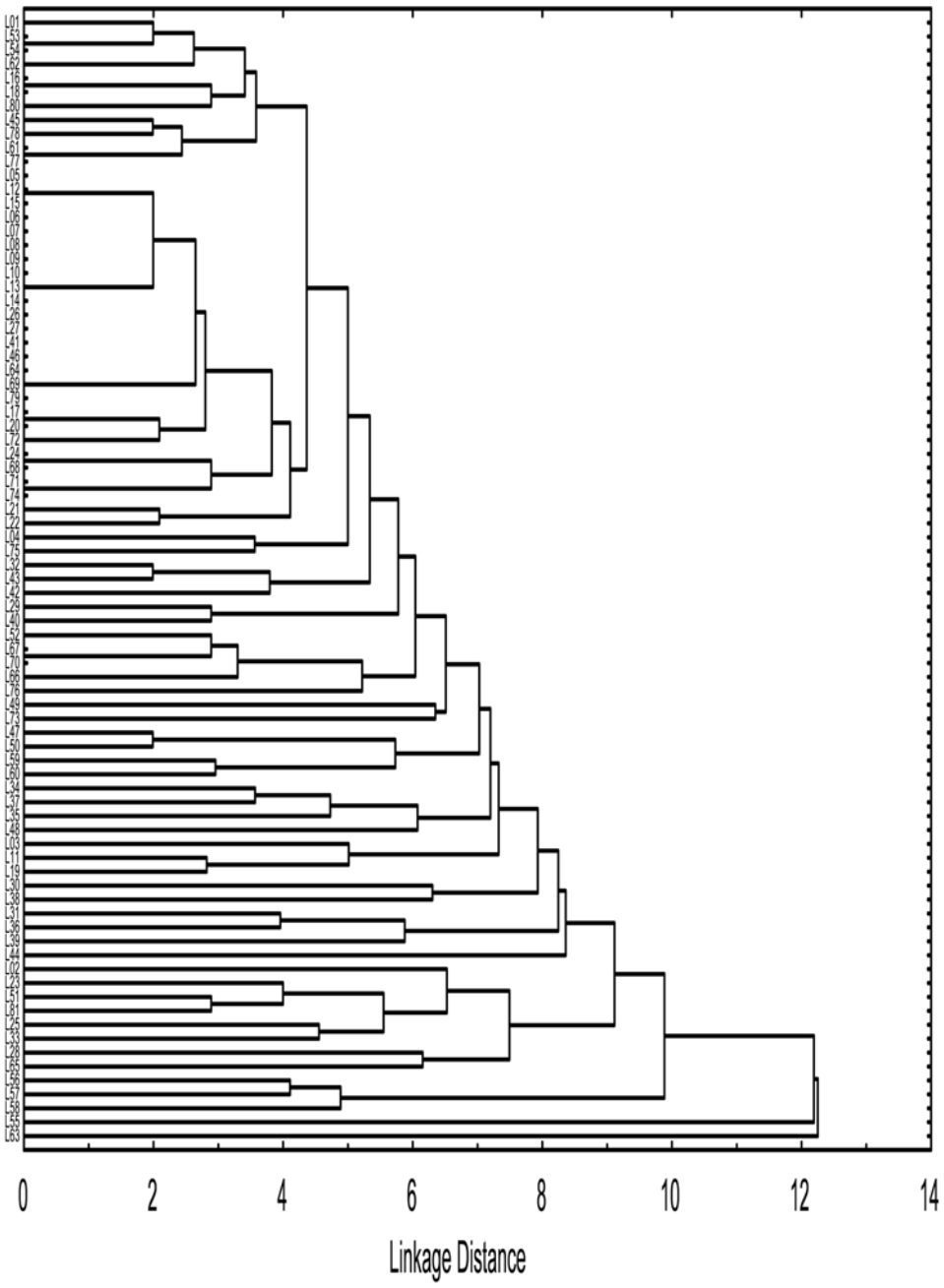


Figure 1. Claster analysis of corn population 1

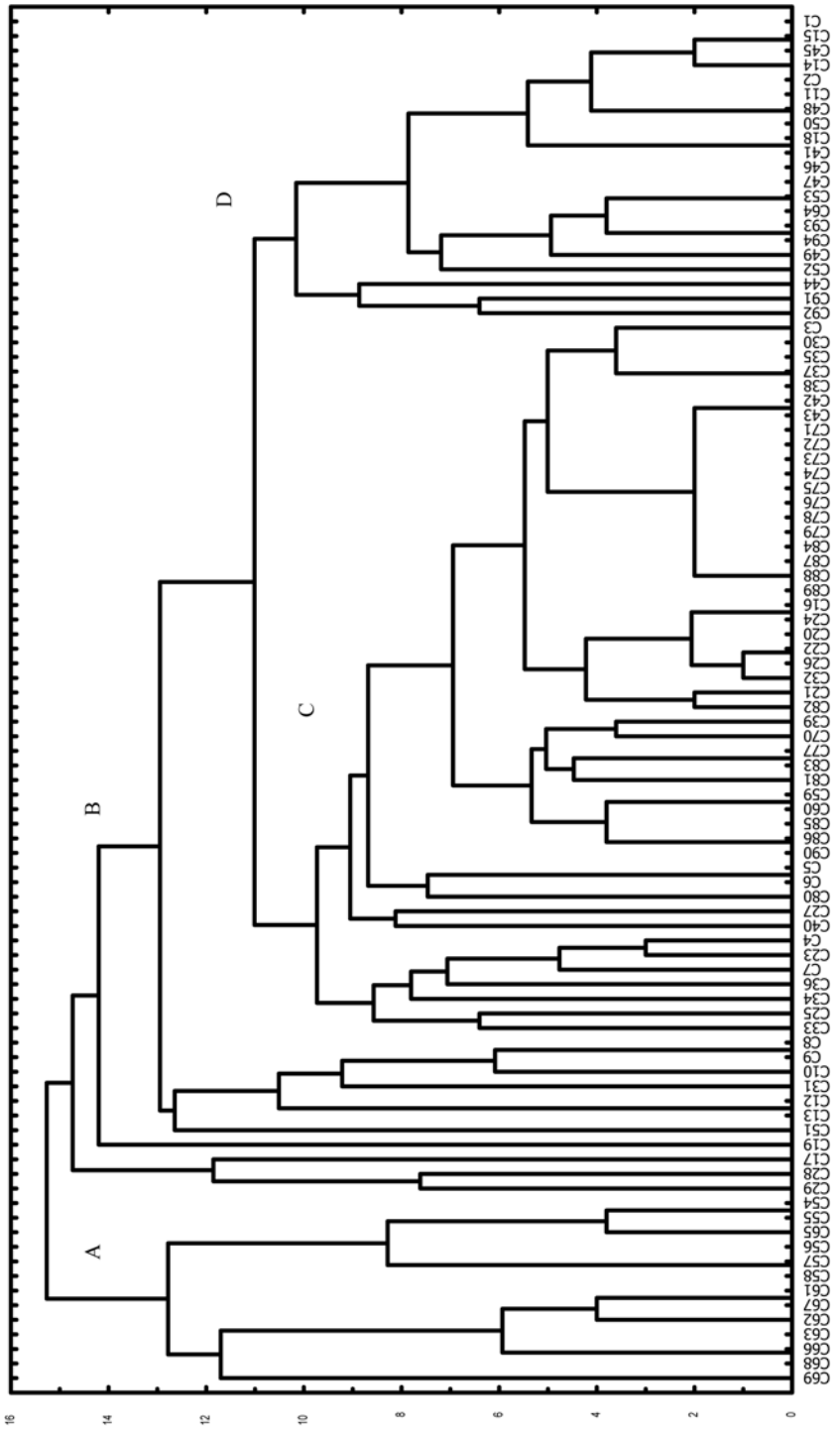


Figure 2. Cluster analysis of corn population 2

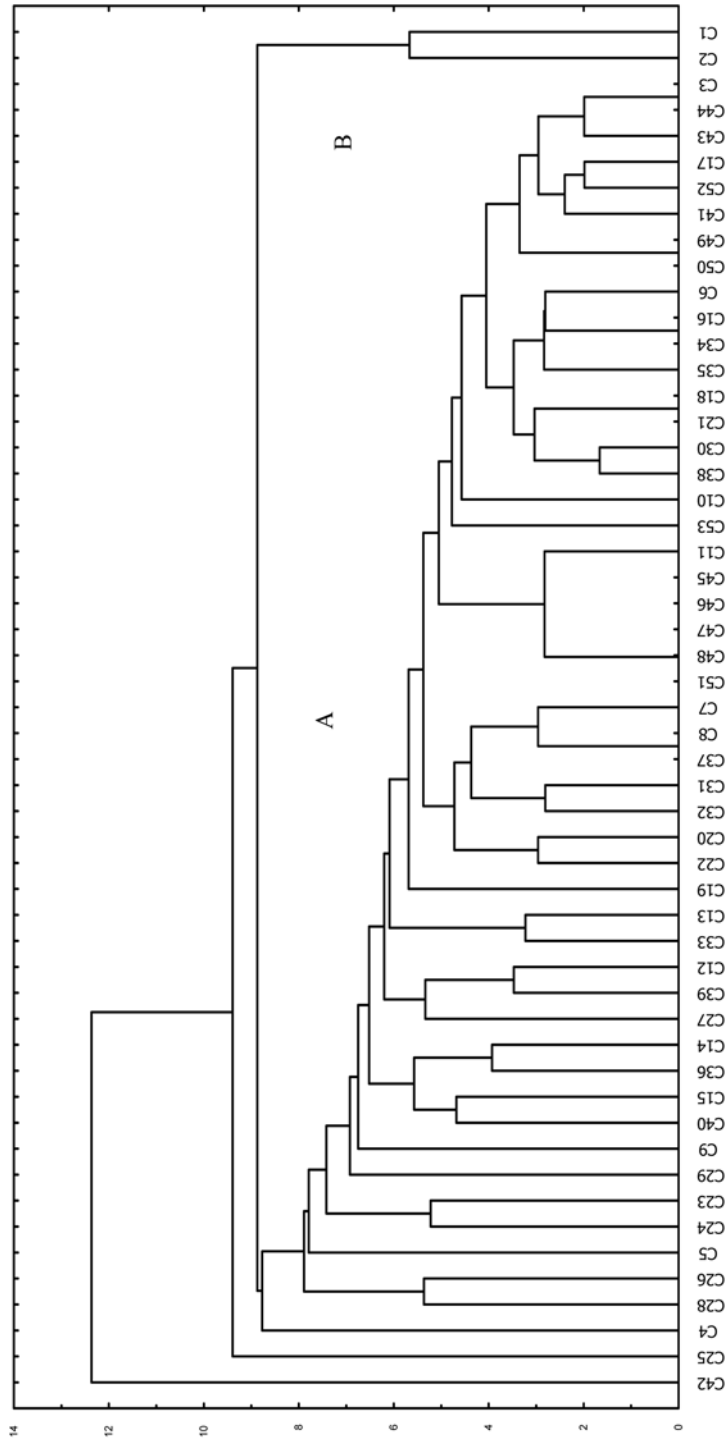


Figure 3. Cluster analysis of corn population 3

The similar frequency of alleles Pgd1—3.8 (0.70) was found in the three analyzed maize populations.

According to T i e et al. (1998), the *Phi* locus in original BSSS population was monomorphic, and the loci Idh1, Pgd1, and Pgd2 each had 3 alleles. In population 1 originating from BSSS, the number of alleles per locus was changed dy breeding, so the loci Pgd1 and Pgd2 had five, and Pgm1 had acquired three alleles.

The different numbers and participation of individual alleles in the populations pointed out their mutual distance and genetic heterogeneity.

The average heterozygosity in populations 1 and 3 was almost the same (0.272—0.274), while in population 2 it was somewhat lower (0.203). Polymorphism of loci in populations 1 and 2 was the same (0.727), and in population 3 it was somewhat higher (0.818).

In the dendrogram presenting mutual relations i. e. distance of inbred lines in population 1, three groups were found (A, B, C), which had uneven numbers of subgroups and lines (Figure 1). Group A consisting of 13 lines was linked to lines from group B at the distance of 4.2. Group C had the largest number of genotypes and a large number of sub-groups (14), which were linked at the distances from 5 to 10. Two indenpendent genotypes, lines L55 and L63, were linked to group C at the distance of 12.

In the dendrogram presenting mutual relations between lines in population 2, four groups (A, B, C and D) were differentiated (Figure 2). Group A consisting of two sub-groups, and group B consisting of linked lines were at the greatest distance of about 15.5. Group C consisting of the largest number of lines was linked to group D at the distance of 11.

In the dendrogram obtained for lines from population 3, two independent genotypes, lines 42 and 25, and two groups of lines (A and B) were distinguished (Figure 3). Line 42 was at the distance of approximately 12.5 from all other lines, while line 25 was at a somewhat smaller distance of 9.3. Group A consisted of a large number of sub-groups, and the lines were mutually linked. Group B consisted of only two lines (1 and 2) connected to group A at the distance of approximately 9.

## CONCLUSION

The obtained results could serve as a criterion for choosing parent lines of heterotic maize hybrids. The chosen lines, as sources of new genetic variability, can be used for the development of new populations. The isozyme analysis can be successfully applied to study the genetic variability, similarity and differences among maize populations.

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# ГЕНЕТСКА ВАРИЈАБИЛНОСТ СЕЛЕКЦИОНОГ МАТЕРИЈАЛА КУКУРУЗА (*ZEA MAYS* L.)

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Резиме

Изучавање варијабилности и дивергентности селекционог материјала је од значаја за оплемењивање и унапређење производње кукуруза јер се у процесима селекције сужава генетска варијабилност материјала и настаје потреба за проналажењем нових извора гена, као што су старе сорте, дивљи сродници и други генотипови који уносе генетску дивергентност. Варијабилност и дивергентност утврђена помоћу изоензима као генетских маркера указују на потенцијални значај одређене гермплазме за селекцију.

