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JUGOSLAVENSKOG DRUŠTVA ZA FIZIOLOGIJU BILJAKA

Tuheljske Toplice, 18.—22. svibnja 1987.

SAŽECI PRIOPĆENJA

EIGHTH MEETING OF THE YUGOSLAV SOCIETY FOR PLANT PHYSIOLOGY

Tuheljske Toplice, 18th—22nd May 1987

ABSTRACTS

JUGOSLAVENSKO DRUŠTVO ZA FIZIOLOGIJU BILJAKA
YUGOSLAV SOCIETY FOR PLANT PHYSIOLOGY

PLENARY LECTURES

PHOTOSYNTHESIS IN VIVO: COMPLEX MEASUREMENTS OF PHOTOSYNTHESIS OF SUNFLOWER

M. PLESNIČAR

(Institute of Field and Vegetable Crops, Novi Sad)

Characteristic reactions of photosynthesis can be measured simultaneously in photosynthetic material, under the influence of variable environmental conditions, by the application of modern nondestructive methods. By simultaneous measurements of gas exchange (CO_2 and O_2), chlorophyll a fluorescence and light scattering in the leaf, isolated protoplasts, and chloroplasts, data were obtained on the interaction of photochemical and biochemical processes and transport mechanisms in photosyntheis (W a l k e r, 1987). Complex measurements of photosynthesis have contributed to a better understanding of regulatory mechanisms of photosynthesis and to an early detection of disorders in plant metabolism caused by stress, high or low temperature, drought, diseases and chemicals.

The results are presented on the simultaneous measurements of photosynthetic oxygen evolution and chlorophyll a fluorescence in the leaves of sunflower (*Helianthus annuus* L.) by the application of a modified oxygen electrode. Complex kinetics of oxygen evolution and chlorophyll *a* fluorescence were measured in the presence of saturating CO_2 as a function of light intensity. Leaves were studied at different stages of plant development. Data were obtained on potential maximum rates and quantum yield of photosynthetic oxygen evolution for some wild varieties, parental lines and NS sunflower hybrids. Early effects of photoinhibition on photosynthetic reactions were detected. The significance of complex measurements of photosynthesis in the course of plant growth and development is discussed in relation to biomass production and yield formation.

Walker, D. A., 1987: The use of the oxygen electrode and fluorescence probes in simple measurements of photosynthesis. Packard Publishing Ltd. Chichester. 1—144.

METABOLISM AND DETOXICATION OF FLUORINE IN PLANT

R. KASTORI AND *N. PETROVIĆ*

(Faculty of Agriculture, Novi Sad)

Although fluorine is not an essential element for plants, its metabolism and effect on them have started to be studied intensively since fluorine became a frequent pollutant, especially in industrial areas.

Among the many physical and chemical properties which distinguish fluorine from other elements, two of them are responsible for its taking part in plant metabolism: the capacity of forming a covalent bond with the carbon atom and the similarity in the size of fluorine atom with the hydrogen atom and the hydroxyl ion. These two properties facilitate the inclusion of fluorine in plant metabolism and its incorporation in some organic compounds such as fluorine acetate and fluorine citrate. The presence of fluorine has been established in lipids of some plants (w--fluorine-fatty acids) and in ribose.

Both organic and inorganic fluorine can be found in plants. The role of organic compounds of fluorine in plant metabolism has not been de-

finitely established. It appears that it functions protectively since it is toxic to mammals and insects. Another hypothesis is that the synthesis of organic compounds including fluorine is a form of plant protection against excess fluorine. On the basis of the specific distribution and metabolism of calcium in many crops grown in conditions of fluorine excess, it is concluded that calcium plays a role in plant detoxication from fluorine.

BIOSYNTHETIC POTENTIAL OF CULTURED PLANT CELLS

M. M. YEOMAN

(Department of Botany University of Edinburgh, Kings Buildings Mayfield road Edinburgh EH9 3JH)

Plants are still a major source of substances used in the food, flavour, fragance and pharmaceutical industries. Cultured plant cells have the potential to produce either by *de novo* synthesis or by the biotransformation of specific precursors, an impressive range of secondary metabolites. However, the yields of these compounds in cultures of plant cells are often very low and in many instances the secondary products characteristic of the intact plant are not present in cultures from that plant. Also the expression of secondary metabolic activity in cultured plant cells varies between cells in a single culture and also with time in a single culture, both throughout a growth cycle and over successive culture periods.[1] Despite these very considerable problems there is an increasing number of reports of yields of particular secondary metabolites in cultures exceeding those of the intact plant and an industrial process in JAPAN is already operating on a commercial scale for the production of shikonin, a purplish-red pigment with mild antiseptic properties, from cultured cells of *Lithospermum erythrorhizon*.[2]

One possible strategy for the development of a process for the commercial production of a selected metabolite is as follows:[3]

1. Selection of high yielding plants (for the product).
2. Establishment of cultures from selected plants.
3. Clonal selection of desired cells.
4. Establishment of a stable cell line with high production capacity.
5. Optimisation of a production medium and process.

Most workers in the field have adopted this strategy up to and including 4. However, the optimisation of a production medium and the manner in which the cells are subjected to that medium may differ quite radically. Basically there are three approaches:

a) to perform the manipulation within the bioreactor in which the cells are grown and then extract the product at the end of the batch. Or

b) to transfer the cells to another similar design of bioreactor for product synthesis and accumulation and then extract the product at the end of this two stage batch process. Or

c) to immobilize the cells within, or attached to an inert matrix and to carry out the production phase, continuously or semi-continuously over an extended period of time during which the product is released to the medium and extracted. The only commercial process so far reported uses approach.

In this presentation a critical appraisal of the approaches will be made paying particular attention to the immobilisation of plant cells and their potential for the production of high value metabolites.[4]

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2 *Balandrin, M. F., Klock J. A., Wurtele E. S., Bollinger W. H.*, 1985: Science 288, 1154.

3 *Heinstein, P.*, 1985: Journal of Natural Products. 48, 1.

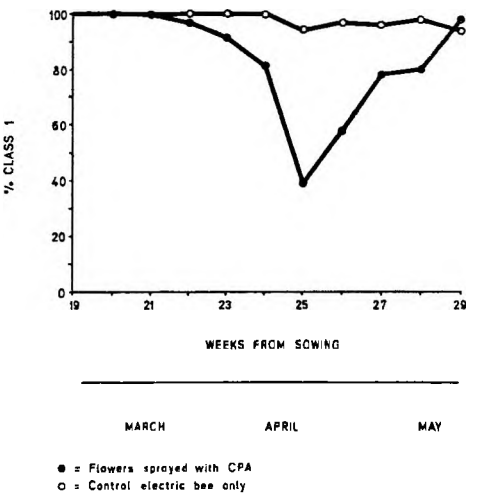
4 *Yeoman M. M.* Ed., 1986: Plant Cell Culture Technology. Blackwell Scientific Publications, Oxford.

HORMONES AND FRUIT QUALITY

A. PICKEN and *M. GRIMMETT*

(Glasshouse Crops Research Institute, Littlehampton, West Sussex)

Growth regulators such as 4CPA and Napthoxy acetic acid are useful for improving fruit set in tomato crops grown outdoors when temperatures may be too low. Research at GCRI has shown that they are not beneficial in overcoming poor fruit set in early heated glasshouse crops when fruit set is not limited by temperature but by low winter light.



In one experiment the overall early (10 week) yield of fruit was not increased by the application of 4CPA to two trusses. However the quality of fruit as indicated by the percentage of Class I was seriously reduced. The graph shows the percentage of Class I fruit at each of the first 11 harvests of an early crop. Fruit quality was most affected in those fruit which had started to swell when light conditions were poorest. There was little loss in quality in fruit formed on young plants or after light levels improved during the spring.

ORGANELLE EXCHANGE

CH. H. BORNMAN

(Cell Biology, Hilleshög Research AB, Landskrona and Department of Plant Physiology, University of Lund, Sweden)

Selection of cybrids (cytoplasmic hybrids) from a heterogeneous population of cells or callus tissues usually involves mechanical or complementation selection techniques. As these techniques may involve chlorophyll deficient or non-allelic mutants, they have little relevance in practical plant breeding.

Existing techniques for the enucleation and evacuolation of protoplasts, as well as for the destruction and/or elimination of chloroplasts, have been adapted in order to produce alloplasmic cybrids in a more direct and controlled manner.

Methods for the substitution of a rapeseed chloroplast genome for that of radish in a male-sterile line of rapeseed will be described. The methods involve use of density gradient centrifugation, inhibition of carotenoid synthesis, pathologic accumulation of protochlorophyllide and electrofusion of cytoplasts and nucleoplasts.

THE ROLE OF SPERMINE IN PROTEIN SYNTHESIS

Ž. KUĆAN

(Department of Chemistry, Faculty of Science, University of Zagreb)

Spermine is known to stabilize the structure of tRNA in solution and in the crystal form. Our previous ESR spectroscopy experiments on spin-labelled yeast $tRNA^{Tyr}$ have shown a marked stabilization of the anticodon region by 5 spermine molecules per tRNA. We have now assayed the effect of spermine on a series of tRNA-involving steps of protein synthesis. The following results were obtained:

(1) Spermine increases the V_m of aminoacylation at low (physiological) Mg^{2+} concentrations; K_m for $tRNA^{Tyr}$ is hardly affected.

(2) The rates of binding of Tyr-$tRNA^{Tyr}$ · EF-Tu · GTP complexes to the A site and of Ac-Tyr-$tRNA^{Tyr}$ to the P site of poly (U_{11}, A)-programmed *Escherichia coli* ribosomes are at least 2.5 fold stimulated by spermine, and so are the rates of binding of the corresponding $tRNA^{Phe}$ derivatives to either poly (U_{11}, A) or poly (U) programmed ribosomes.

(3) Optimal saturation (> 90%) of both sites is reached at spermine/tRNA rations of 3 for $tRNA^{Phe}$ and 5 for $tRNA^{Tyr}$. In contrast, only 9 and 16% saturation of the A and P sites, respectively, was reached with $tRNA^{Tyr}$ derivatives in the absence of spermine.

(4) The mRNA-independent binding to both sites is decreased by spermine to less than 1%.

(5) Finally, the messenger-free, EF-Tu and EF-G-dependent polymerization of lysine from Lys-$tRNA^{Lys}$ is drastically reduced while the poly(A)-directed polimerization is stimulated by spermine.

(6) The effect of spermine on misreading of UUU codons *in vitro* is currently being examined.

All these results suggest that spermine plays an essential role in a series of macromolecular interaction leading to the correct incorporation of an amino acid into protein, at the same time preventing some unspecific or erroneous interactions.

ON THE BIOCHEMISTRY OF THE VOLUME REGULATION OF GUARD CELL PROTOPLASTS

H. SCHNABL

(Institut für Landwirtschaftliche Botanik der Universität Bonn, Meckenheimer Allee 176, 5300 Bonn, FRG)

The opening of stomata requires energy which is probably ATP, synthesized in chloroplasts in light or in mitochondria in the absence of illumination. In order to investigate the energy state of guard cell protoplasts in detail, the changes in the levels of total cellular ATP/ADP were determined during the K^+-induced volume increase of protoplasts in the absence of light (Hampp and Schnabl, 1984). The uptake of K^+--ions by the guard cell protoplasts was coupled with the increase of the ATP/ADP ratio, the stimulation of phosphoenolpyruvate carboxylase activity (S c h n a b l et al., 1982) and the decrease in the K_m for phosphoenolpyruvate within 3 min of swelling (Kottmeier and Schnabl, 1986). Therefore, the final product of the carboxylation step, malate, was proposed to act as a substrate for respiration.

Since the data given by H a m p p and S c h n a b l (1984) were total cellular pools of metabolites, a subcellular compartmentation in plastids, mitochondria and the supernatant was required. A technique for rapid fractionation of guard cell protoplasts from *Vicia faba* was developed including i) selection of suitable markers (plastids: light-dependent oxygen evolution; mitochondria: fumarase; cytoplasm: phosphoenolpyruvate carboxylase, ii) rapid quenching of metabolism and iii) methods for determining metabolite concentrations. Using these techniques, we report the distribution of energy pool sizes and malate in plastids, mitochondria and the supernatant (ATP: 3% in plastids, 31% in mitochondria, 65% in the supernatant; total amount 2.2 fmol protoplast^{-1}; malate: 35% in the mitochondria, 60% in the supernatant; total amount 270 fmol protoplast^{-1}). On the basis of this technique alterations in adenylate pool sizes and malate during the volume changes of guard cell protoplasts were determined in order to ascertain the participation of the oxidative phosphorylation and the role of malate as a possible substrate for respiration during volume changes of guard cell protoplasts.

A GENETIC APPROACH TO THE STUDY OF PHOTOTROPISM AND GEOTROPISM IN *ARABIDOPSIS THALIANA*

K. POFF

(Plant Research Lab. East Lansing, Michigan State Univ. USA)

The goal of this work is to develop a genetic system for the analysis of sensory physiology in plants. *Arabidopsis thaliana* has been chosen for many reasons, including its rapid growth, small size, relatively small genome size, and small amount of repetitive DNA.

Screening procedures have been designed and used to isolate mutants with altered shoot phototropism or altered shoot geotropism. The gravitropic response has been tested for those mutants with altered phototropism. Two strains exhibit altered phototropism and normal geotropism. Thirty three strains exhibited altered phototropism and altered geotropism. In addition, 105 strains have been identified with altered shoot geotropism. In one of these strains the shoots grow randomly with respect to the gravitational vector. However the strain exhibitis normal shoot phototropism and normal root geotropism.

These results demonstrate the feasibility of developing a genetic system for sensory physiology. In addition the data are consistent with a transduction sequence in which shoot phototropism and shoot geotropism share a common pathway, differing at the sensory inputs, but clearly indicate that shoot geotropism has a least one mutable step not critacal for root geotropism.

MECHANISMS OF TRANSPORT PROCESSES IN THE PLANT MEMBRANE

Č. RADENOVIĆ

(Maize Research Institute »Zemun Polje«, Biophysical Laboratory, P. O. Box 89, 11080 Belgrade, Yugoslavia)

A critical analysis is given of the transport processes integrally connected with life functions of the plant cell, organs and organism, which include material, bioenergetic, informational, regulational and thermal dependence. A view is expounded that the ways of transport processes are different and that they occur with several alternatives; they are described with several different mechanisms: passive transport processes, relieved diffusion, ways of active transport (ferments — phosphotransferase system, oxido-reduction ystem, ATP-ase ion transport, co-transport and ionophores). The electrophysiological apect of the above transport processes is particularly analyzed. Cell membranes are emphasized as the central site of many transport processes. Interdisciplinary approach in studying cell membrane and transport processes in it are stressed. In the forthcoming period increasing attention is expected to be paid to the role and function of cell phases (cell wall, cytoplasm and vacuole) in rgulating the courses and mechanism of total transport processes.

Results of long-term studies of bioelectric potentials (Ψ, mV), both of cell phases and membranes are presented. They include data on ion activities; K^+, Na^+, Cl^- and their distribution in the cell. Numerous results on membrane potential oscillations on plasmalema and tonoplast are discussed.