Генетска варијабилност три популације кукуруза је одређена на бази полиморфизма седам ензимских система. У анализираних 228 линија на локусима ензима нађено је од 2 до 5 алела, а највећи полиморфизам су показали локуси *Pdg1* и *Pgd2*. Просечна хетерозиготност локуса у популацијама је била од 0,203 до 0,274, а полиморфизам је варирао од 0,727 до 0,818.

Добијени резултати могу послужити оплемењивачима као један од критеријума у избору родитељских парова у циљу добијања хетерозиса код хибрида кукуруза или као извори нове генетске варијабилности за стварање нових популација кукуруза.

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# GENETICALLY MODIFIED SOYBEAN PLANTS AND THEIR ECOSYSTEM

ABSTRACT: Transgenic plants are developed by introgressing new genes using methods of molecular genetics and genetic engineering. The presence of these genes in plant genome is identified on the basis of specific oligonucleotides, primers, and the use of PCR (Polymerase Chain Reaction) and DNA fragments multiplication. Genetically modified plants such as soybean constitute a newly created bioenergetic potential whose gene expression can cause disturbance of the biological balance ecosystem, soil structure and soil microbiological activity.

Genetically modified plants may acquire monogenic or polygenic traits causing genetic and physiological changes in these plants, which may elicit a certain reaction of the environment including changes of microbiological composition of soil rhizosphere.

The aim of introgressing genes for certain traits into a cultivated plant is to enhance its yield and intensify food production. There are more and more genetically modified plant species such as soybean, corn, potato, rice and others and there is a pressure to use them as human food and animal feed.

Genetically modified soybean plants with introgressed gene for resistance to total herbicides, such as Round-up, are more productive than non-modified, herbicide-sensitive soybeans.

KEY WORDS: GMO, PCR, transgenic plants, ecosystem

## INTRODUCTION

Genetically modified organisms (GMO) are created all over the world, which possess new, desirable, previously non-existing traits. Presently, however, GMOs are suspected of possible negative effects on the environment and humans (Kalaitzandonakes, 1999; Metcalfe et al., 1996). Because

of that, biotechnology and genetic engineering are closely controlled and regulated in this and other countries.

Applications of modern biotechnology show a significant potential in improving agricultural productivity, reducing poverty and enhancing food security in developing countries.

Techniques of genetic engineering, such as introgression of gene for new and improved existing traits, are tools used by breeders for development of transgenic plants. These plants are made artificially, without natural pollination. The introgressed genetic sequence may come from another related plant or from a completely different species. An example for the latter case is transgenic Bt corn which produces a protein with insecticidal properties on account of a gene received from a soil bacterium (M i r e l e s, 2000). Plants containing new genes are called genetically modified, or GM crops, although all plants are in fact GM originating from genetic modifications of their wild progenitors. Transgenic plants of soybean and corn contain introgressed genes for tolerance to herbicides, pesticides, insecticides, etc. A soybean variety has been developed which is tolerant to glyphosate, the active substance in the non-selective herbicide „Roundup". Soybean resistance to this herbicide was introduced via *Agrobacterium* sp. gene fragment of cauliflower mosaic virus (promoter) which contains peptide sequence for Roundup resistance (V a n H o e f et al., 1998). This gene fragment produces synthase — the enzyme responsible for soybean tolerance to „Roundup". On the other side, non-transformed soybeans are highly sensitive to this herbicide (D e l a n n a y et al., 1995; P a d g e t t e et al., 1995).

Transgenic soybean lines with high tolerance to this herbicide maintain high and stable yields upon herbicide treatment. Introduction of GM plants into an environment disturbs the existing biological balance. Biological divergence between genotypes in the ecosystem is increased, and so is the divergence between this ecosystem and other ecosystems which originally had been much closer to it.

In EU countries, testing of GM plants during growing season or after it, but in any case prior to the use for processing and food production, is obligatory. These tests serve to protect humans against potential risks coming from genetically modified plants. In the case of non-modified crops, fields that exhibit phenotypic modifications are inspected because such modification may indicate possible tainting with GM plants.

The aim of this investigation was to determine relations between GM plants and ecosystem to which they belong. Soil microbiological activity was analyzed in fields under GM soybeans to determine the effect of these plants on changes of the environment.

## MATERIAL AND METHODS

Modified (+) and unmodified (–) soybean plants with intact roots and soil around them were sampled in 14 locations. Soil samples were taken aseptically from the depth of 0—30 cm. Genetic identification of soybean plants was do-

ne in DNA extracts using the PCR method (polymerase chain reaction) on the basis of specific primers 35S promotor and NOS3" terminator (Van Hoef et al., 1998; Meyer et al., 1996; Wurz and Willmund, 1997). Qualitative presence of the introgressed gene was determined on the basis of the PCR method and amplification of DNA fragments. After identification of plants, soil analyses were done.

Determination of microbial number in the soil was done using plate counts. The following groups of microorganisms were determined: total number of microorganisms on soil agar (Pochon and Tardieux, 1962), number of fungi on the Czapek-Dox agar (Sharlau, 2000), number of azotobacters on the Fiodorov agar (Anderson, 1958), and the number of ammonifiers on the nutritious agar (Torlak).

## RESULTS AND DISCUSSION

The PCR method determines whether a genetic transformation has been made or not. New quality traits obtained by transformation are the result of physiological processes which, in their turn, are controlled by certain genes. The objectives of gene manipulation are increased efficiency of plant production and improved protection of the environment and humans.

The numbers of bacteria and fungi were high in both soil variants (+GM crops, –GM crops), without significant differences between them (Table 1).

Tab. 1 — General microbiological activity of soil

| No. | Soil sample* | Total number of bacteria ($10^7$ $g^{-1}$ abs. dry soil) | Total number of fungi ($10^4$ $g^{-1}$ abs. dry soil) |
|---|---|---|---|
| 1 | 1 +GM | 81.37 | 46.50 |
| 2 | 1 –GM | 108.65 | 20.37 |
| 3 | 10 $km^2$ +GM | 69.36 | 35.87 |
| 4 | 10 $km^2$ –GM | 128.88 | 23.01 |
| 5 | 3 +GM | 57.41 | 12.75 |
| 6 | 3 –GM | 38.70 | 34.40 |
| 7 | 3/1 +GM | 54.30 | 15.20 |
| 8 | 3/1 –GM | 44.68 | 25.53 |
| 9 | 5 +GM | 44.65 | 10.63 |
| 10 | 5 –GM | 53.65 | 12.87 |
| 11 | 6 K +GM | 130.20 | 6.97 |
| 12 | 6 K –GM | 92.48 | 7.11 |
| 13 | 8 +GM | 113.93 | 15.95 |
| 14 | 8 –GM | 226.78 | 24.69 |

* +GM — sample of soil in which GM plants were grown.
* –GM — sample of soil in which non-GM plants were grown.

The presence of azotobacters was high in all samples, which was characteristic for the tested soil type, except that somewhat lower values were determined in samples 8, 9 and 10, but these reductions were too small to indicate any soil disturbance (Table 2).

Tab. 2 — Microorganisms of the nitrogen cycle

| No. | Soil sample* | Azotobacter ($10^2$ $g^{-1}$ abs. dry soil) | Ammonifiers ($10^7$ $g^{-1}$ abs. dry soil) |
|---|---|---|---|
| 1 | 1 +GM | 11.04 | 51.15 |
| 2 | 1 –GM | 54.89 | 24.90 |
| 3 | 10 $km^2$ +GM | 34.08 | 74.14 |
| 4 | 10 $km^2$ –GM | 25.89 | 25.31 |
| 5 | 3 +GM | 34.55 | 48.90 |
| 6 | 3 –GM | 29.03 | 43.01 |
| 7 | 3/1 +GM | 16.29 | 23.89 |
| 8 | 3/1 –GM | 2.12 | 57.44 |
| 9 | 5 +GM | 7.97 | 25.51 |
| 10 | 5 –GM | 6.43 | 34.33 |
| 11 | 6 K +GM | 40.68 | 41.85 |
| 12 | 6 K –GM | 33.19 | 59.28 |
| 13 | 8 +GM | 43.86 | 102.54 |
| 14 | 8 –GM | 20.77 | 44.90 |

* +GM — sample of soil in which GM plants were grown.
* –GM — sample of soil in which non-GM plants were grown.

The number of ammonifiers was optimal in most of the analyzed samples (Table 2).

Interactions between GM and non-GM plants were not found (B e r i n g e r, 2000).

## CONCLUSION

The transgenic DNA or proteins from transgenic crops can be detected in plant material and processed food. Potential effects of GM crops on ecosystems and environment are of special significance and must be observed.

The results obtained in this study showed that for the GM and non GM soybean plants caused no change in the soil microbiological activity. It seems to indicate that GM plants do not cause large changes in the biological balance of the environment in which they grow.

Transgenic DNA has been deemed to be safe for consumption as it is made up of the same building blocks as plant genomic DNA. Transgenes should remain stable from generation to generation and GM plants should not disturb the biological balance of the environment. Some authors have considered the

benefits and risks of transgenic crops in great detail, which can be of importance for further biotechnological advancement.

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# ГЕНЕТСКИ МОДИФИКОВАНЕ БИЉКЕ СОЈЕ И ЊИХОВ ЕКОСИСТЕМ

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Резиме

Методе молекуларне генетике уведене у генетски инжињеринг учествују у програму трансгених биљака, насталих увођењем нових гена, који су у основи тражених особина. Њихово присуство у биљном геному се идентификује на бази специфичних олигонуклеотида, прајмера, коришћењем PCR (Polymerase Chain Reaction), методе умножавања ДНК фрагмената. Генетски модификоване биљке, као што је соја, постају новонастали биоенергетски потенцијал за средину у којој се развијају, чија експресија гена може изазвати поремећај биоравнотеже, екосистема, структуре земљишта, његове микробиолошке активности.

Генетски модификоване биљке могу бити са уведеним моногеним или полигеним својствима, што доводи до њихових генетско-физиолошких промена и одређених реакција у средини у којој се налазе. Сматра се да организми са новонасталом генетском структуром могу утицати на измену био-равнотеже средине у којој се налазе, као што су промене хемијског и микробиолошког састава у ризосфери земљишта, које ће условити низ измена његове биоенергетске способности.

Увођење гена за одређене особине биљака има за циљ повећање њиховог приноса и интензивнију производњу хране. Отуда је све већи број модификованих биљних врста, као што су соја, кукуруз, кромпир, пиринач и друге, које имају велико учешће у исхрани људи и животиња, због чега је неопходна контрола таквих биљних врста и средине у којој се оне налазе.

Генетски модификоване биљке соје, са унетим геном за резистентност на тотални хербицид, као што је Round up, доприноси њиховој већој продуктивности, за разлику од немодификованих биљака, које не подносе овакву врсту хербицида. Међутим, особина новоунета у геном биљке може имати различите последице по околину у којој се налази, што је пре свега у вези са циљем њеног настанка, као што су отпорност соје на тотални хербицид, Round up и њен однос са средином.

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# PHOTOSYNTHETIC PROPERTIES OF ELITE ERECT LEAF MAIZE INBRED LINES AND THEIR CONTRIBUTION TO SEED PRODUCTION IMPROVEMENT*

ABSTRACT: A hypothesis that elite erect leaf maize inbred lines are characterized by properties of an efficient photo-model and that as such are very desirable in increasing the number of plants per unit area (plant density) in the process of seed production has been confirmed in the present study.

The properties of the observed elite erect leaf maize inbred lines were based on the effects and characteristics of thermal processes of delayed chlorophyll fluorescence occurring in their thylakoid membranes. The temperature dependence of the delayed chlorophyll fluorescence intensity, the Arrhenius plot for the determination of phase transitions (critical temperatures) and activation energy are the principal parameters of the thermal processes.

Based on the obtained results on photosynthetic properties it was also possible to estimate the tolerance and adaptation of elite erect leaf maize inbred lines to high temperatures and drought.

KEY WORDS: maize inbred lines, erect leaves, maize seed, photosynthetic model, thermal and photosynthetic processes, thylakoid membrane, delayed chlorophyll fluorescence

## INTRODUCTION

This study considers two evident facts from the most recent history of maize breeding and seed production in our country. The first one is with a plus sign, based on the results of maize breeding and seed production that have been intensively developing during the last 50 years. As a result of these activities, approximately a thousand grain and silage maize hybrids were developed; at the same time, conditions necessary for a highly developed seed production had been provided: drying and processing plants, choice of land, irri-

* This paper is contributed to the memory and long reminiscence of a countenance and deeds of Dr. Jovan Smiljaković's.

gation systems, sufficient quantity of basic seed and qualified staff (Duvick, 1984; Trifunović, 1986; Ivanović et al., 1995; Radenović and Somborac, 2000). Regardless of such a colossal success in maize breeding and seed production, eagerness and enthusiasm of the total research body did not slow down. The search for new methods and exact approaches in the completion and enrichment of the research within maize breeding and seed production was continued. The other fact, with a minus sign, is related to the interrelation between photosynthesis and maize seed production. Although photosynthetic processes are highly productive in their intensity, very complex in their nature, and vastly studied in their scientific actuality, their application in maize seed production is still insignificant. Such a state of affairs is probably a consequence of the existence of several functional interrelations that unify conformational and dynamic changes within chloroplasts and their thylakoid membranes on the one hand, and the effects of environmental stress factors on them on the other.

Delayed chlorophyll fluorescence (DF) can be phenomenologically described as an occurrence of luminescence (bioluminescence) within the red range of the visible spectrum produced by plant systems, bacteria, algae and higher plants (maize), immediately after their intermittent illumination (Radenović, 1992, 1994, 1997). DF was discovered by Strehler and Arnold (1951). Important studies, especially those conducted over last 20 years (Jursinic, 1986; Veselovski and Veselova, 1990; Marković et al., 1993, 1996) revealed a direct connection between DF and the photosynthetic processes, in which DF was considered an unavoidable indicator — a sensitive „probe" for experimental photosynthetic and bioluminescence studies (Radenović et al., 1994a, 1994b; Radenović and Jeremić, 1996; Marković et al., 1987, 1999).

The objective of the present study was to determine general and photosynthetic properties of elite erect leaf maize inbred lines, which serve as an efficient photo-model, using a non-invasive photosynthetic and bioluminescence method in maize breeding and seed production (Radenović et al., 2000, 2001a, 2001b, 2002a, 2002b).

## MATERIAL AND METHODS

A brief survey of the studied elite erect leaf maize inbred lines is given in Table 1. The material selected for this study was grown at the experiment field of the Maize Research Institute, Zemun Polje.

During July and August, maize plants were brought from the field to the laboratory during morning hours (between 7 a. m. and 8 a. m.). During sampling in the field, plants were transversely cut at the ground internode. In the laboratory, plants were internode-lengthwise placed in water. Two hours prior to the bioluminescence experiment, the plants were kept under the black ball glass. A segment of ear intact leaves (or leaves just above the ear) was taken from such plants and placed into a chamber of the phosphoroscope (Figure 1) and kept in the dark for at least 15 minutes, and then DF was measured. The

non-invasive photosynthetic bioluminescence method used to measure DF is presented in Figure 1. This block scheme of the bioluminescence method was developed at the Maize Research Institute, Zemun Polje. Measurements of changes in DF intensities were performed after a method that had been described, both in principle and detail, in previous papers (Radenović, 1992, 1994, 1997; Radenović et al., 2001a, 2001b, 2002a, 2002b; Marković et al., 1996).

Table 1. Description of elite erect leaf maize inbred lines

| Ordinal number | Designation of inbred lines | FAO maturity group | Origin | The axil between ear leaf and stalk | Ear leaf area ($cm^2$) |
|---|---|---|---|---|---|
| 1 | ZP PL 16 | 700 | BS13(S2)CO | 31.60° | 5260.30 |
| 2 | ZP PL 111 | 600 | Iowa SSS | 32.10° | 4986.80 |
| 3 | ZPL 773 | 650 | B73 rec | 31.98° | 4904.10 |
| 4 | ZP PL 121 | 600 | Mo17 x 25-10-1 | 32.50° | 5320.20 |

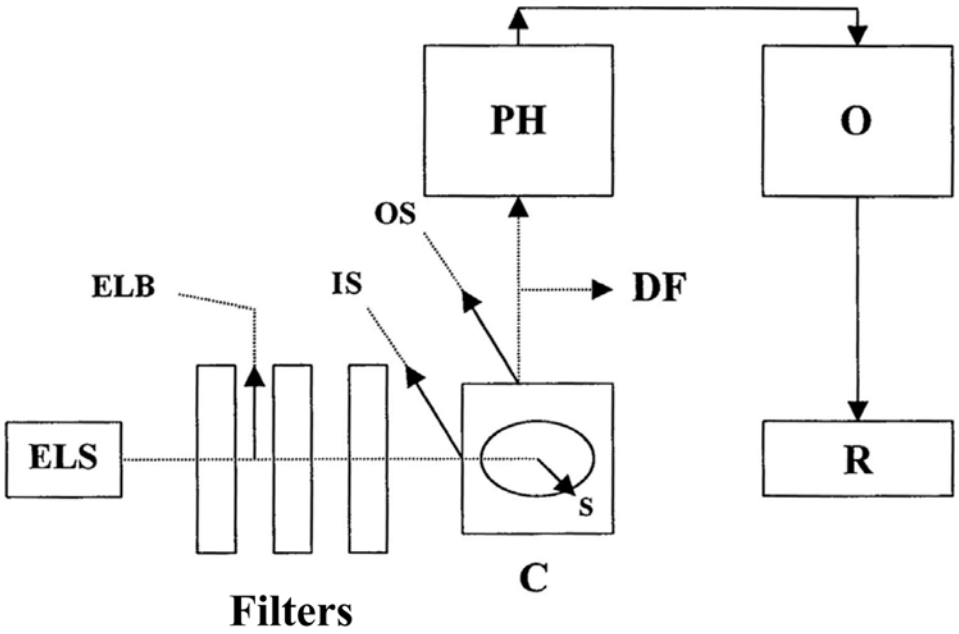


Figure 1. Experimental setup of the method and measuring equipment for delayed chlorophyll fluorescence: C — dark chamber with a sample stand; s — sample (leaf segment), filters, ELS — excitation light source, PH — photo-multiplier; O — oscilloscope, R — printer, ELB — excitation light beam, DF — luminescent light, IS — input chamber slot, OS — output chamber slot

## RESULTS

The results of photosynthetic properties of the elite erect leaf maize inbred lines, as optimal photo-models, are presented through five sections.

### 1. *The measure of the axil and leaf area of the elite erect leaf maize inbred lines*

A specially designed protractor was used to measure the axil of each leaf of the elite erect leaf maize inbred plants. The results of the measurements of the axils (ranging from 31.60° to 32.50°) between the ear leaves and the stalks are presented in Table 1. Average leaf areas of the elite erect leaf maize inbreds are also presented in Table 1. It is clear that the leaf area has no properties that would particularly distinguish the studied elite erect leaf maize inbred lines.

### 2. *Parameters of thermal processes of the delayed chlorophyll fluorescence in the studied elite erect leaf maize inbred lines*

A detailed study on thermal processes of chlorophyll DF was carried out for the elite erect leaf maize inbred lines. These processes were characterized by time parameters in regard to the duration of conventionally sampled segments: **a**, **b**, **c**, **d**, **e**, **f** and **g** on the thermal curve of chlorophyll DF (Figure 2). The results on time parameters of the chlorophyll DF thermal curve for the stated segments are presented in Table 2.

Table 2. Duration (in seconds) of segments on the thermal curve of delayed chlorophyll fluorescence for elite erect leaf maize inbred lines

| Segment of thermal curve | Segment designation | MAIZE INBRED LINE | | | | Temperature range for establishing thermal curve (°C) |
|---|---|---|---|---|---|---|
| | | ZPPL 16 | ZPPL 111 | ZPL 773 | ZPPL 121 | |
| Stationary level of DF intensity | a | >130 | >130 | >130 | >130 | 15—45 |
| Initial increase of DF intensity | b | 460 | 488 | 496 | 448 | 15—45 |
| Linear increase of DF intensity | c | 150 | 162 | 166 | 138 | 15—45 |
| Maximum level of DF intensity | d | 58 | 66 | 52 | 56 | 15—45 |
| Linear decrease of DF intensity | e | 132 | 120 | 126 | 138 | 15—45 |
| Decelerated decrease of DF intensity | f | 119 | 102 | 96 | 88 | 15—45 |
| Minimum level of DF intensity | g | 26 | 22 | 18 | 24 | 15—45 |

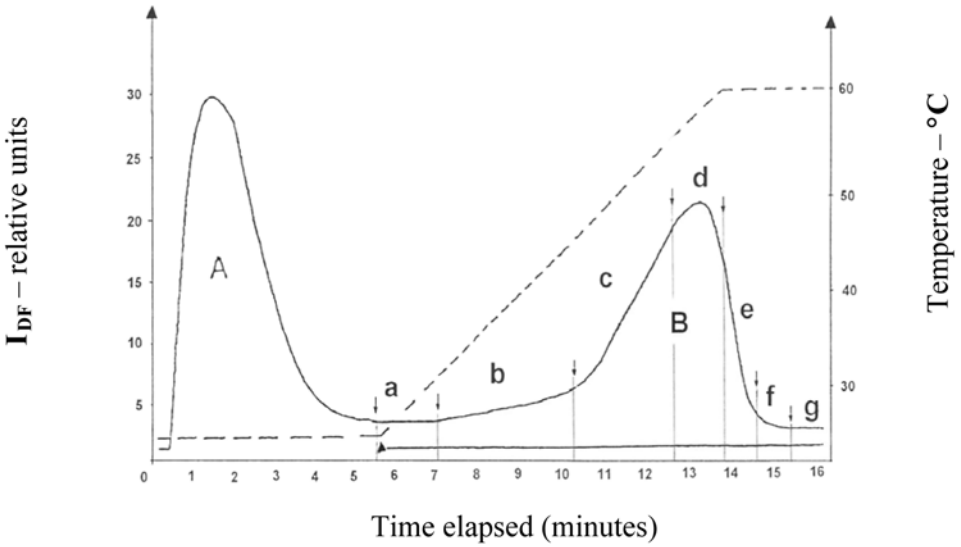


Figure 2. Schematic presentation of typical changes of chlorophyll DF intensities in the intact leaves of the observed elite erect leaf maize inbred lines (solid line) and changes of temperatures (dashed line): curve **A** indicates induction processes of chlorophyll DF, while curve **B** encompasses thermal processes of chlorophyll DF. Segments on the thermal curve (**a, b, c, d, e, f, g**) are interception points in which conformational and functional changes in the thylakoid membrane occur

### 3. *Temperature dependence of the delayed chlorophyll fluorescence intensity for the thylakoid membrane of the elite erect leaf maize inbred lines*

Figure 3a, b, c, d presents the changes in the intensity of the stationary DF level in the function of temperatures ranging from 15°C to 45°C in the thylakoid membrane of the following elite erect leaf maize inbred lines: ZPPL 16, ZPPL 111, ZPL 7739 and ZPPL 121.

### 4. *The Arrhenius plot for the determination of conformational changes in the thylakoid membrane of the elite erect leaf maize inbred lines*

Figure 4a, b, c, d presents results of conformational changes that occurred in the thylakoid membrane of the elite erect leaf maize inbred lines. These changes were caused by the temperature impact on the intact leaf segment of the following erect leaf maize inbred lines: ZPPL 16, ZPPL 111, ZPL 7739 and ZPPL 121.

Figure 3a

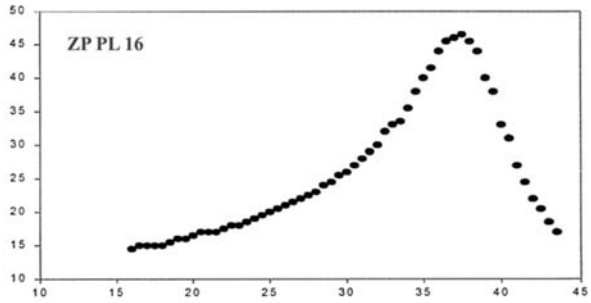


Figure 3b

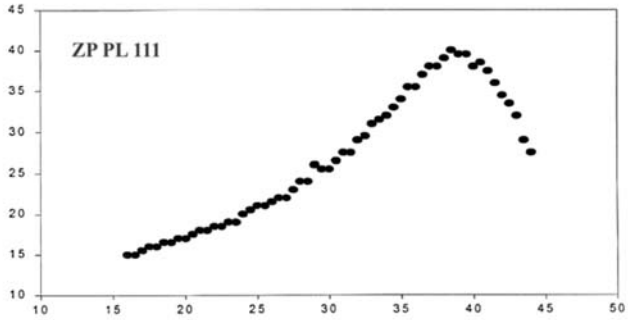


Figure 3c

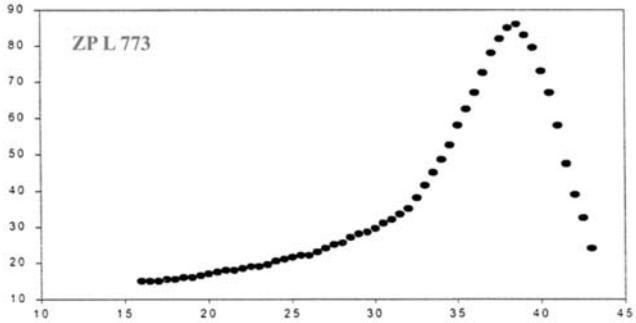


Figure 3d

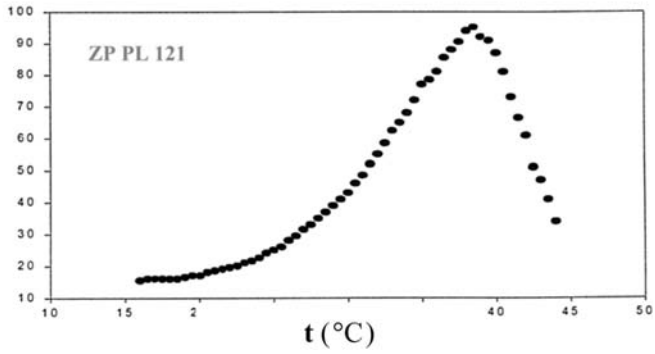


Figure 3 a, b, c, d. Changes of the intensity of the stationary chlorophyll DF level in dependence of the temperature in the thylakoid membrane of intact leaves of the elite erect leaf maize inbred lines