Evidence is provided on the existence of oscillatory ion transport mechanism through the plant membrane. The osciallatory ion transport mechanism through the plant membrane is based on the modern view of the cell membrane structure, and even more so of the mobility of membrane components, primarily of the character of lipid complex mobility; its mobility may be lateral. rotational. translatory. vibrational and oscillatory.

Our hypothesis on the oscillatory mechanism of transport processes through the cell membrane was published in several of our papers and was especially supplemented and described in our recent papers.

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BIOGENESIS AND METABOLISM OF INDOLEACETIC ACID IN PLANTS

V. MAGNUS

(»Ruđer Bošković« Institute, Zagreb)

Indoleacetic acid is formed in plant metabolism from tryptophan. The free hormone equilibrates with a number of conjugates, and it is degraded by catabolic enzymes to yield indolic and non-indolic metabolites.

Research in this laboratory has been focused on the function of indoleethanol in the biogenesis of the growth hormone. That alcohol was shown to be subject to reversible conjugation, in a similar fashion as indoleacetic acid. Out of the esters and glycosides formed, the compounds examined so far sustained elongation growth of stem segments and they could also be used as sources of auxin in plant tissue culture. Amino acid conjugates of indoleacetic acid may also be used as such sources of auxin: they even appear to have certain advantages in practical work.

THE POSSIBILITIES OF LASER RED LIGHT APPLICATION IN PLANT PRODUCTION

G. VASILEVSKI, N. GAJDADŽIEV

(Faculty of Agriculture — Skopje WO MEGO — 18. Noemvri, Gostivar)

Light as an energy source manifests significant effects on seed and plant with three aspects: thermal energy, photosynthesis, photomorphogenesis. The sun light that reaches the earth is not homogeneous but consists of various wave lengths i.e. colours. The photosynthetic active radiation for plants is considered the light of wave lenghts from 390 to 750 nm.

Plants with their phytochromic, phytohormone and fermentative systems absorb of light. Consequently, the bioenergetic potentiality increases and stimulates biochemical and physiological processes.

The theoretical and practical hypothesis referring to the red light activity on the growth and development of plants, have been used to include the laser red light into the group of stimulators. The application of laser light in plant production is based on seeds and plants capability for light absorption, its transformation and utilisation in biomass creation.

The mechanism of laser, monochromatic, coherent and polarised red light activities, results from the possibilities of photons »pumping« of plant cells, i.e. of structural bioplasm elements.

The current experience of laser red light application in field-crop, vegetables, fruit growing and viticulture has shown very good results in the stimulation of plant growth and development, as well as in the yield quantity and quality.

Session 1.

PHOTOSYNTHESIS, RESPIRATION METABOLISM

TEMPERATURE ACCLIMATION OF PHOTOSYNTHESIS IN HOLLY (*ILEX AQUIFOLIUM L.*)

F. BATIČ
(Inštitut za gozdno in lesno gospodarstvo yu-61000 Ljubljana, Večna pot 2)

Holly (*Ilex aquifolium* L.) is one of the few evergreen tree species growing in the temperate climate of Europe. Leaves of the holly can stay on the tree up to eight years. The continuous area of the species is confined to more humide Atlantic regions. The survival of the species deeper in the continent is limited by low winter temperatures and with increasing aridity to the South.

The annual pathway of photosynthesis and its temperature acclimation was measured on holly plants from natural habitat (Uzmani, 650 m) and on plants transplanted into the Botanical Garden of Ljubljana (cca 270 m). Photosynthesis was measured on intact plants outside and on detached holly twigs in the laboratory under controlled conditions. Seasonal acclimation of photosythesis to temperature was determined by temperature compensation point of photosynthesis and by freezing experiments of holly leaves. Photosynthesis was measured by infra-red gass analysis except in field determinations of net photosynthesis where colorimentric method was used.

The results of the measurements show that holly was photosynthetically active throughout the winter. The intensity of photosynthesis is low (maximum is only 6 mg $CO_2 dm^{-2} h^{-1}$) and its seasonal temperature acclimation is specific. In the winter time the plants were photosynthetically active to —7(—9)°C. The recovery of photosynthesis after low temperatures was quick provided that periods of frost were not too long. The ability of acclimation to low temperatures was also confirmed by freezing experiments of holly leaves. In the winter time the ability of leaves to undercool was higher and they froze at lower temperatures withouth greater damages.

The research was carried out at the Institute of Biology, Edvard Kardelj University, Ljubljana.

EFFECT OF VARIED N-NUTRITION ON THE CHLOROPHYLL CONTENT IN THE LEAVES AND THE TOTAL N, PROTEINS, THEIR FRACTIONS, PHOSPHORUS AND POTASSIUM CONTENT IN THE RICE GRAIN

D. POP-MIHAJLOVA and *M. GORGIEV*
(Institut of Rice, Kočani and Faculty of Sciences, Skopje)

This field experiment was carried out according to »Cade« method on alluvial soil presowed with rice. The effect of 70, 80, 90, 100 and 110 kg/ha N applied before sowing and 70 kg/ha N applied before sowing and 10, 20, 30, 40 and 70 kg/ha N applied during the earing period was tested.

With the increase of N-content in the soil from 70 to 110 Kg/ha N, t. e. 140 kg/ha, there was a notable increase of the number of rice plants over one sq. metre, their size, the relative chlorophyll content in the leaves dry substance, phosphorus and potassium content in the gran's dry substance as well as the quantity of grains and straw. When N was applied once, namely before sowing, the 100 kg/ha N-nutrition re-

sulted in highest quantities of grains and straw, and when N was applied twice, i. e. before sowing or during the earing period, best results were noted with 110 kg/ha N-nutrition. Compared with the single application, the double application gave better effects — larger plants with high relative chlorophyll content in the leave's dry substance.

In addition, only the double N-nutrition resulted in safe and significant increase od proteins in the relative content. The highest increase of protein content in the grain's dry substance, 41.2% was noted when N-nutrion was increased from 70 to 140 kg/ha. The increased soil N--nutrition resulted in increased relative content of albumens, globulines, glutelines and compound proteins, whereas the prolamines content did not show any notable changes.

During the grain riping period the content of albumens, compound proteins (in rice plants once nourished with N), phosphorus and potassium decreased, whereas the content of globulines and glutelines (particularly in rice plants once nourished with N) increased.

In the Monticelli rice type the grain appeared to have the highest percentage of compound proteins content, the content of glutelines, albumins and globulines was lower and the content of prolamines was the lowest.

ON THE ANATOMICAL AND PHYSIOLOGICAL FEATURES OF LEAVES OF SOME ORNAMENTAL PLANTS

N. PLAVŠIĆ-GOJKOVIC, M. BRITVEC, Z. GREGOV and *K. DUBRAVEC*
(Institute for Plant Protection; Department for Agricultural Botany, University of Zagreb)

Comparative investigations of anatomical features and transpiration (Tr) of the leaves of *Diospyros lotus* L. (W. Asia, China), *Catalpa bignonioides* Walt. (N. America) and *Reynoutria japonica* Houtt. (Japan) were carried out.

Tr of leaves was determined by Stock's method in natural conditions of the Maksimir park in Zagreb. Anatomic features of the leaf blades were studied. The degree of stomata opening was determined by infiltration method (ethanol, xylol). Measurements of microclimatic factors were performed using standard techniques.

It has been found that the leaves of the species investigated are dorsiventral and hypostomatic with the exception of *Reynoutria japonica*, which had a negligible stomata density (0.90/mm^2) on the upper epidermis. The leaf blades were of different thicknesses *(Catalpa bignonioides* — 165 μ; *Reynoutria japonica* — 215μ; *Diospyros lotus* — 216μ). A double layer palisade tissue was found for *Catalpa bignonioides* species. Stomata density was in the range from 89 *(Reynoutria japonica)* to 258 *(Catalpa bignonioides)* per mm. The degree of stomata opening varied during the daytime.