Figure 4a

Figure 4b

Figure 4c

Figure 4d

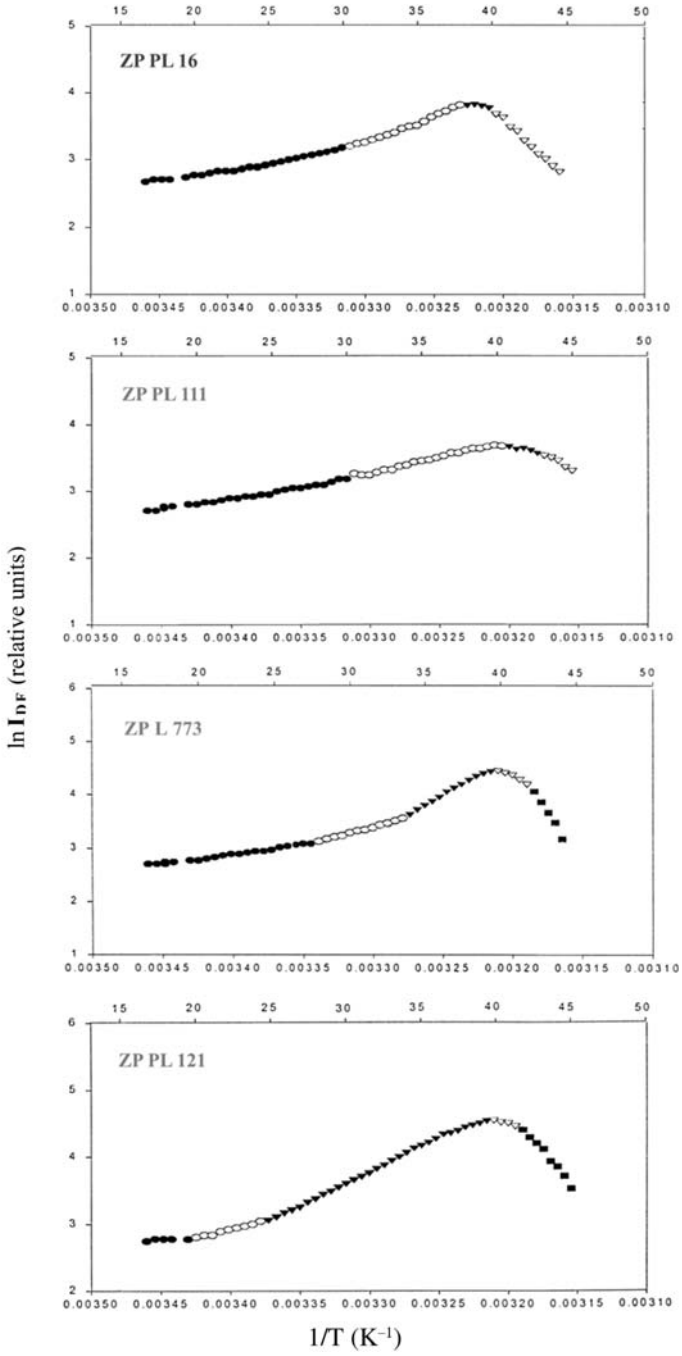


Figure 4 a, b, c, d. Arrhenius plot for a change of the logarithm of intensity of chlorophyll DF in dependence of the reciprocal values of temperatures on the thylakoid membrane of the elite erect leaf maize inbred lines

### 5. *Activation energies and critical temperatures in the thylakoid membrane of the elite erect leaf maize inbred lines*

Activation energies and temperatures of phase transitions (critical temperatures) in the thylakoid membrane of the elite erect leaf maize inbred lines ZPPL 16, ZPPL 111, ZPL 7739 and ZPPL 121 are presented in Table 3.

Table 3. Activation energies and temperatures of phase transitions in the thylakoid membrane of the studied elite erect leaf maize inbred lines

| ZPPL 16 | | ZPPL 111 | | ZPL 773 | | ZPPL 121 | |
|---|---|---|---|---|---|---|---|
| Ea (kJ mol$^{-1}$) | $t_{p.t.}$ (°C) | Ea (kJ mol$^{-1}$) | $t_{p.t.}$ (°C) | Ea (kJ mol$^{-1}$) | $t_{p.t.}$ (°C) | Ea (kJ mol$^{-1}$) | $t_{p.t.}$ (°C) |
| — | 18.0 | — | 18.5 | — | 18.6 | — | 18.6 |
| 28.1 | 29.0 | 26.7 | 28.9 | 29.7 | 26.8 | 6.5 | 24.0 |
| 65.3 | 36.5 | 29.0 | 39.8 | 56.3 | 33.6 | 42.6 | 38.0 |

## DISCUSSION

As already mentioned, the second half of the 20$^{th}$ century will be remembered, recognized and hardly excelled for the great success achieved in maize breeding and seed production. This immense activity was based on a very broad and complex program of maize breeding and seed production. Its goal was clear and concrete — to provide the highest possible grain yield in the newly developed maize hybrids and to provide a sufficient amount of quality seed for the domestic and foreign markets. The number of plants per area unit kept growing ever since 1980. This trend in maize breeding was referred to as „plant density" program and it most directly affected further yield increase of seed and commercial maize. In addition, a subprogram on maize breeding and seed production of erect leaf maize inbred lines was established. In pursuance of our hypothesis it was considered that these inbreds were closest to the projected photosynthetic model. These two subprograms of maize breeding and seed production were not only complementary, but also they were considerably expanded. Their implementation led to new results in both, maize breeding and seed production. New hybrids with high grain and silage yields were developed (Ivanović and Stojnić, 1992; Ivanović et al., 1995; Kojić, 1993; Trifunović, 1986; Duvick, 1984). Furthermore, seed production volume and quality were improved, reaching the amount of 70,000 tons (Selaković, 1999). In view of the above, it was quite expected for the breeding and seed production program of erect leaf maize inbred lines to be further expanded. A large number of erect leaf maize inbred lines, including the inbreds ZPPL 16, ZPPL 111, ZPL 773 and ZPPL 121 that were the objects of the present study, was in fact developed in an attempt to achieve the envisaged objective and to confirm the proposed hypothesis on the photosynthetic model.

It was necessary to characterize in detail the photosynthetic properties of the elite erect leaf maize inbred lines (Table 1). For that purpose, a non-invasive photosynthetic-bioluminescence method was applied (Radenović et al., 2001a, 2001b, 2002a, 2002b). The application of this method provided the characterization of the studied elite erect leaf maize inbred lines in relation to their resistance to both drought and high temperatures.

Actually, the temperature dependence of DF in all of the studied elite erect leaf maize inbred lines is, to a certain extent, characterized only by typical points on the thermal curve (Figure 2). The first typical point occurred at the contact point of segment **a** and segment **b** and it was related to the lowest critical temperature at which the initial change in DF intensity was observed. The second typical point occurred at the contact point of segment **b** and segment **c** and it was related to linear monotony and the angle of the rising part of the DF intensity. Throughout the duration of this typical point, evident changes in the structure of the thylakoid membrane occurred. The third typical contact point reflected a smaller or greater rotundity of DF intensity peaks. The „breaking" conformational changes occurred at two interception points of segments **c** and **d** and segments **d** and **e**. The fourth typical contact point was related to the linear monotony and the inclination angle of the declining part of the DF intensity. This segment bore the last conformational changes that had occurred in the thylakoid membrane. These changes can hardly be described as characteristic for the of functioning of a living leaf segment. The typical points designated **f** and **g** almost had no physiological role at all.

The observed typical points (Figure 2) can be considered as points that characterize elite erect leaf maize inbred lines, especially as these points are also points of possible conformational and functional changes in the thylakoid membranes. This statement is in accordance with literature data (Vučinić et al., 1982; Radenović, 1992, 1994; Radenović et al., 2001a, 2001b, 2002a, 2002b; Marković et al., 1987).

All critical temperatures at which even the slightest conformational changes occurred in the thylakoid membranes of the studied elite erect leaf maize inbred lines were determined by the Arrhenius criterion and the linearization of the DF temperature dependence. The value of critical temperatures (°C), their frequency and intermediate distance on the thermal curve, characterized the elite erect leaf maize inbred lines in respect to their resistance and adaptation to high temperatures. The Arrhenius criterion is based on the existence of straight lines. Each Arrhenius straight line represents its activation energy (Ea). The interception point of two straight lines is determined by a critical temperature. Each critical temperature is preceded by a certain value of activation energy and then it is followed by another value of activation energy (Radenović 1985, 1997; Marković et al., 1993, 1996; Radenović et al., 2001a, 2001b, 2002a, 2002b). The values of activation energies in the rising and declining part of the thermal curve are explained by smaller or greater conformational changes that occur in the molecules of the thylakoid membranes with the temperature increase. Due to such changes these molecules become more reactive and thereby gain an additional energy that is used in the recombining process of DF occurrence (Table 3). This study was an attempt to

show that there are elite erect leaf maize inbred lines having properties of an efficient photosynthetic model and that as such, they are very desirable in the process of contemporary selection and seed production.

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# ФОТОСИНТЕТИЧНЕ КАРАКТЕРИСТИКЕ ЕЛИТНИХ САМООПЛОДНИХ ЛИНИЈА КУКУРУЗА СА УСПРАВНИМ ПОЛОЖАЈЕМ ЛИСТОВА И ЊИХОВ ДОПРИНОС У УНАПРЕЂЕЊУ ПРОИЗВОДЊЕ СЕМЕНА

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Резиме

У овом раду потврђује се наша хипотеза да елитне самооплодне линије кукуруза са усправним положајем листова имају својство ефикасног фотосинтетичног модела и да се, као такве, у семенарству успешно користе при повећавању броја биљака на јединици површине (густина биљака). Ова хипотеза доказана је егзактном применом неинвазивног фотосинтетично-биолуминисцентног метода са закаснелом флуоресценцијом хлорофила, погодног за оцену ефикасности фотосинтетичног модела.

Добијене фотосинтетичне карактеристике елитних самооплодних линија кукуруза: ZPPL 16, ZPPL 111, ZPL 773 и ZPPPL 121, са усправним положајем листова, засноване су на ефектима и природи промена закаснеле флуоресценције хлорофила које се одигравају у њиховим тилакоидним мембранама. Главни показатељи су температурна зависност интензитета закаснеле флуоресценције хлорофила, Аренијусов критеријум за утврђивање фазних прелаза (критичне температуре) у тилакоидним мембранама и енергије активације.

Утврђене фотосинтетичне карактеристике још омогућавају да се проучаване елитне самооплодне линије кукуруза са усправним положајем листова оцене и на њихову толерантност и адаптацију према деловању високих температура као и суше.

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# STUDY OF SUGARBEET GENOTYPE RESPONSE TO NITROGEN NUTRITION USING LEAF ANALYSIS

ABSTRACT: Nitrogen rates and NPK ratios applied in the study had different effects on total nitrogen and nitrate contents in the laminas and petioles of the studied sugarbeet genotypes. Amount of nitrates, especially in the petioles, can be considered the most reliable indicator for determining sugarbeet plant supply with nitrogen. Differences in nitrate content found among the genotypes indicate the necessity of knowing the genetic specificity in nitrogen nutrition for individual genotypes.

KEY WORDS: sugarbeet, genotypes, nitrogen rates, nitrate test, total and nitrate nitrogen

## INTRODUCTION

Sugarbeet develops large organic mass (especially in intensive farming), which, among other things, requires that sufficient amounts of essential nutrients, particularly nitrogen, be provided (Petrović and Milošević, 1988). However, excessive use of nitrogen may often have undesirable effects, not only in sugarbeet but in other crops as well (Burba, 1977). The subject of excessive nitrogen use received much attention recently, in view of its economic and environmental implications (Kastori and Petrović, 2003). Similarly, problems related to nitrogen use in sugarbeet growing have come under scrutiny in last few decades. Many experiments have shown that excessive nitrogen use degrades the technological properties of sugarbeet and often has an unfavorable effect on leaf to root mass ratio (Prośba-Bialczyki et al., 2001). In this connection, optimal plant supply with essential elements during the growing season plays a significant role in obtaining high, good quality yields of sugarbeet roots. This is even more so when it is taken into account that genetic potentials for beet yield and root quality come to full fruition only in conditions of harmonious plant nutrition. Significant efforts are therefore

being made to develop maximally reliable methods for assessing soil fertility, i. e., the level of plant supply with essential elements. A number of methods are used today for this purpose such as chemical soil analysis, various biological methods, etc. In our conditions, the most commonly used method is chemical soil analysis. Lately, however, chemical analysis of plant material, i. e., certain plant organs, gains importance in assessing plant supply with essential elements.

In light of the specific importance nitrogen has in the formation of sugarbeet yield and root quality, the present paper will discuss the justifiability of determining total and nitrate nitrogen concentrations and using quick nitrate-test methods in the assessment of sugarbeet plant supply with nitrogen.

## MATERIALS AND METHODS

Sugarbeets used in the study had been grown in a long-term trial with four replicates established at the Rimski Šančevi experiment field of the Institute of Field and Vegetable Crops in Novi Sad back in 1965 using a randomized block design and an experimental unit size of 200 $m^2$. Four crop species per annum (wheat, sugarbeet, maize and sunflower) have been grown in the trial and rotated in a particular sequence. Nitrogen, phosphorus ($P_2O_5$) and potassium ($K_2O$) were applied at 50, 100 and 150 kg per hectare. Besides these amounts, there were also treatments in the trial with different ratios of the three elements. Entire phosphorus and potassium doses and one half of the nitrogen dose were applied in the fall, and the other half of nitrogen was applied in the spring prior to sowing.

The present study included only the treatments with increasing nitrogen rates and treatments with moderate and/or excess nitrogen, phosphorus and potassium amounts (Table 1).

Table 1. Rates and composition of N, P and K applied (kg $ha^{-1}$)

| N°. | N | $P_2O_5$ | $K_2O$ |
|---|---|---|---|
| 1 Control | 0 | 0 | 0 |
| 2 | 50 | 50 | 50 |
| 3 | 100 | 50 | 50 |
| 4 | 150 | 50 | 50 |
| 5 | 100 | 100 | 100 |
| 6 | 150 | 100 | 100 |
| 7 | 150 | 150 | 150 |

Three sugarbeet genotypes were used in the trial: NS-Hy-1R, NS-Hy-8R and Sara. Cultural practices recommended for commercial sugarbeet production in the local agroecological conditions were used in the course of the growing season.