Minimal Tr value was measured in *Diospyros lotus* (2.88 mg/g/min.) while the highest value was determined for *Reynoutria japonica* (21.25 mg/g/min). The transpiration dynamics was considerably influenced by the changes of the microclimatic factors. Tr curves had single maxima.

On the basis of these results it is possible to conclude the following:

— According to the Gračanin's Tr intensity classification *Diospyros lotus* has medium (4.62 g/g), *Catalpa bignonioides* has high (5.76 g/g) whereas *Reynoutria japonica* has very high Tr intensity (8.06 g/g).

— Correlation between Tr intensity and stomata density was not found.

CHANGES IN THE PLANKTON PRIMARY PRODUCTION AND CHLOROPHYLL *a* IN THE SPECIAL ZOOLOGICAL RESERVE OF KOPAČKI RIT

J. HORVATIĆ

(Pedagoški fakultet, University of Osijek)

The plankton primary production (Knöpp 1960) as well as the concentration of the chlorophyll *a* (Strickland and Parsons 1965) were investigated during the spring and summer periods in 1984, on the localities of Kopačko Lake, Sunčani and Hulovski Canal.

According to vertical water layers the greatest plankton primary production takes place in the surface water of all localities investigated. The summer values of the plankton primary production in the surface layer were higher by 24.8%, at a depth of 0.5 m by 3.15% and at a depth of 1 m by 8.83% in comparison with those of the spring period in 1984.

Regular increase in the chlorophyll *a* concentration from the spring (8.83 μg dm^{-3}) towards the summer (20.77 μg dm^{-3}) was obvious at the localities of Kopačko Lake and Hulovski Canal. Maximal summer concentrations of the chlorophyll *a* were conditioned by the maximum development of the representatives of Chlorophyta as well as by the water flowering due to Cyanobacteria.

PHYSIOLOGY OF HIGH-LYSINE BARLEY

Z. LUTHAR and I. KREFT

(Agronomy Department, Biotechnical Faculty, E. Kardelj, University Ljubljana, 61001 Ljubljana, Yugoslavia)

High-lysine gene *lys-3a* has pleiotropic effects such as shrivelled seeds, altered endosperm enzyme contents and/or activity, altered endosperm ultrastructure, larger embryo, smaller average kernel weight, lower content of starch in endosperm, higher content of sugars in endosperm, lower germinative faculty, lower net flow of photosynthates into kernels and altered growth of roots.

It is possible that the *lys-3a* barley gene, at some stage of ripening, by the altered enzyme content and/or activity, partially blocks the conversion of sugar to starch, and that the high concentration of sugars in the endosperm prevents the unloading of photosynthates from the transport mechanisms. Furthermore, these factors may also lower the endosperm starch content, cause shrivelled seeds and smaller kernel mass and lower the yield. It has been proposed that a yield improvement of high-lysine barley could be achieved by a higher number of kernels per unit of field area. It is not possible to increase the number of kernels per unit of field area. It is not possible to increase the number of kernels per unit of land area above a certain level, which may be reached in modern high-yielding barley cultivars, without an enchroachment into the regulation of barley plant morphogenesis. There is a need to construct a new barley ideotype, taking into acount the properties of high-lysine genes.

EFFECT OF GROWING AREA AND APPLICATION RATES OF NITROGEN ON THE CHARACTERISTICS OF LEAF AREA OF SAGE (*SALVIA OFFICINALIS* L.)

E. KOTA and R. KASTORI

(Faculty of Agriculture, Novi Sad)

Laef area is of great importance for the yield of cultivated plants in general, but especially in those plants whose leaves are economically useful, as is the case with sage. Considering this fact, we tested the effect of five levels of nitrogen in field conditions as well as the effect of twelve vegetative area sizes on the following leaf area indexes: leaf area index (LAI), leaf area density (LAd), leaf area ratio (LAR) and specific leaf area (SLA) in the first and second years of growing of sage.

The following conclusions were made according to the results: when the size of vegetative area was decreased and the application rates of nitrogen were increased LAI was increased in both years. When the size of the vegetative area was decreased and the application rates of nitrogen were increased LAd value increased, while the LAR value decreased in both cases. When the size of the vegetative area increased, the SLA value decreased, and when the application rates of nitrogen increased, the SLA value also increased.

LAI, LAd, LAR and SLA values were highest when 120 N kg/ha were applied. The yield of the herb was also the highest.

THE ROLE OF GLYCOLITIC ENZYMES IN ANAEROBIC METABOLISM OF CORN

M. ZLOKOLICA

(Faculty of Agriculture Institute of Field and Vegetable Crops Novi Sad)

Inadequate oxygen provision in plants causes changes in the synthesis of glycolitic enzymes. Enzymes are directly related to anaerobic plant metabolism and its adaptation to such stress as anaerobiosis.

Changes in the synthesis of glycolitic enzymes due to a partial lack of oxygen are observed in the homozygous line, in the primary root of corn seedlings. Induced anaerobic proteins (ANP) were labelled with *in vivo* S methionine and analyzed by electroporesis on 2D—PAA gel using autoradiography. The identification of proteins was made on the basis of the standard molecular weight.

The synthesis of ANP was quantitatively and qualitatively different from the synthesis of proteins in aerobic conditions. Of almost twenty synthetized proteins, the following proteins were identified: ADH 1, ADH 2, Fr. 1, 6 dif. aldolase, Phos. hex isomerase (PHI 1) and sacharose synthetase (SS). Gene expression for SS 1 isoenzyme in anaerobic induced root of homozygous line was marked, while in the »shrunken« mutant of corn it was absent.

On the basis of ANP synthesis as a gene product, we observed that this group of genes was perfectly coordinated and responsible for plant survival in anaerobic conditions.

NET ASSIMILATION RATE IN NS SUNFLOWER HYBRIDS AND THEIR PARENTAL LINES IN THE COURSE OF PLANT DEVELOPMENT

Z. SAKAČ, D. SAFTIĆ, T. ĆUPINA and M. PLESNIČAR

(Institute of Field and Vegetable Crops, Novi Sad)

The aim of this work was to determine net assimilation rate and growth parameters for six sunflower genotypes at different stages of their development.

Sunflower plants (*Helianthus annuus* L.) were grown in the experimental field of the Institute of Field and Vegetable Crops, Novi Sad. Following genotypes were studied: hybrid NS-H-43 and its parental lines OCMS-22 (♀) and RHA-SNRF (o), hybrid NS-H-26-RM and its parental lines CMS-HA-V-8931-3-4 (♀) and RHA-58 (o). Leaf area and dry mass of leaves and other plant parts for six plants of each genotype were measured once a week in the course of vegetation.

The results obtained show significantly higher values for the leaf area, dry mass productions, and total dry mass of hybrid plats in relation to their parental lines at all examined stages of development. Net assimilation rate, determined as the increase of dry mass per unit leaf area per day, was highest at initial stages of development for all examined genotypes. Leaf weight ratio (LWR) increased to the 25th day and then decreased in the course of vegetation, in all genotypes. Specific leaf area (SLA), after an initial rise, remained constant for some time. SLA then increased in NS-H-43 and decreased in its parental lines at the stage of flowering. Leaf area ratio (LAR) showed similar tendencies as leaf weight ratio and reflected a decreased accumulation of photosynthates in the leaves in the process of plant senescence.

Relative growth rate (RGR) decreased in the course of vegetation, while crop growth rate (CGR) increased till full anthesis and then decreased in all genotypes examined. Crop growth rate was higher for the hybrids as compared with their parental lines.

LIGHT, DAY MASS PRODUCTION AND YIELD OF NS SUNFLOWER HYBRIDS AND THEIR PARENTAL LINES

T. ĆUPINA, M. PLESNIČAR, Z. SAKAČ, D. SAFTIĆ, D. MIHAJLOVIĆ and F. ACH

(Institute of Field and Vegetable Crops, Novi Sad)

Sunflower hybrid NS-H-43 and its parent lines OCMS-22 (♀) and RHA-SNFR (o), and hybrid NS-H-26-RM and its parent lines CMS-HA-V-8931-3-4 (♀) and RHA-58 (o) were investigated in this experiment. The experiment was conducted at the experimental field of the Institute of Field and Vegetable Crops. The size of the elementary experimental plot was 126 sq. m.

We studied the dynamics of dry mass production per plant and per unit area. The distribution of dry mass over different plant parts in the course of vegetation was studied too. The efficiency of utilization

of photosynthetically active radiation (PAR) per sunflower plant/genotype was calculated as the following ratio:

$$\frac{\text{Chemical energy of dry mass (calorimetric measurement)}}{\text{Energy of incident PAR}}$$

Photosynthetically active radiation on the level of the crop was calculated on the basis of global radiation measurements in sunflower crop, from emergence to the physiological maturity, i. e., from May to September. Efficiency of solar energy utilization was expressed in % per plant and per plant part. Special attention was paid to the calculation of efficiency of light energy utilization in seeds in the course of their development and filling.

The results obtained show significant differences among the hybrids examined and their parent lines in respect to dry mass production per plant parts and the entire plant. Likewise, efficiency of light energy utilization was different for plant parts and genotypes examined. The hybrids were more efficient users of photosynthetically active radiation per plant than the lines. This indicator had an increasing trend from the stage of head forming to wax maturity.

ORIGIN OF ASSIMILATED CARBON IN SOME TERRESTRIAL AND SUBMERGED MOSSES ACCORDING TO ISOTOPE ANALYSES

D. SRDOČ and *E. MARČENKO*
(Ruđer Bošković Institute, Zagreb)

The origin of carbon in samples of mosses *Cratoneurum commutatum* (Hedw). Roth and *Neckera crispa* (L.) Hedw. was determined from isotope ratios using mass spectrometry for ^{13}C *vs* ^{12}C ratio and a proportional counter for the measurement of the radioactive isotope ^{14}C. The distribution of carbon isotopes depends on the source of carbon dioxide and on isotope fractionation process occurring in the course of photosynthesis. Isotope ratios are constant for atmospheric carbon dioxide, but subject to considerable variation for carbon dioxide dissolved in fresh water; they were studied previously for carbon dioxide from either source in the region of the Plitvice Lakes.

Such isotope rations were now determined for the moss *Cratoneurum commutatum* which grows in the area of waterfalls and karst springs and, for comparison, for the terrestrial moss *Neckera crispa* and for a representative sample of terrestrial higher plants. As the values obtained for *Neckera crispa* and C_3 — cormophytes growing in the same area (e. g. *Fagus silvatica* L. and *Picea excelsa* (Lam.) Lk. were not substantially different, it is concluded that this terrestrial moss assimilates carbon dioxide from the atmosphere using the Calvin-Benson cycle (C_3-plants). It is reasonable to assume that the aquatic moss *Cratoneurum commutatum* uses the same pathway of photosynthesis. If this is true, then it follows from our isotope analyses that *Cratoneurum* takes up carbon dioxide dissolved in water, that it does not split the bicarbonate ion and that it does not utilize atmospheric carbon dioxide even when exposed to the atmosphere for prolonged periods of time. These results provide a background for explaining the process of calcium carbonate precipitation in fresh waters, and they are of more general significance as a contribution to the physiology and ecology of mosses.

A STUDY OF THE ROLE OF INDIVIDUAL LEAVES IN THE COURSE OF SUNFLOWER (*HELIANTHUS ANNUUS* L.) DEVELOPMENT

D. SAFTIĆ, M. PLESNIČAR, T. ĆUPINA and Z. SAKAČ

(Institute of Field Vegetable Crops, Novi Sad)

It is well known that photosynthetic activity of individual leaves *in vivo* depends on the developmental stage of the plant and on the position of the leaf measured. We studied the role of individual leaves by analyzing growth parameters during the ontogenesis of sunflower (*Helianthus annuus* L.).

Six sunflower genotypes were used: hybrid NS-H-43 and its parental lines OCMS-22 (♀). RHA-SNRF (o), and hybrid NS-H-26-RM with its parental lines CMS-HA-V-8932-3-4 (♀), RHA-58 (o). Six fieldgrown plants were excised once a week for measurements of leaf area and dry mass production of individual leaves.

The dynamics of leaf area growth as well as the rate and duration of leaf growth for each individual leaf were calculated. Differences were observed not only among individual leaves of each plant but also among leaves in the same position on the genotypes examined. Hybrids have larger leaf areas and leaf growth rates than their parental lines, but differences among hybrids and their paternal lines were smaller than differences among hybrids and their maternal lines.

Plant leaf area, which reaches its maximum size during anthesis, differed in hybrids and their parental lines. The total number of leaves per plant was different too. The accumulation of organic substances in leaves mostly followed the dynamics of leaf area growth. Specific leaf weight of young leaves can be twice that of the leaves which have achieved their maximum area.

The results obtained offer a basis for future investigations of plant photosynthetic activity during ontogenesis.

DEVELOPMENT OF THE PHOTOSYNTHETIC APPARATUS IN MAIZE CHLOROPLASTS — CYTOCHEMICAL STUDIES

M. WRISCHER

(Ruđer Bošković Institute, Zagreb)

The localization of photosynthetic activity during chloroplast differentiation of maize leaves was followed cytochemically *in situ* at ultrastructural level. For detection of the activity of photosystem I (PS I) the photooxidation of DAB (diaminobenzidine) was used and for detection of the activity of photosystem II (PS II) the photoreduction of TCNBT (thiocarbamyl nitroblue tetrazolium).

The activity of PS I could be detected already in the primary thylakoids of plastids from young cells at the leaf base. On the contrary, the activity of PS II was observed not earlier than in the first grana regions of both young mesophyll and bundle sheath chloroplasts. During further differentiation of bundle sheath chloroplasts, parallelly with the disappearance of grana, the photoreduction of TCNBT weakened.

In grown up mesophyll and bundle sheath chloroplasts all thylakoids were DAB positive, although the inner thylakoids of the grana were

often much less stained. The osmiophilic reaction products were always deposited inside the thylakoids. Diformazan — reaction product of the photoreduction of TCNBT appeared interthylakoidally mostly in grana regions of grown up mesophyll chloroplasts. In fully grown bundle sheath chloroplasts this reaction was very weak.

SEASONAL CHANGES IN CHLOROPLASTS OF BLACKBERRY LEAVES

Z. MODRUŠAN and M. WRISCHER

(Ruđer Bošković Institute, Zagreb)

The ultrastructure, pigment content and photosynthetic activity of chloroplasts in blackberry leaves (*Rubus fruticosus* L. s. l.) were studied during spring, summer, autumn and winter. Changes in chloroplast ultrastructure were quantitatively determined by morphometric analysis.

The ultrastructure of young spring leaves shows characteristics of the young leaf tissue with the maximum value of photosynthetic activity. The fine structure of these leaves changes parallelly with their development and maturation through summer and autumn. Chloroplasts of the summer leaves have a well developed thylakoid system with big grana, high content of pigments and normally high photosynthetic activity. Reduction of the thylakoid system, enlargement of plastoglobules, fall of pigment content and photosynthetic activity are features of chloroplasts in the autumn leaves, so these leaves decay gradually.