Average samples of young and photosynthetically most active leaves were taken in late July in order to determine plant supply with nitrogen and

plant response to different nitrogen nutrition treatments. Plant nitrogen supply and genetic specificity for nitrogen nutrition were determined based on total and nitrate nitrogen contents, by the quick nitrate test method. Total nitrogen was determined by the Kjeldahl method, nitrate content by spectrophotometry using phenol disulphonic acid. The quick nitrate test was performed directly in the experimental plot using diphenyl amine as the reagent. In order to make sure the results are as reliable as possible, the indicators were analyzed separately for laminas and petioles. This approach makes it possible to see which particular indicators in which plant part (laminas or petiole) are the most accurate indication of sugarbeet supply with nitrogen.

The results were statistically processed by the analysis of variance for a factorial trial.

## RESULTS AND DISCUSSION

In sugarbeet, as in most other plant species, nitrogen has a favorable effect on yield. However, excess and/or inadequate nitrogen application may significantly reduce sugarbeet root quality (B u r b a, 1977). Sugarbeet requirement for nitrogen is considerably higher than in most other cultivated crop species, which is why there are only limited possibilities for determining the optimum supply level with this element by chemical soil analysis and/or the $N_{min}$ method. Because of this, leaf analysis and the quick nitrate test method have been used for this purpose as of late (A r m s t r o n g, 1985; P e t r o v i ć and U b a v i ć, 1989).

The lowest total nitrogen content in laminas and petioles was found in the control (Table 2). Total nitrogen content was visibly influenced by higher nitrogen rates and changed NPK ratios. The study results showed that the dependence of total lamina nitrogen on the nitrogen content in the soil was not very pronounced. Conversely, total petiole nitrogen was influenced much more by the increasing nitrogen rates and NPK rates and ratios (Table 2). Total nitrogen content ranged from 3.0 to 3.5% in the laminas and from 1.2 to 1.5% in the petioles. This indicates optimal supply with nitrogen (B e r g m a n n and N e u b e r t, 1976) of all the genotypes under study in all the treatments except control. Our results thus suggest that there is a need for choosing more adequate procedures for determining sugarbeet nitrogen supply, especially because excess nitrogen in the soil may cause excessive total nitrogen accumulation in the plant in general and laminas in particular (P e t r o v i ć and M i l o š e v i ć, 1988).

Table 2. Effect of NPK rates and ratio on total nitrogen content in laminas and petioles of different sugarbeet genotypes (in % dry matter)

| Treatment (B) | Genotype (A) | | |
|---|---|---|---|
| | NS-$H_y$—1R | NS-$H_y$-8R | Sara |
| | *Lamina* | | |
| 1. | 2.73 | 2.85 | 2.65 |
| 2. | 3.54 | 3.34 | 2.81 |
| 3. | 3.59 | 3.57 | 4.29 |
| 4. | 3.74 | 4.09 | 4.00 |
| 5. | 3.55 | 4.13 | 4.05 |
| 6. | 3.59 | 4.00 | 4.12 |
| 7. | 3.73 | 4.37 | 4.33 |
| LSD 5% | A 0.31 | B 0.24 | AB 0.38 |
| | *Petiole* | | |
| 1. | 0.92 | 1.10 | 0.80 |
| 2. | 1.19 | 1.24 | 1.15 |
| 3. | 1.50 | 1.34 | 1.41 |
| 4. | 1.72 | 1.73 | 1.44 |
| 5. | 1.42 | 1.45 | 1.55 |
| 6. | 1.55 | 1.67 | 1.65 |
| 7. | 1.40 | 1.64 | 1.90 |
| LSD 5% | A 0.14 | B 0.10 | AB 0.18 |

In sugarbeets, nitrates are found mostly in the above-ground plant parts, especially in petioles. Of the total nitrates accumulated in sugarbeet plants, 55% is found in petioles, 30% in laminas and only 15% in the root (B u r b a, 1977). Numerous authors are of the opinion that nitrate concentration in laminas is one of the most reliable indicators of plant supply with nitrogen in sugarbeet (K a s t o r i and P e t r o v i ć, 1993).

In our study, the nitrogen rates and NPK ratios applied significantly influenced the nitrate contents of the sugarbeet genotypes studied. The amount of nitrates in laminas and especially in petioles depended significantly not only on nitrogen rate but also on NPK ratio as well as on the genotype (Table 3).

Tab. 3. Effects of NPK rates and ratios on nitrate content in laminas and petioles of different sugarbeet genotypes (mg $NO_3$/kg dry matter)

| Treatment (B) | Genotype (A) | | |
|---|---|---|---|
| | NS-$H_y$—1R | NS-$H_y$-8R | Sara |
| | *Lamina* | | |
| 1. | 342 | 456 | 416 |
| 2. | 415 | 470 | 423 |
| 3. | 833 | 566 | 452 |
| 4. | 2315 | 1945 | 1380 |
| 5. | 822 | 694 | 620 |
| 6. | 2296 | 1637 | 1275 |
| 7. | 2133 | 1355 | 1465 |
| LSD 5% | A 101 | B 72 | AB 128 |
| | *Petiole* | | |
| 1. | 385 | 268 | 316 |
| 2. | 419 | 384 | 383 |
| 3. | 554 | 478 | 482 |
| 4. | 1613 | 2033 | 1019 |
| 5. | 665 | 787 | 614 |
| 6. | 1901 | 3389 | 1645 |
| 7. | 2358 | 2146 | 1601 |
| LSD 5% | A 122 | B 78 | AB 136 |

The study results also indicated that the genotypes involved had a specific response not only to nitrogen nutrition but probably to nitrogen uptake, distribution and metabolism as well. In the treatments with increasing nitrogen rates, the highest nitrate contents in laminas and petioles were observed in NS-Hy-1R and NS-Hy-8R, respectively. The reduced nitrate portion in laminas and petioles caused by increased potassium and phosphorus rates also suggests that the technological quality of sugarbeet roots is significantly affected by well balanced levels of the essential elements in the nutrient substrate. Many studies have confirmed that a petiole nitrate content of 1,000 $mg \cdot kg^{-1}$ dry matter six to eight weeks before sugarbeet root lifting represents the critical concentration. Having in mind this and the findings from the present study, we can conclude that the optimum nitrogen supply for the genotypes under study is attained with 120 $kg\ N \cdot ha^{-1}$.

Justification for the use of the quick nitrate test method in estimating plant nitrogen supply can be found in many studies, such as, for instance, Halvorson et al. (1975) or Petrović and Ubavić (1989). In many cases, just like in the present study (Tables 3 and 4), positive correlations have been found between the nitrate contents in dry petioles and in xylem sap of fresh petioles.

Table 4. Determination of nitrogen supply in sugarbeet genotypes by diphenyl amine test (on a scale of 0 to 3)

| Treatment | Genotype | | |
|---|---|---|---|
| | NS-$H_y$-1R | NS-$H_y$-8R | Sara |
| 1. | 0.0 | 0.0 | 0.0 |
| 2. | 0.3 | 0.2 | 0.3 |
| 3. | 1.4 | 1.3 | 1.2 |
| 4. | 1.9 | 2.1 | 1.8 |
| 5. | 1.4 | 1.5 | 1.4 |
| 6. | 2.6 | 2.8 | 2.6 |
| 7. | 3.0 | 3.0 | 2.5 |

## CONCLUSIONS

Three sugarbeet genotypes were studied in field conditions for the effects of nitrogen rate and fertilizer NPK ratio on total and nitrate nitrogen contents in laminas and petioles. The obtained results showed the following.

The nitrogen rates and NPK ratios studied had no significant effect on total nitrogen content, which makes the use of this procedure for determining plant supply with nitrogen less justified.

Nitrate content in laminas and, especially, in dry and fresh petioles depended significantly on plant nitrogen supply. With this in mind, we can conclude that sugarbeet supply with nitrogen can be determined reliably based on nitrate level or nitrate test results. The results also show the necessity of knowing the genetic specificity in nitrogen nutrition of individual genotypes.

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## ИСПИТИВАЊЕ РЕАКЦИЈЕ ГЕНОТИПОВА ШЕЋЕРНЕ РЕПЕ НА ИСХРАНУ АЗОТОМ ПОМОЋУ ЛИСНЕ АНАЛИЗЕ

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Резиме

У пољским условима на стационираном вишегодишњем огледу код три генотипа шећерне репе испитан је утицај растућих доза азота и однос садржаја N, P и K на удео укупног и нитратног азота у лискама и лисним дршкама. Добијени резултати указују да испитиване дозе азота, те односи N, P и K нису значајније утицали на садржај укупног азота у лискама и лисним дршкама код испитиваних генотипова шећерне репе. Стога се може закључити да је коришћење садржаја укупног азота као показатеља обезбеђености шећерне репе у овом елементу недовољно поуздано.

Садржај нитрата у лискама, нарочито у лисним дршкама, видно је зависио не само од испитиваних доза азота, него и од односа N, P и K. На основу неведеног може се закључити да удео нитрата у листовима, посебно у лисним дршкама, може да се сматра поузданијим показатељем у утврђивању оптималне обезбеђености ове врсте у азоту. Разлике у садржају нитрата између испитиваних генотипова, при њиховој различитој обезбеђености у азоту, оправдано указују на потребу познавања генетске специфичности појединих генотипова шећерне репе при примени азота.

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# VARIABILITY OF ANATOMICAL-PHYSIOLOGICAL TRAITS IN BLACK LOCUST CLONES (*Robinia pseudoacacia* L.)

ABSTRACT: Variability within *R. pseudoacacia* species represents an important factor in selection of fast-growing genotypes. Therefore, it is important to identify superior individuals according to their anatomical and physiological traits.

This paper presents the results of a study of genotype variability of the main leaf anatomical (frequency, length and width of stomata, leaflet thickness among veins, leaflet thickness on the main vein, mesophyll thickness, length and width of vascular bundle of main vein) and physiological (leaf area, photosynthetic pigments content and content of N, P, K, Ca, Na) parameters among five clones of *Robinia pseudoacacia* L.

Significant interclonal variations were observed in the investigated parameters. Clone R-56 had the highest N, P, and K concentrations, the largest mesophyll volume and the highest pigment concentration. We concluded that the clone R-56, although without a remarkable leaf area, possesses the ability for high photosynthetic production. The results are going to be used in further work on selection.

KEY WORDS: *Robinia pseudoacacia*, leaf anatomy, mineral nutrition, photosynthesis, respiration, variability introduction

## INTRODUCTION

Black locust (*Robinia pseudoacacia* L., *Fabaceae*) is a hardwood with a high juvenile growth rate that is adaptable to a wide range of soils, environments and weather conditions. This species grows on a large variety of soils in the temperate regions of the world. Although the species is endemic to the USA, it is planted on more than 3 million hectares in other countries. It is se-

cond only to the many *Eucalyptus* species that have been planted worldwide (Geyer and Bresnan, 1991; Führer and Jaró, 1996). In Serbia and Montenegro, black locust has been planted over a large area.

The black locust is crooked and thorny tree rich in extractives, but these disadvantages are relatively minor. Its only major drawback is the susceptibility to locust stem borer (*Megacyllene robiniae*). Silvicultural and biotechnological methods have shown promise in minimizing this problem (Hanover, 1991)

One of the most attractive features of the black locust is a high juvenile growth rate (height), 2—6 cm/day, which places it amongst the most rapidly growing plants (Kamdem et al., 1995).

The ever-increasing shortage of high quality wood on the market, potential for mechanical processing of black locust (Klašnja and Kopitović, 1994; Kopitović and Klašnja, 1994), development of conversion processes, potential for high biomass production in short rotations (Bongarten et al., 1992), utilization in bee-keeping by increasing the duration of the period and abundance of flowering (Guzina and Tomović, 1997), have enhanced interest in black locust cultivation (Tomović et al., 1997).

Since little attention has been given to potential improvement of the black locust by selection and hybridization, a long-term research program has been initiated at Poplar Research Institute in Novi Sad aiming at the best possible utilization of black locust genetic potential for timber production. The research within the long-term programs of black-locust breeding has been based on the establishment of a series of experimental plantations from the progenies of the selected populations and individual trees. The study results will enable the production of the selected nursery stock for mass production of seedlings and rooted cuttings. The program includes the study of some anatomical and physiological characters of black locust leaves and the variability of these characters. The results are going to be used in further selection work.

This paper presents the results of the research of interclonal variability of the main leaf anatomical (frequency, the length and width of stomata, leaflet thickness among veins, leaflet thickness on the main vein, mesophyll thickness, height and width of vascular bundle of main vein) and physiological (leaf area, photosynthetic pigments content, rates of photosynthesis and dark respiration, and content of N, P, K, Ca, Na) parameters among five clones of *Robinia pseudoacacia* L.

## MATERIALS AND METHODS

A trial was initiated in the spring of 1992 at the Poplar Research Institute (location: 45°17’ N, 19°53’ E, elevation 76 m) in three replicates with 30 plants on the fluvisol soil type. Black locust seedlings (1/1) were planted at a distance of 2.5 x 3 m, and the following clones were chosen for the trial: R-54-1 (characterized by a straight and full trunk), R-56 (average trunk growth, low technical quality, but abundant flowering), R-115 (rapid growth, extremely straight trunks), and R-135 (straight, full trunk, and monopodiality). The stan-

dard, the most widely planted black locust, was produced from non-clonal commercial seed. All analyses were done on 18-year-old plants.

Ten randomly chosen samples of fifty completely developed and light--exposed leaves taken from randomly chosen individual trees were used. Samples were taken in the summer of 2000.

### *Leaf anatomical characteristics*

Fully developed pinnately compound leaves from the upper crown were used for analysis of anatomic characteristics.