New leaves which grow up in the late summer and early autumn remain on the shrubs through the winter and finish their lifespan in late spring. These leaves are exposed to low temperatures and short days through the period from autumn till winter and thus they are adapted to temperatures below 0°C. The winter leaves have large chloroplasts and high content of pigment but their photosynthetic activity is relatively low. These chloroplasts of winter leaves have a well developed thylakoid system and numerous plastoglobules in abundant stroma. Chloroplasts of overwintered leaves have a reduced thylakoid system and numerous starch grains as well as large plastoglobules in the stroma. The overwintered leaves decay in late spring.

BREAKDOWN OF CHLOROPHYLL IN THE LICHEN THALLUS OF *HYPOGYMNIA PHYSODES* (L.) NYL. AS AIR POLLUTION INDICATOR

F. BATIČ

(Inštitut za gozdno in lesno gospodarstvo yu-61000 Ljubljana, Večna pot 2)

The breakdown of chlorophyll *a* in the thallus of epiphytic lichen *Hypogymnia physodes* (L.) Nyl. was used as a physiological indicator of air pollution in the Slovenian forest dieback survey carried out in 1985.

The chlorophyll *a* concentration in the lichen thallus was compared with parameters like condition of epiphytic lichen vegetation on the

forest dieback research plots, with sulphur content of lichen thallus and spruce needles and with the degree of damage to the observed forest stands.

Very low chlorophyll *a* content in the lichen thallus, especially in the lichen samples collected in 1986 indicates the influence of air pollution. Sulphur content in the lichen thallus is higher in comparison with that in spruce needles, which clearly suggests air pollution by sulphur dioxide. The most damaged lichen thalli with very low chlorophyll content usually do not contain much sulphur. The amount of destroyed chlorophyll in the lichen thalli, its sulphur content and damages to the thallus (chlorosis, necrosis, regenerative thallus growths) are not in best correlation, either with the state of epiphytic lichen vegetation on the forest dieback research plots or with the degree of forest stand damage.

DYNAMICS OF THE CONTENT OF PHOTOSYNTHETIC PIGMENTS IN DIFFERENT WHEAT CULTIVARS

Ž. S. STANKOVIĆ, M. R. SARIĆ, B. KRISTIĆ and S. ZATEZALO

(Institute of Biology, Faculty of Sciences, Novi Sad)

The results of chlorophyll and carotenoid contents in individual leaves at earing and milk-waxy maturity in 15 wheat cultivars (1) Novosadska rana 2; 2) Partizanka; 3) Balkan; 4) Osječanka, 5) Super Zlatna; 6) Kragujevčanka 56; 7) Skopljanka; 8) Jugoslavija; 9) Posavka; 10) Zvezda; 11) Mačvanka; 12) Baranjka; 13) Zelengora; 14) Nizija, and 15) Žitnica) are presented. The cultivars under investigation were characterized by the same growing season while their genetic origin was different. Plants were grown under greenhouse conditions where optimum mineral nutrition, temperature, and humidity were coupled. Also, plants were grown in the field with optimum cultivation measures.

The results obtained show that pigment content depended on the cultivar included in the investigation, developmental phase and growing conditions. The highest chlorophyll content in plants grown in greenhouse was recorded in cvs. 9, 4, 7 and 1, whereas the lowest in cvs. 5, 6, and 10 at both phases analyzed. In all cultivars investigated chlorophyll content, at milk-waxy maturity, was higher or the same when compared with earing, with the exception of cv. 15 where it was lower. Also, significant differences were recorded in chlorophyll content between individual leaves. In contrast to chlorophyll, carotenoid content in the leaves analyzed varied to a greater extent between individual cultivars. The highest carotenoid content was recorded in cvs. 1, 4, and 11, and the lowest in cvs. 5 and 14. In particular, differences were evident between the developmental phases. In most cultivars, carotenoid content was lower at milk-maxy maturity than at earing. Such differences were particularly evident in cvs. 1, 14, 9, 11, and 15. On the contrary, cvs. 8, 6, and 4 exhibited increased carotenoid content at milk-waxy maturity.

Pigment content in plants grown in field was lower than in plants grown under greenhouse conditions. Also, pigment content was significantly lower at milk-maxy maturity than at earing. The highest pigment content was found in the second leaf. An extremely high chlorophyll content, at earing, was found in cvs. 7 and 1 while that of carotenoid only in cv. 7.

FORMATION OF CHLOROPHYLL-PROTEIN CHLOROPLAST COMPLEXES UNDER RED LIGHT

D. MILIVOJEVIĆ and D. MARKOVIĆ
(INEP, Institute for the Application of Nuclear Energy, Zemun)

Chlorophyll-protein complexes of maize and black pine chloroplasts were isolated by electrophoresis and classified as: complex I or P_{700} chlorophyll protein; complex II or lightharvesting chlorophyll a/b protein; and, complex III or free pigments.

Maize and black pine plants were grown under the controlled conditions of white (380—700 nm) and red light (600—700 nm) of equal intensity (1.45 W/m^2).

PAGE discontinual SDS electrophoresis was applied according to the method of Anderson et al. (1978).

The results obtained by electrophoretic isolation of the chlorophyll-protein complexes bound to the photosystems I and II of the maize and black pine chloroplasts indicated differing adjustment of the chloroplasts to the influence of red light.

Judging by the percent of light-harvesting chlorophyll a/b protein in the total pigments of thylakoid membranes, under red light the maize chloroplasts reacted as a shadowtype chloroplast, whereas the black pine plants could not adjust in the same manner.

ISOLATION AND PURIFICATION OF MAIZE ENDOSPERM MITOCHONDRIA

V. HADŽITAŠKOVIĆ-ŠUKALOVIĆ and Ž. VUČINIĆ
(Maize Research Institute, POBox 89-Zemun, Yugoslavia)

The aim of our work was to isolate and purify immature endosperm mitochondria from previously frozen plant material, for subsequent use in studies of some mitochondrial enzymes.

Mitochondria were isolated by differential centrifugation and further purified through a Percoll gradient. They were identified by using marker enzymes (succinate:cytochrome c oxidoreductase, NADH:cytochrome c oxidoreductase, malate dehydrogenaze and fumarase). Of the 15 mg protein placed on the Percoll gradient, 2 mg were found in the fraction containing whole mitochondria, and this fraction had low catalase contamination. However, due to freezing, such mitochondria had no intact external membrane, and the intactness of the internal membrane was less than 50% (assayed by NADH:cytochrome c oxidoreductase and malate dehydrogenase, respectively).

Electron microscopy of such mitochondria showed them to be to a large extent broken and contaminated by other membranes and slightly by amyloplasts. Oxygen uptake monitored by O_2 polarography showed that the electron transport chain of such mitochondria was nonfunctional (0.1—0.2 $\mu mol\ O_2$. mg. $protein^{-1}.hr^{-1}$ with succinate, malate and NADH and P/O ratio of 1).

Thus, such mitochondria could be only of limited use in the studies of some of the membrane bound enzymes. Isolation from fresh material, or partial use of the oil extraction technique prior to freezing, is recommended.

INVESTIGATION INTO THE PHOTOSYNTHETIC ACTIVITY OF DIFFERENT INBRED LINES AND HYBRIDS OF SUGAR BEET UNDER WATER STRESS

*M. PETROVIĆ and Ž. STANKOVIĆ**

(Institute of Field and Vegetable Crops, Faculty of Agriculture and Institute of Biology, Faculty of Sciences, Novi Sad)

The results of water stress effect upon the photosynthetic activity of isolated chloroplasts and intact leaves in individual parent components and F_1 hybrids of sugar beet are presented. Investigations were performed under controlled conditions by the method of water cultures. Polyethylene glycol (PEG 6000) was used to provoke water stress. The osmotic pressure of solution was — 10 bars. 9-weeks old plants were exposed to stress for 72 h.