Stomata number per $mm^2$ and their length and width on adaxial and abaxial epidermis prints produced by a modified Wolf method were determined (Marjanović and Krstić, 1998). A small brush was used to apply a thin layer of colorless nail polish to leaflet parts suitable for epidermis print sampling. After a few minutes, a clear sticky tape was used to remove prints, which were put on slides for microscope observations. Stomata number in each of ten vision fields and stomata size of 20 stomata per leaflet were determined.

A freezing microtome was used for cross sectioning of leaflet middle part. Standard microscopic measurements of preparations with a micrometric ocular inserted into an „Olympus" light microscope were applied to analyze the following characteristics: leaflet thickness at 1/4 width, mesophyll thickness, thickness of the main vein, height and width of vascular bundle of main vein, vessels, palisade cells, adaxial and abaxial epidermis cells, and cuticle thickness of adaxial epidermis.

### *Leaf physiological characteristics*

The concentrations of mineral element in leaves were analyzed. Plant material dried at 100°C was milled prior to testing. Nitrogen concentration was determined by the standard micro Kjeldahl method (Nelson and Sommers, 1973). Concentration of total phosphorus was determined spectrometrically by the ammonium-vanadate-molybdate method. Concentrations of potassium and sodium were determined by the flame photometric method. Calcium in the stock solution was determined with an atomic absorption spectrophotometer (Sarić et al., 1990). The leaf mineral element concentration was expressed as mg $g^{-1}$ dry matter.

The concentration of photosynthetic pigments was determined spectrometrically (DU Series 60 Spectrophotometer) using the Wettstein method (Wettstein, 1957; Lichtentaler and Wellburn, 1983).

Net photosynthesis and dark respiration were determined polarographically, using an oxygen electrode (Walker, 1989). The rate of photosynthesis was evaluated by the quantity of released oxygen ($\mu molO_2 cm^{-2} h^{-1}$). The dark respiration was determined by the quantity of absorbed oxygen ($-\mu molO_2 cm^{-2} h^{-1}$). Leaf segments used for analysis were taken from fully formed, unda-

maged leaves, avoiding main veins, and suspended in a buffer pH 7.6—7.8 containing 10 mM $NaHCO_3$. The rate of photosynthesis was measured under complete white light saturation, while the rate of respiration was observed in the dark (P a j e v i ć et al., 1999a).

Leaf area of pinnately compound leaves was determined using a leaf area meter apparatus LiCOR 3000 (USA).

The obtained results were statistically processed by the analysis of variance, with LSD-test for means separation, for the level of significance of p = 0.05. Significance of the average values was determined by the Duncan test.

## RESULTS

### *Anatomical parameters*

#### Stomata number and size

The average stomata number per $mm^2$ in the analyzed black locust clones was considerably larger on the abaxial than on the adaxial side of leaves, 205 and 18 per $mm^2$, respectively (Table 1).

The stomata number on the adaxial side was between 10 (R-135) and 28 (R-56) per $mm^2$ while on the abaxial side it was between 125 (R-135) and 245 (R-115) per $mm^2$. A comparison of the stomata number variations on the adaxial and abaxial sides showed that the variability was larger on the abaxial epidermis. When compared with the standard, the analyzed clones had a somewhat larger stomata number on the adaxial side and a smaller stomata number on the abaxial side.

Table 1. Leaflet stomata number and size for five black locust clones

| Clone | Number/$mm^2$ | | Size on adaxial epidermis (μm) | | Size on abaxial epidermis (μm) | |
|---|---|---|---|---|---|---|
| | Adaxial epidermis | Abaxial epidermis | Length | Width | Length | Width |
| Standard | 11 b | 239 a | 15.3 c | 7.1 a | 11.8 a | 6.3 a |
| R-56 | 28 a | 190 b | 16.0 bc | 6.3 b | 10.2 b | 4.8 bc |
| R-135 | 10 b | 125 c | 17.5 a | 6.1 b | 10.5 b | 4.6 c |
| R-54-1 | 25 a | 228 ab | 16.2 bc | 7.2 a | 12.6 a | 6.0 a |
| R-115 | 16 b | 245 a | 16.5 ab | 6.8 a | 10.5 b | 5.1 b |
| Average | 18 | 205 | 16.3 | 6.7 | 11.1 | 5.3 |

Note: Means with the same letter within columns did not differ significantly at pP within columns did lumns did.

Adaxial stomata were larger than abaxial ones in all analyzed clones. Largest stomata were found in the leaflets of the standard and clone R-54-1, the smallest in clone R-56. The most conspicuous interclonal differences were found for the adaxial stomata length and the abaxial stomata width.

## Leaflet structure

Anatomically, leaflets of the black locust clones are dorsiventral. Their single-layer epidermis has a relatively thick cuticle with verrucose thickenings. The abaxial epidermis is subpapillar. Nonglandular uniseriate trichomes composed of 2—3 short basal cells and an elongated curved terminal cell that is easily separated from basal cells occur in the epidermis. They are numerous on the abaxial epidermis.

The mesophyll is differentiated into palisade and spongy tissues where the former is composed of single-layer elongated cells arranged at the right angle below the adaxial epidermis. A layer of considerably shorter cells is found below them. The spongy tissue is composed of only 2—3 layers of cells which positioned at the right angle with the epidermis. A layer of large, chlorophyll-free, water storage cells designated as hypodermics occurs below the abaxial epidermis (M e t c a l f e and C h a l k, 1957). The palisade and hypodermal tissues contain numerous cells, which are larger than the surrounding ones and which are filled with an orange tanniferous content. Ca-oxalate crystals are visible in certain mesophyll cells.

The main vein is round and conspicuous abaxially. A large vascular bundle with fornicate sclerenchyma tissue along its phloem portion and a small group of sclerenchyma fibers occur along the xylem. Only a few layers of chlorophyll-free parenchyma cells are found around the vascular bundles. Subepidermally, colenchyma was present in the main vein. In the phloem portion, large cells filled with tanniferous content are visible.

## Leaflet and mesophyll structure

The investigated clones were characterized by a relatively small leaflet thickness, ranging from 128 μm (R-135) to 165 μm (standard clone and R-56) (Table 2).

The thickest mesophyll was found in the standard (154 μm), the thinnest in clone R-54-1 (99 μm).

## Parameters of the main vein

The average thickness of the leaflet main vein was 359 μm (Table 2). The differences among the clones were insignificant. The largest difference in main vein thickness existed between the standard (385 μm) and clone R-54-1 (320 μm). The variations in bundle height were somewhat larger than those in bundle width. When compared with the other investigated clones, the standard had the largest main vein. There were no significant differences in the height and width of the bundle tracheae of the main vein among the investigated clones. The average height and width values were each 13.4 μm.

Table 2. Leaflet anatomical characteristics (m) for five black locust clones

| Clones | Thickness among veins | Mesophyll thickness | Thickness on main vein | Main vein vascular bundle | | Vessel diameter | |
|---|---|---|---|---|---|---|---|
| | | | | Height | Width | Height | Width |
| Standard | 165 a | 154 a | 385 a | 246 a | 296 a | 13.8 a | 13.0 a |
| R-56 | 165 a | 141 a | 382 a | 255 a | 275 a | 13.1 a | 12.8 a |
| R-135 | 128 b | 103 b | 328 a | 213 ab | 248 a | 13.0 a | 13.1 a |
| R-54-1 | 131 b | 99 b | 320 a | 187 b | 266 a | 13.2 a | 13.0 a |
| R-115 | 161 a | 132 a | 378 a | 226 ab | 302 a | 13.9 a | 14.9 a |
| Average | 150 | 126 | 359 | 225 | 277 | 13.4 | 13.4 |

Note: Means with the same letter within columns did not differ significantly at pP within columns did lumns did.

## Leaflet cell size

The highest palisade tissue cells (48 μm) were found in the standard clone, the shortest (33 μm) in clone R-54-1. Cell width was between 6.8 μm (R-56) and 8.2 μm (R-115) (Table 3). The palisade tissue cells of the investigated clones were more variable in height than in width.

The largest height of the adaxial epidermal cells was found in the standard (13.2 μm), the largest width in clone R-56 (19.9 μm). The smallest cells were found in clone R-54-1. Cuticle thickness of the adaxial epidermis was between 1.9 μm (R-56) and 3.6 μm (R-54-1).

The abaxial epidermis, which was almost papillose, had larger cell height than cell width. The largest cells of the abaxial epidermis were found in the standard clone, the smallest in R-56. Some interclonal differences were observed in the height of the abaxial epidermis cells and in the adaxial cuticle thickness (Table 3).

Table 3. Leaflet cells size (m) for five black locust clones

| Clone | Palisade cells | | Adaxial epidermis cells | | Abaxial epidermis cells | | Cuticle thickness |
|---|---|---|---|---|---|---|---|
| | Height | Width | Height | Width | Height | Width | (adaxial) |
| Standard | 48.0 a | 7.4a | 13.2 a | 15.3 a | 16.1 a | 12.3 a | 2.3 ab |
| R-56 | 42.0 ab | 6.8a | 11.1 a | 19.9 a | 11.5 b | 12.3 a | 1.9 b |
| R-135 | 47.8 a | 7.4a | 13.1 a | 18.3 a | 12.8 ab | 12.7 a | 3.2 ab |
| R-54-1 | 33.0 b | 7.3a | 11.4 a | 15.4 a | 14.8 ab | 13.2 a | 3.6 a |
| R-115 | 43.9 a | 8.2a | 12.0 a | 15.3 a | 11.3 b | 13.9 a | 3.2 ab |
| Average | 42.9 | 7.4 | 12.2 | 16.8 | 13.3 | 12.9 | 2.8 |

Note: Means with the same letter within columns did not differ significantly at pP within columns did lumns did.

## Physiological parameters

The average leaf area of a single pinnately compound leaf was between 49.74 $cm^2$ (R-115) and 81.48 $cm^2$ (standard). The investigated clones showed smaller leaf areas than the standard (Table 4).

The highest dry leaf mass was obtained with the standard clone. The smallest value was obtained with clone R-115.

The smallest specific dry leaf mass was found in clone R-135 (76 mg $cm^{-2}$), the greatest in the standard clone (101 mg $cm^{-2}$).

Table 4. Some morphological and physiological characteristics of black locust leaves

| Clone | Leaf area of whole compound leaf ($cm^2$) | Dry mass of 10 leaves (g) | Specific mass of dry leaves (mg/$cm^2$) |
|---|---|---|---|
| Standard | 81.48 a | 8.26 a | 101 |
| R-56 | 61.84 bc | 5.42 b | 87 |
| R-135 | 73.52 ab | 5.64 b | 76 |
| R-54-1 | 62.27 c | 5.66 b | 90 |
| R-115 | 49.74 d | 4.80 b | 96 |
| Average | 65.77 | 5.96 | 90 |

Note: Means with the same letter within columns did not differ significantly at pP within columns did lumns did.

## Concentration of photosynthetic pigments

The highest concentration of photosynthetic pigments was obtained with clone R-56 (Table 5). According to the pigment concentration, all clones were classified into two groups, i. e., the group with the highest pigment concentration (R-56) and the group that included all other clones. The chlorophyll a:b ratio was about 5:1.

Table 5. Concentration of photosynthetic pigments (mg/g dry matter) and rates of photosynthesis and respiration ($\mu molO_2 cm^{-2} h^{-1}$) for five black locust clones

| Clone | Chl. *a* | Chl. *b* | Chl *a*+*b* | Carotenoids | Photo-synthesis | Dark respiration |
|---|---|---|---|---|---|---|
| Standard | 3.75 b | 0.83 ab | 4.64 b | 3.33 b | 2.01 c | 1.77 b |
| R-56 | 4.62 a | 0.94 a | 5.56 a | 3.99 a | 3.83 ab | 2.98 ab |
| R-135 | 4.04 ab | 0.85 ab | 4.88 ab | 3.52 ab | 3.19 bc | 4.18 a |
| R-54-1 | 3.48 b | 0.63 bc | 4.12 b | 3.14 b | 4.69 a | 3.90 a |
| R-115 | 3.55 b | 0.57 c | 4.11 b | 3.27 b | 2.77 bc | 3.12 ab |
| Average | 3.89 | 0.76 | 4.66 | 3.45 | 3.30 | 3.19 |

Note: Means with the same letter within columns did not differ significantly at pP within columns did lumns did.

## Photosynthesis and dark respiration

The data related to the rate of photosynthesis and dark respiration are summarized in Table 5. The highest rate of photosynthetic oxygen evolution was obtained in clone R-54-1 (4.69 $\mu molO_2 cm^{-2} h^{-1}$). Clones R-135 and R-54-1 could be separated from the others by the highest dark respiration (–4.18 and –3.9 $\mu molO_2 cm^{-2} h^{-1}$, respectively).

## Concentration of macronutrients and Na

The differences among the clones in the concentrations of the investigated elements are presented in Table 6.

Table 6. Foliar concentration of N, P, K, Ca and Na (mg $g^{-1}$ dry matter) for five black locust clones

| Clone | N | P | K | Ca | Na |
|---|---|---|---|---|---|
| Standard | 28.67 b | 0.55 b | 4.75 a | 22.00 b | 0.65 a |
| R-56 | 30.42 a | 0.71 a | 4.83 a | 23.00 b | 0.70 a |
| R-135 | 28.03 b | 0.53 b | 2.67 bc | 34.00 a | 0.75 a |
| R-54-1 | 26.48 c | 0.41 c | 3.33 b | 31.00 a | 0.72 a |
| R-115 | 24.47 d | 0.47 bc | 2.50 c | 25.83 b | 0.62 a |
| Average | 27.61 | 0.53 | 3.62 | 27.16 | 0.69 |

Note: Means with the same letter within columns did not differ significantly at pP within columns did lumns did.