The results obtained show that the rates of ATP synthesis in noncyclic and cyclic phosphorylation were aproxymately 3.5 times lower than that of the control plants. Similar effects were observed in $^{14}CO_2$ fixation, i. e. photosynthesis rate. The effect of water stress upon photosynthetic activity depended also on the lines and hybrids investigated. The highest inhibition was obtained in the lines 4n MM-206 and 4n MM-214 and their hybrids, whereas the lowest in the line 4n MM-213 and the hybrid NS-Hy 194.

On the basis of the results obtained one can conclude that certain correlation occurs between the photosynthetic activity and other physiological parameters (water potential, RWC, water loss rate, etc.), used as criteria for evaluating plant resistance to drought.

EFFECT OF DCMU ON CONCENTRATION OF ATP IN SPINACH CHLOROPLAST DURING PHOTOSYNTHESIS

S. VELJOVIĆ, LJ. JOVANOVIĆ, V. JANJIĆ and R. STANKOVIĆ-KALEZIĆ

(INEP, Institute of Pesticide and Environmental Protection, ZEMUN)

The effect of catalytic amounts of DCMU on stimulation of cyclic electron flow in intact spinach chloroplasts has been investigated. This effect was characterized by measurement of chlorophyll *a* fluorescence and concentration of ATP. O_2 evolution was followed simultaneously.

The light-induced decline of chlorophyll *a* fluorescence in intact isolated chloroplasts is controlled mainly by the oxidation status of the primary acceptor Q (q_Q quenching) and the high energy state of the thylakoid membrane, pH (q_E quenching). q_Q and q_E are largely determined by the availability of ATP and NADPH and their turnover rates. An addition of 0.13 μM DCMU induced the P-S decline which was faster than in the control. The increase observed of the level of ATP pointed to the stimulated cyclic electron flow. In the presence of 0.15 μM DCMU the characteristic transient in the secondary fluorescence kinetics was observed. This relaxation of fluorescence quenching was in correlation with the consumption of ATP in the reactions of the RPP cycle. Thus, DCMU as a poising agent can stimulate cyclic photophosphorylation and enhance the level of ATP in intact isolated chloroplasts.

Session 2.

MINERAL NUTRITION

THE ROLE OF Fe IN PRIMARY NITROGEN ASSIMILATION

N. PETROVIĆ and R. KASTORI
(Faculty of Agriculture, Novi Sad)

The assimilation of N is significantly affected by plant provision with microelements. Mo is a constituent of nitrate reductase (NR) that Mn and Cu occur as constituents or cofactors of enzymes which catalyzes the reduction of NO_3 to NH_4. Considering that Fe is a constituent of feredoxine, cytochrome and numerous Fe proteins, significant for NO_3 reduction, we found it interesting to study the effect of Fe-deficiency and its different concentrations on NR activity.

The experiments were carried out with sugarbeet plants in semi-controlled conditions in the glasshouse. Plants were grown in water cultures on full nutrient solution for three weeks. After that, they were transferred to the nutrient solution containing 1 to 500 mg $Fe.dm^{-3}$. In studying the effect of Fe deficit, after three weeks of growing, plants were transferred to the nutrent solution without Fe.

The following conclusions may be drawn:

Both the deficit and excess of Fe significantly decreased NR activity in sugarbeet leaves and roots.

The most favorable effect of Fe on NR activity, NO_3 content and chloroplast pigment was observed with Fe concentration of 20 and 50 $mg.dm^{-3}$.

NR activity and NO_3 concentration were significantly decreased in leaves and absolute and relative increase in roots by Fe-deficiency.

On the basis of the results obtained we may conclude that Fe stress conspicuously affects NR activity.

FOLIAR NUTRITION OF STRAWBERRY PLANTS (c. v. *ZENGA ZENGANA*) WITH Fe, Zn AND NATURAL PLANT GROWTH BIOREGULATOR

Đ. JELENIĆ and V. LICINA
(Faculty of Agriculture, INEP, ZEMUN)

These investigations concern some aspects of intensive Fe, Zn foliar nutrition of strawberry plants, with variants which include combined application of Fe, Zn and natural plant growth bioregulator NPGB»2G«. Its bioregulator effect upon the physiological growth processes is based on the activity of allelopathic compounds which were extracted by biotechnological methods from weed species, especially from *Agrostema githago*, L. We tried to observe some changes in the mineral composition of treated plants and changes in the activity of the root system if they had been induced. Experiments were done in the following conditions of growth: a) plants were grown in water medium; b) in a complete nutrient solution; c) in a nutrient solution deficient in Fe and Zn. The results were statistically evaluated.

In the variants with the application of iron, we got variations of Fe content at a high level according to its quantity in strawberry organs and between plants, so we were not able to observe precisely what kind of effect simultaneous application of Fe and bioregulator NPGB»2G« produced. However, we obtained very significant differences in combin-

ed treatment with Zn and bioregulator versus control. This treatment was more efficient in increasing Zn level in the leaves than single treatment with zinc.The changes in the root activity which had to be a consequence of foliar nutrition were mostly not observed.

ALUMINIUM AND IRON AS TOXIC AGENTS TO MAIZE GROWTH IN ACID SOILS OF EAST CROATIA AND BOSNIA

V. KOVAČEVIĆ, B. BERTIĆ, I. ŽUGEC, V. VUKADINOVIC and V. KATUŠIĆ
(Agricultural Institute, Faculty of Agriculture, University of Osijek)

It was estimated that about 23% of the world's arable land sustains mineral stress as the major limitation of food production. In the case of strong disorders, the plants show specific symptoms of stress. Thus, retardation of growth and chlorosis (yellowish-green and violet color of the leaves) appeared during early growth stage of maize (stage of 6—9 leaves) especially during 1986. The cold and wet weather was a promoting factor of this disorder.

Since both chlorotic and normal plants existed on the same plot, a comparative analysis was made. Twenty-eight soil and 28 maize (top part) samples were taken from seven typical plots.

In general, chlorotic maize was grown in the soil of lower pH value compared with normal maize (pH in ln KCl = 3.95 and 4.35, respectively). Also, contents of exchangeable Ca and plant available P were lower in the soil with chlorotic maize (68 and 100 mg Ca; 15.7 and 19.7 mg P_2O_5 in 100 g, respectively), while Fe and Al contents (ln HCl extraction) were similar (2993 and 2878 ppm Fe, 1108 and 1156 ppm Al, respectively).

Chlorotic maize was significantly shorter and lighter in weight compared with normal maize (26 and 65 cm; 2.24 and 15.76 g, respectively), while Al and Fe contents were higher (1824 and 387 ppm Al, 1808 and 374 ppm Fe, respectively) and P content was lower (0.30 and 0.47%, respectively) than in the normal plants.

Our results indicate that these disorders were induced by lower P contents and toxic concentrations of Al and Fe in maize plants.

DROP-BY-DROP NUTRITION OF TOMATO ON VARIOUS MEDIA IN GREENHOUSE CONDITIONS

K. SPASOVSKI, T. AVRAMOVSKI
(Institute for Soil Research, Skopje)

In experiments of tomato nutrition in the greenhouse of Lozar-ZIK, Titov Veles, Estrela tomato was grown on the following media: 1. Grahovo peat, as control; 2. Ohrid peat; 3. inert matter of volcanic origin; 4. inert matter + Ohrid peat, 2:1.5; 5. hydroponic medium, and 6. soil.

The ratios of $N:P_2O_5:K_2$ were 1:0.5:1.1. Nutritive solution was applied once a day, and water several times depending on the temperature in the greenhouse. Dropping lasted 5 — 10 minutes with a concentration of 1 — 2 millisimences depending on the development stage of the tomato plants.