Nitrogen concentration was between 24.47 mg $g^{-1}$ (R-115) and 30.42 mg $g^{-1}$ (R-56). The N concentration found in clone R-56 was significantly higher than those found in the other clones. Potassium content was between 2.50 (R-115) and 4.83 mg $g^{-1}$ (R-56). The average phosphorus content amounted to 0.53 mg $g^{-1}$, with the minimum and maximum values ranging between 0.41 mg $g^{-1}$ (R-54-1) and 0.71 mg $g^{-1}$ (R-56). According to the calcium content, the clones were classified into two groups, with clones R-135 and R-54-1 having significantly higher calcium concentrations than the others. The minimum and maximum values for calcium were between 22.00 mg $g^{-1}$ and 34.00 mg $g^{-1}$ (variability of 35%). The leaf sodium concentration was low, showing no statistically significant differences among the investigated clones.

## DISCUSSION

Variability within *R. pseudoacacia* species represents an important factor in the selection of fast-growing genotypes. Therefore, it is absolutely essential to define individual cultivars according to their physiological traits like net photosynthetic rate per unit leaf area, stomatal conductance, chlorophyll content and total leaf area, particularly when significant correlation between the

photosynthetic rate x total leaf area and growth traits is taken into account (Mebrahtu and Hanover, 1991). There are abundant data showing the possibility of improving the growth of the black locust by selection and breeding for large leaf area, high rates of net photosynthesis, and low rates of dark respiration (Mebrahtu et al., 1991). The selection aimed at the development of high-yielding varieties (clones) requires the knowledge of leaf anatomy. Leaf anatomy, stomata characteristics, and the organization of the photosynthetic and vascular tissues are important traits (Fromard et al., 1995). Surveys of variability of these traits are focused on the selection of genotypes with desirable traits.

The clone leaflets are amphystomatic while their stomata are of the anomocytic type (Metcalfe and Chalk, 1957). Number and size of stomata rely upon plant species, leaf position, leaf portion, and the environmental conditions. Orlović (1993, 1996) reported the existence of positive correlations between the stomata number on one side and plant growth parameters on the other in poplar species. Of the investigated clones, R-115 showed an increased total number of smaller stomata. Such a number of stomata per area unit and their small dimensions are characteristic for plants adapted to arid conditions (Janković, 1990).

The lower epidermis anatomy of leaflets of the investigated clones and the presence of leaf cells filled with tanniferous content are characteristic for a large number of *Leguminosae* genera. The presence of hypodermis is noteworthy when drought adaptability is discussed.

Since palisade and spongy tissues are most interesting from the production aspect, blade thickness and mesophyll thickness undoubtedly point to the photosynthetic traits and biomass production (Orlović, 1993). Genotype R-56 had significantly highest values of blade and mesophyll thickness compared with all other genotypes except the standard.

The size of cells of the photosynthetic tissue is also important. The inner photosynthetically active area size increases as the cell size decreases. An earlier investigation confirmed that the more productive poplar clones have small cells in the photosynthetic tissue (Orlović et al., 1994). Of the investigated clones, clone R-56 showed the narrowest and consequently the most numerous cells in the palisade tissue, taking into consideration their arrangement in a layer, thus forming the greatest photosynthetically active area. Also, this clone had the largest number of adaxial stomata, providing $CO_2$ to its palisade tissue. The above points to a possibility of significant photosynthetic activity. Genotype R-56 which showed the thickest blade also had a smaller leaf area size than the remaining genotypes. Since plant leaf area size significantly affects the photosynthetic capacity and therefore productivity, it points to a significant role of thickness, i. e., mesophyll volume, in photosynthetic productivity (Ceulemans and Saugier, 1991).

The synthesis and accumulation of organic matter are achieved by the process of photosynthesis, and thus the influence of factors determining plant productivity could be better assessed if photosynthetic potential of individual leaves is determined (Bugbee and Salisbury, 1988; Pajević et al., 1999b). Investigations of genotypic variation in photosynthesis and transport

include both physiological and anatomical factors. The structural organization of leaves represents a significant factor for interpretations of these processes (Kubinová, 1994; Syvertsen et al., 1995). According to the results in this study, it seems that clone R-56 possesses the ability for high organic productivity, as confirmed by the high photosynthetic rate. Compared to the others, clone R-56 developed the thickest leaves, with the highest mesophyll volume. This clone was also characterized by the highest concentration of photosynthetic pigments. According to Kebede et al. (1992), the structural and functional organization of the vascular tissue is significant from the aspect of assimilate transport and final yield. Clone R-56 possesses well-developed main vein vascular bundles, just like the standard. Nevertheless, it is difficult to reliably define genotypic specificity for photosynthetic activity since this process is significantly affected by many factors. Austin (1993) reported that difficulties in standardizing the status of a photosynthetic organ are due to its age, time of measurement, and some abiotic factors, so the possible differences could not be ascribed exclusively to the genotype.

Although photosynthesis has been the focus of much measurement and modeling, little is known about plant respiration (Mitchell et al., 1999), a process essential for the construction of new tissues and for the maintenance of existing tissues (Bolstad et al., 1999). Increased efficiency of respiration is the main objective in defining genotypes with desirable traits (Penning de Vries, 1975). Compared with the rate of photosynthesis, interclonal divergence was more pronounced for dark respiration rate. Overall, leaf respiration ranged between –1.77 (standard) and –4.18 (R-135) $\mu molO_2 cm^{-2} h^{-1}$. Compared with standard, the other clones were divided into two groups. Highest respiration rates were recorded in clones R-135 and R-54-1. Studying interspecific and environmentally induced foliar respiration variations in deciduous tree species, including *Robinia pseudoacacia*, Mitchell et al. (1999) revealed considerable interspecific variability. These authors also reported that respiration rate varied vertically through the canopy (with light gradient). Hence, these factors, among others, must be taken into consideration when studying leaf respiration.

The mineral composition of genotypes from the same habitat and environmental conditions is frequently specific, representing a genotypic characteristic (Sarić and Loughman, 1983). Therefore, the determination of mineral composition in selection and breeding programs offers a more detailed picture of the ability of an individual clone to assimilate the available nutrients.

Differences are present in physiological and biochemical properties of macronutrients (Kastori, 1983). The requirements for macroelements and microelements have been studied in detail in field crops, whereas only limited data are available on the locust tree.

Nitrogen (N) is necessary for the formation of important components of proteins and chlorophyll, thus directly affecting plant photosynthetic performance. The supply of N to the forest trees relies upon factors controlling organic matter decomposition and the release of mineral N (Harris and Riha, 1991). Also, *R. pseudoacacia* is N-fixing plant (leguminous tree) and the amo-

unt of N taken up and accumulated in the shoots of a plant significantly increases with inoculation (Rohm and Werner, 1992; Turk et al., 1993).

By comparing the obtained results on the concentration of individual elements in the locust leaflets with those of woody plants like plum, apple, and pear (Bergman and Henbert, 1976), correspondence may be established for N and Na, whereas significantly lower Ca and P contents were reported for the locust tree. The characteristic concentration ratios of individual elements in the locust tree (N:P = 10:1 and Ca:K = 5:1) are different (N:P = 50:1 and Ca:K = 10:1 in most of the studied clones and Ca:K = 5:1 in the standard and R-56). The extremely high leaflet N accumulation in clone R-56 concurrent with a high P accumulation give priority to the selection of genotypes having favorable leaf area and mass, i. e., leaf specific mass since contents N and P, as biogenic elements, significantly affect the leaf area and mass. In addition, N and P concentrations in the leaf tissue vary throughout the growing season (Vogel and Dawson, 1993).

Phosphorus concentrations in different plant species range from 0.05 to 1.0%. An extremely low value, amounting to 0.05% on the average, was obtained in the surveyed leaflets of the black locust genotypes. The effect of abiotic factors on P accumulation in leaves is significant, particularly in the leguminous trees where legume rhizosphere and acidification and consequently an increased N and P availability to crop trees are present (Gillespie and Pope, 1990). In spite of that, significant genotypic specificity of leaf P concentration was evident.

Field and vegetable crops and the fruit plants have the potassium content between 1 and 2.5%. Forest species (fir, pine, and birch) possess considerably lower potassium concentrations ranging from 0.1 to 1.0%. Irrespective of clone, the obtained potassium concentration in black locust leaves was 0.36%. K and Cl ions in leaf cells considerably affect turgor shift and together with Ca considerably affect the structure and function of cell walls (Moysset et al., 1991).

Calcium uptake relies on a large number of abiotic factors (Wiersum, 1979). The effect of Ca on the soil physical and chemical properties is important since it influences the acidity and P availability. Reduced Ca causes an increased N2-fixing capacity of a symbiont (Liu and Deng, 1991). A complex physiological role of Ca primarily implies the maintenance of the structure and function of cellular membrane, enzyme activation, and so on. Total Ca in plants ranges from 0.5 to 3.0% in dry mass. Plant Ca content does not show actual plant requirements for this element. Instead, a large amount of Ca, in old leaves in particular, is bound as Ca-oxalate. The investigated black locust clones showed a high leaf Ca of 2.7% on the average.

Sodium belongs to the group of useful elements since its presence may have a favorable impact on the growth and development of cultivated plants (sugarbeet, etc.) while no data are available for forest plants like the black locust. The recorded average Na concentrations in black locust leaves were quite low, amounting to 0.068%.

## CONCLUSION

Of the investigated black locust clones, clone R-56 was distinguished for the highest N, P, and K accumulation, the largest mesophyll volume and the highest pigment concentration. We concluded that clone R-56, although without a remarkable leaf area, possesses the ability for high photosynthetic production. Selection of potential parents in a plant breeding program requires wide and comprehensive physiological and anatomical analyses of available genotypes.

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# ВАРИЈАБИЛНОСТ АНАТОМСКО-ФИЗИОЛОШКИХ ОСОБИНА КЛОНОВА БАГРЕМА (*Robinia pseudoacacia* L.)

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Резиме

Варијабилност унутар врсте *R. pseudoacacia* L. представља важан фактор у селекцији генотипова који се карактеришу брзим растом и великом органском продукцијом. Стога је веома важна идентификација супериорних генотипова на основу њихових анатомских и физиолошких особина.

Овај рад представља резултате истраживања генотипске варијабилности главних анатомских параметара (фреквенција, дужина и ширина стома, дебљина лиске између нерава, дебљина лиске на главном нерву, дебљина мезофила, дужина и ширина проводног снопића главног нерва) и физиолошких особина (лисна површина, садржај фотосинтетичких пигмената и садржај N, P, K, Ca, Na) код пет клонова багрема (*Robinia pseudoacacia* L.). Утврђена је значајна варијабилност проучаваних параметара међу клоновима. Клон R-56 је имао највећу концентрацију N, P и K, дебљину мезофила и концентрацију пигмената. Закључили смо да клон R-56 поседује особине које омогућавају високу органску продукцију. Добијени резултати биће употребљени у селекцији.

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# NUTRITION OF PHEASANTS UNDER CONDITIONS OF INTENSIVE AGRICULTURAL PRODUCTION IN THE VOJVODINA PROVINCE

ABSTRACT: We examined the content of crops in 672 pheasants of both sexes. The pheasants have been shot at all seasons of the year in different hunting grounds in the Vojvodina Province. Out of total number of pheasants, 145 were shot in the spring, 128 in the summer, 229 in the fall and 179 in the winter. The examined pheasant crops contained mostly the seeds of weeds and grasses (62.2%), culminating in the fall (67.7%), and green feed (61.6%), culminating in winter and spring (79.8% and 70.4%, respectively), while the other kinds of feed were found in a considerably smaller number of pheasants. According to the average number of consumed particles, the seeds of weed and grass were in the first place (374 pheasants), culminating in the fall (431 pheasants), while the numbers of particles of other feeds were considerably lower, from 78% to 93%. According to quantity, corn was in the first place with the annual average of 8.4 g that culminated in the winter (11.3 g). The average proportion between the herbal and animal parts in feed was 99.6 : 0.4 g in favor of the herbal feed, with seasonal variations from 99.2 : 0.8 g in the summer to 99.9 : 0.1 g in the winter. Analyzing herbal proportions in feed, we saw that generative plant parts prevailed over the vegetative parts (92.0 g and 7.6 g, respectively). The percentage of animal parts present in the rations of pheasants was reduced over last 30 years from 33.5% to 0.4%.

KEY WORDS: intensive agriculture, nutrition, pheasant

## INTRODUCTION

It is known that one of the basic factors that influences game populations in hunting grounds located in level open lands is the availability of different feed resources, of both herbal and animal origin. However, the meliorative actions and modern cultural practices applied in recent decades have changed the steppe into intensively cultivated agricultural land. Major agricultural crops like wheat, corn and barley have been replaced by crops that are more interesting for the market such as sugarbeet, sunflower and soybean, which has caused reductions in the number of herbal species. Intensive land management restrains the production of secondary plants (weeds) in favor of economically more important plants used for nutrition of humans and domestic animals.

Additionally, agrochemical practices (application of agricultural chemicals and mineral fertilizers) tend to reduce the production of components of game feed (Hanuš and Fišer, 1983; Jovanović, 1998; Točka, 1983; Zabloudil, 1984, 1986) like insects, mollusks and worms. Some authors think that the modern reorganization of plant production may be defined as a major change of the environment for the wild game (Libosvar, 1955).

The intensive plant production caused reductions in the numbers of different game species in the hunting grounds of the Pannonian plain (Havasi and Varadi, 1988). Therefore, we decided to examine the nutrition of pheasants in the Vojvodina Province under the prevailing ecological conditions in order to assess possibilities of helping the game by improving management practices in the hunting grounds.

## MATERIAL AND METHODS

The study lasted for 10 years (1990—1999). The study material were the contents of crops of 672 adult pheasants shot at all seasons of the year in different hunting grounds of the Vojvodina Province. The contents of crops were examined in 145 pheasants in the spring, 128 in the summer, 229 in the fall and 179 in the winter. The contents were investigated in laboratory. For every season we examined the kind of feed (proportion of animal and plant parts), number of particles (pieces), quantity of various feeds and seasonal changes in feed composition. The obtained results were analyzed and tabulated.

## RESULTS AND DISCUSSION

Table 1 presents the annual and seasonal frequencies of consumption of various feeds. It can be seen that the seeds of weeds and grasses were consumed by the majority of the pheasants (62.2%) throughout the year, varying from 56.5% in the summer and 67.7% in the fall.

Table 1: Seasonal frequency of intake of certain feeds in the period 1990—1999

| Component | Season | | | | | | | | Total | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Spring | | Summer | | Autumn | | Winter | | | |
| | № | % | № | % | № | % | № | % | № | % |
| Green plant particles | 102 | 70.4 | 61 | 47.6 | 131 | 57.2 | 120 | 70.8 | 414 | 61.6 |
| Weed and grass seeds | 85 | 58.6 | 72 | 56.5 | 155 | 67.7 | 106 | 62.3 | 418 | 62.2 |
| Wheat | 9 | 6.2 | 22 | 17.2 | 18 | 7.8 | 13 | 7.6 | 62 | 9.2 |
| Barley | 13 | 8.9 | 12 | 9.3 | 11 | 4.8 | 5 | 2.9 | 41 | 6.1 |
| Corn | 27 | 18.6 | 27 | 21.1 | 105 | 45.8 | 45 | 26.5 | 204 | 30.4 |
| Other cultivated plants | 7 | 4.8 | 3 | 2.3 | 7 | 3.1 | 3 | 1.8 | 20 | 3.0 |
| Sunflower | 3 | 2.1 | 15 | 11.7 | 27 | 11.8 | 2 | 1.2 | 47 | 7.0 |
| Fruits and roots | — | — | 4 | 3.1 | 16 | 6.9 | 10 | 5.9 | 30 | 4.5 |
| Animal feed particles | 81 | 55.8 | 33 | 25.8 | 41 | 17.9 | 5 | 2.9 | 160 | 23.8 |

Green feed, i.e., vegetative plant parts, were in the second place according to consumption frequency, with the annual value of 61.1% and seasonal variations from 47.6% to 70.8%. These findings were in accordance with the results of Tarasenko and Jovanović (1981), Havasi and Varadi (1988), and partially in accordance with the findings of Točka (1983).

Fruits (blackberries, elderberries, mullberries, etc.) and roots of some plants were found in only 4.5% of the crops, 3.1% in the summer, 6.9% in the fall and 5.9% in the winter.

Coleopterans, ant eggs and larvae, fly eggs, worms and other insects were found in the crops of 23.8% of the pheasants. These findings were in accordance with the findings of Havasi and Varadi (1988), Točka (1983) and Tarasenko and Jovanović (1981) (Table 2).

Table 2: Average number of particles (number of seeds) in feed consumed per season in the period 1990—1999

| Component | | Spring | Summer | Fall | Winter | Year |
|---|---|---|---|---|---|---|
| Green plant particles | Average | 47 | 30 | 22 | 44 | 31 |
| | Variation | 1—134 | 3—96 | 2—98 | 3—168 | 1—168 |
| Weed and grass seeds | Average | 112 | 301 | 431 | 108 | 347 |
| | Variation | 1—918 | 2—511 | 1—453 | 4—862 | 1—918 |
| Corn | Average | 25 | 12 | 34 | 38 | 31 |
| | Variation | 1—107 | 1—49 | 1—159 | 1—182 | 1—182 |
| Wheat | Average | 29 | 102 | 53 | 4 | 51 |
| | Variation | 2—73 | 2—538 | 1—94 | 3—138 | 1—538 |
| Barley | Average | 93 | 29 | 87 | 5 | 71 |
| | Variation | 5—212 | 2—181 | 4—136 | 3—45 | 2—212 |
| Sunflower | Average | 45 | 48 | 89 | 9 | 78 |
| | Variation | 4—62 | 2—241 | 1—360 | 1—150 | 1—360 |
| Other cultivated plants | Average | 23 | 37 | 14 | 3 | 49 |
| | Variation | 14—324 | 2—58 | 1—16 | 1—5 | 1—324 |
| Fruits and roots | Average | — | 6—32 | 3—94 | 2—38 | 2—94 |
| | Variation | — | 6—32 | 3—94 | 2—38 | 2—94 |
| Animal feed particles | Average | 15 | 13 | 2 | 2 | 11 |
| | Variation | 1—207 | 1—269 | 1—44 | 2—8 | 1—269 |

It can be seen in table 2 that the largest average number of particles per year consisted of the seeds of weeds and grasses (347 seeds), which ranged from 431 to 108. These findings were in agreement with those of Nagy (1972), Hanuš and Fišer (1983), Tarasenko and Jovanović (1981) and Točka (1983).

The average numbers of seeds of cultivated plants (wheat, barley, corn, sunflower, soybean and broomcorn) found in the crops were considerably lower and these could be found mainly during the times of seeding and harvest.

The average number of particles of green feed, i.e., vegetative plant parts, was highest in winter and spring (44 and 47, respectively). This confirmed the findings of Nagy (1972) and Tarasenko and Jovanović (1981).

Plant fruits and roots were found in the crops during summer, fall and winter, which was in accordance with the findings of Havasi and Varadi (1988).

The average numbers of particles of animal origin were highest in spring and summer; the amounts of this kind of feed found during fall and winter were practically negligible. These findings were in according with the findings of most of the authors that had performed similar examinations in areas of intensive agricultural production (Havasi and Varadi, 1988; Nagy, 1972; Šelmić and Adamović, 1959; Tarasenko and Jovanović, 1981; Točka, 1983) (Table 3).

Table 3: Average weight of consumed feed (in grams) for the period 1990—1999

| Component | | Spring | Summer | Fall | Winter | Year |
|---|---|---|---|---|---|---|
| Green plant particles | Average | 2.1 | 1.1 | 1.2 | 2.2 | 1.3 |
| | Variation | 0.1—0.6 | 0.1—14.1 | 0.1—5.1 | 0.1—8.5 | 0.1—14.1 |
| Weed and grass seeds | Average | 1.4 | 3.2 | 15.8 | 1.3 | 1.7 |
| | Variation | 0.1—6.5 | 0.1—14.6 | 1.7—24.7 | 0.1—22.5 | 0.1—24.7 |
| Corn | Average | 7.0 | 4.2 | 7.7 | 11.3 | 8.4 |
| | Variation | 0.1—29.1 | 0.6—33.4 | 0.1—34.3 | 0.2—60.8 | 0.1—60.8 |
| Wheat | Average | 1.3 | 7.3 | 2.9 | 0.7 | 2.5 |
| | Variation | 0.1—2.5 | 0.1—15.1 | 0.1—3.5 | 0.1—4.0 | 0.1—15.1 |
| Barley | Average | 3.5 | 1.2 | 5.7 | 0.2 | 3.0 |
| | Variation | 1.3—9.4 | 0.1—4.8 | 0.2—8.3 | 0.1—20.4 | 0.1—20.4 |
| Sunflower | Average | 3.6 | 3.3 | 10.3 | 2.0 | 7.2 |
| | Variation | 0.1—7.2 | 0.1—16.3 | 0.1—22.5 | 0.1—88.6 | 0.1—88.6 |
| Other cultivated plants | Average | 0.9 | 0.2 | 0.6 | 0.3 | 0.5 |
| | Variation | 0.3—18.7 | 0.1—1.6 | 0.1—1.0 | 1.1—3.2 | 0.1—18.5 |
| Fruits and roots | Average | — | 12.4 | 16.5 | 1.2 | 3.2 |
| | Variation | — | 1.3—19.2 | 0.3—22.5 | 0.1—9.7 | 0.1—22.5 |
| Animal feed particles | Average | 0.6 | 0.8 | 0.2 | 0.1 | 0.4 |
| | Variation | 0.1—4.2 | 0.1—4.8 | 0.1—1.8 | 0.1—0.3 | 0.1—4.8 |

According to the average annual quantitative data shown in this table, the first group consisted of corn (8.4 g) and sunflower (7.2 g) although these amounts were far behind the number of seeds of weeds and grasses. These findings were in accordance with the results of Tarasenko and Jovanović (1981) for the Vojvodina Province and the results of Havasi and Varadi (1988) for the Hungarian plain. The second group consisted of wheat (2.5 g), barley (3.0 g) and roots (3.2 g). The third group consisted of seeds of weeds and grasses (0.17 g) and green feed (1.3 g). These findings were in accordance with those of Tarasenko and Jovanović (1981), Havasi and Varadi (1988), Libosvar (1955), Nagy (1972), Točka (1983) and Zabloudil (1984, 1986). The last group consists of animal feed, with the annual average of only 0.4 g.

Table 4 shows the results of the analysis of seasonal changes in the feed of adult pheasants.

Table 4: Seasonal composition of feed of adult pheasants

| Season | Green plant feed | Of which | | Animal feed |
|---|---|---|---|---|
| | | Generative parts | Vegetative parts | |
| Spring | 99.4 | 95.6 | 3.8 | 0.6 |
| Summer | 99.2 | 91.6 | 7.6 | 0.8 |
| Fall | 99.8 | 89.9 | 9.9 | 0.2 |
| Winter | 99.9 | 96.9 | 3.9 | 0.1 |
| Year | 99.6 | 92.0 | 7.6 | 0.4 |

While feeds of herbal origin were present throughout the year (99.6%), the feed of animal origin was present in the nutrition of pheasants from the hunting grounds in the Vojvodina Province with only 0.4%. Generative parts predominated in the feeds of plant origin. Those were mostly seeds; flowers and buds were preset in small quantities.

Analyzing the pheasant crop contents in this study, it could be noticed that the nutrition of pheasants became phytophagous for the entire year, evidently due to the previously described environmental changes. These changes in the living environment were so chaotic and fast in the last decade that the game could not adapt to them (Libosvar, 1955). The intensity of these changes is illustrated by the facts presented in Table 5.

Table 5: Changes in the proportion of plant and animal feeds observed in hunting grounds over the period of 30 years

| Year and author | 1959 Šelmić | 1976 Hanuš-Fišer | 1981 Tarasenko et al. | 1988 Havasi et al. | 1991 Our findings |
|---|---|---|---|---|---|
| Green plant feed | 66.5 | 75.5 | 91.7 | 98.6 | 99.6 |
| Animal feed | 33.5 | 24.5 | 8.3 | 1.4 | 0.4 |

The studies of the changes in the type of feed, performed over last 30 years by Šelmić and Adamović (1959) and Tarasenko and Jovanović (1981) in the Vojvodina Province, Hanuš and Fišer (1983) in Slovakia and Havasi and Varadi (1988) in Hungary, confirmed that in the period from 1959 to 1991 the average annual proportion of the animal part in the pheasant feed dropped from 33.5% to only 0.4%. It means that the pheasants may be considered as having become phytophages. This fact certainly influences the life and survival of pheasants in the Vojvodina hunting grounds. In the first weeks of their life, young pheasants are given feed of animal origin, so the current situation influences the actual annual growth rate of pheasants in nature. In the case of adult pheasants, which have to satisfy all their needs by consuming plants, it has not been determined how this undifferentiated nutrition influences their growth and change in body weight. This problem should be addressed by experts in this field.

## CONCLUSION

Contents of crops of 672 adult pheasants of both sexes were examined over the 10-year period (1990—1999). Laboratory analyses of the contents produced the following results.

The crops of the majority of the pheasants mostly contained the seeds of weeds and grasses (62.2%), culminating in the fall (67.7%), and green feed (61.6%), culminating in winter and spring (70.8% and 70.4%, respectively). The other feeds were found in a considerably smaller number of pheasants (Table 1).

The annual average number of consumed feed particles was 8.4 g, culminating in the winter (11.3 g) although the number of field crop seeds was about 90% lower than the number of weed and grass seeds (Table 3).

The average annual proportion between plant and animal parts in feed was 99.6 : 0.4 in favor of plant feed, with the seasonal variations from 99.2 : 0.8 g in the summer to 99.9 : 0.1 g in the winter (Table 4).

The presence of feed of animal origin in pheasant rations was reduced over last 30 years from 33.5% to 0.4%. This is an indication of an unfavorable ecology of the hunting grounds of the Vojvodina plain with regard to pheasant nutrition.

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# ИСХРАНА ФАЗАНА У УСЛОВИМА ИНТЕНЗИВНЕ РАТАРСКЕ ПРОИЗВОДЊЕ У ЛОВИШТИМА ВОЈВОДИНЕ

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Резиме

У току 10 година испитиван је садржај вољке код 672 фазана оба пола. Фазани су одстрељивани током целе године на разним ревирима ловишта Војводине, и то 145 фазана током пролећа, 128 у току лета, 229 током јесени и 170 током зиме. Утврђено је да се у вољци током године код највећег броја фазана налази семе корова и трава (62,2%) са кулминацијом у јесен (67,7%) и зелена храна (61,6%) са кулминацијом у току зиме (70,8%) и пролећа (70,4%), док се остале врсте хране налазе код знатно мањег броја фазана. По просечном броју конзумираних честица на првом месту се налази семе корова и трава (374) са кулминацијом у јесен (431), док је број честица других врста хране мањи за 78% до 93%. По маси на првом месту је кукуруз са годишњим просеком 8,4 g и кулминацијом у току зиме (11,3 g). Однос између биљног и анималног дела хране износи у просеку 99,6:0,4 g, са сезонским варијацијама од 99,0:0,8 g у току лета до 99,9:0,1 у току зиме у корист биљне хране. У биљном делу хране преовлађују генеративни делови биљака (92,0 g) над вегетативнима (7,6 g). Проценат заступљености анималног дела хране у оброку фазана смањен је током последњих 30 година са 33,5% на 0,4%.