The media and 3 tomato plants were placed in poly-ethylene purses (8,000/ha). The hydroponic medium was placed on open poly-ethylene sheets containing a nutritive solution and tomato plants.

The experiments were conducted from September 1981 to February 1982 (autumn-winter period) and from February to the end of June (winter-spring).

The experiments gave the following results:

1. In autumn-winter conditions, the best yields of tomato fruits were 63,074 kg/ha on Ohrid peat, and during the winter-spring period on inert medium + Ohrid peat, amounting to 63,683 kg/ha.
2. The autumn-winter tomato was of a low grade quality in comparison to the winter-spring yields, regarding C-vitamin and sugar contents in the fruits.
3. Tomato grown on soil under the same drop-by-drop nutrition conditions received too much water in comparison with other fruits, while the fruits raised on the inert medium received too little water.
4. Uneven wetting of the hydroponic medium in plastic soil requires a solid base and a small inclination. The fruits mature about a week earlier.

SEASONAL CHANGES OF NITROGEN CONTENT IN LEAF AND FRUIT OF THE PLUM cv. POŽEGAČA AND cv. COUNT ALTHANS GAGE

M. BAŠOVIĆ, Z. ČMELIK and N. MICIĆ
(Agricultural Faculty, Sarajevo)

In order to obtain accurate information about seasonal changes in nitrogen content in leaf and fruit of the plum »Požegača« and »Count Althans Gage« the following was investigated:

— changes of N concentration in the dry mass of the leaf, fruit and certain tissues of the fruit
— acumulation of N in whole fruit and their tissue.

On the basis of the experimental data it can be concluded:

— N concentration was highest in young leaves, fruits and certain tissues of the fruits. The concentration of N decreased while the leaves getting older and during the growth and maturing of the fruit. The highest decrease of N was found in the stone and the smallest in the leaf and seed. The tendency of N decreasing was similar in both varieties of plum.

— N in whole fruit, fruit flesh and seed was accumulated proportionally with the increase of the dry matter. In the stone, N content was unchanged throughout the period of growth and development. It was clear that N accumulation in average fruits was higher in 'Count Althans Gage', because of its size than in fruits of »Požegača«.

A STUDY OF THE ROLE OF POTASSIUM IN NUTRITION OF THE MAIZE HYBRID SK-701

D. STOJANOVIĆ, M. VUČKOVIĆ and M. ĐURĐEVIĆ
(Institute for the Application of Nuclear Energy, INEP, Zemun)

The hybrid SK-701 falls within a group of hybrids with higher drought-resistance than some other varieties. As potassium is one of

the elements responsible for hydrophilicity of the cell, i.e. water binding in the plant cells, this study examined effects of potassium fertilizer on the changing levels of this element in SK-701 kernel during its development from milky phase until physiological maturity. To this end an experiment with the hybrid was set up on soil with medium supply of nitrogen, but well supplied with phosphorus and potassium. The experiment was carried out in two alternatives: a treatment with addition of 60 kg K_2O/ha and the control with no potassium added.

Compared with the control the analysed kernel samples that were taken from the potassium-treated lots once a week for six weeks demonstrated a 10% increase in potassium level. It was also demonstrated that the kernel potassium level correlated with the moisture content of the kernel. Besides, the potassium level in the exposed portion of the plant on the potassium-treated lots was increased in comparison to the controls. Such information, therefore, indicates that SK-701 hybrid responds to the introduction of potassium into the soil which is, otherwise, well provided with this element. The data obtained may be of significance for nutrition purposes, i.e. the production of the hybrid.

RELATIONSHIP BETWEEN UPTAKE OF PHOSPHORUS BY YOUNG MAIZE PLANTS AND THE CONTENT OF INORGANIC AND ORGANIC PHOSPHORUS FRACTIONS IN A CALCAREOUS CHERNOZEM SOIL

S. BLAGOJEVIC, M. JAKOVLJEVIĆ and M. PETROVIĆ

(Faculty of Agricultural Science, Belgrade, Yugoslavia)

The purpose of this investigation was to determine which fractions of inorganice and organic phosphorus in a calcareous chernozem soil could significantly contribute to phosphorus nutrition of young maize plants. Soil samples were taken from a long-term field experiment carried out by Maize Research Institute in Zemun Polje. The content of inorganic phosphorus fractions was determined by the method of Chang-Jackson while organic phosphorus fractions were determined according to the modified procedure of Bowman and Cole. The latter procedure separates organic phosphorus fractions according to their activity in phosphorus cycle. A greenhouse experiment was carried out with three maize plants per pot and it lasted for a period of 28 days after which phosphorus was determined in the plants. Uptake of phosphorus by maize plants (in mg. P_2O_5 per not) was compared with the content of various forms of inorganic and organic phosphorus in the soil.

Significant correlation was found between phosphorus uptake and the following fractions of inorganic phosphorus: aluminium phosphates, iron phosphates, water soluble and weakly bound phosphates. Organic fractions in general do not significantly contribute to phosphorus nutrition of young maize plants. Only labile fraction of organic phosphorus gives significant correlation ($r = 0.625$) with the phosphorus uptake by maize plants. A mathematical model where uptake of phosphorus (in mg P_2O_5 per pot) was given as a function of the content of certain phosphorus fractions in the soil was determined.

EFFECT OF DIFFERENT PHOSPHORUS CONCENTRATIONS ON BARLEY PHOTOSYNTHESIS

S. ZATEZALO, M. KUMMEROVA and Ž. S. STANKOVIĆ

(Institute of Biology, Faculty of Sciences, Novi Sad)

Inorganic phosphorus plays an important role in regulation of plant photosynthesis. Phosphorus influences ATP/ADP ratio, redistribution of photosynthetic products (triosephosphate) between starch and sucrose biosynthesis, activity of enzymes acting in metabolism of photosynthetic products, and enzyme activity in various metabolic processes influencing photosynthesis directly or indirectly. Therefore, particular attention has been devoted to the role of phosphorus in plant metabolism. The complex role of phosphorus however, in physiologic-biochemical processes in plants, frequently provokes disagreement among scientists, in particular when its optimum concentrations in photosynthetic processes are discussed.

The paper deals with the influence of different phosphorus concentrations on total CO_2 assimilation in leaves, isolated protoplasts and chloroplasts, and on certain other parameters acting in this process.

The experiments were conducted by using barley plants grown in nutrient solutions with different phosphorus concentrations [$P_{1/8}$ (3.77 mg/l), $P_{1/2}$ (15.5 mg/l), (control — 31 mg/l). P_2 (162 mg/l) and P_3 (124 mg/l)]. Plants were grown under greenhouse conditions for 30 days, then harvested before chemical analyses were carried out.

Our results shown that as phosphorus concentration in a nutrient solution increased, the photosynthetic activity of leaves, isolated protoplasts and chloroplasts also increased. Increase in photosynthetic activity, however, did not always correlate with other parameters investigated. For example, chlorophyll and carotenoid contents were approximately equal in all variants of phosphorus nutrition, with the exception of the lowest concentration where pigment contents were considerably lowered. Similar results were obtained when dry mass per plant as analyzed. Consequently, irrespective of an increase in the photosynthetic rate, lower phosphorus concentrations ranging from 15.5 to 31.0 mg/l may satisfy demands for optimum organic matter production.

THE INFLUENCE OF N AND K RATIO ON N, P, K AND PIGMENT CONCENTRATIONS IN YOUNG WHEAT PLANTS

V. VUKADINOVIĆ and T. TEKLIĆ

(Faculty of Agriculture, Osijek)

Two wheat varieties (Žitarka and Slavonija) were grown for 30 days in sand culture. Nitrogen (factor A) and potassium (factor B) were added in amounts of 25 and 100 mg. Variety was treated as factor C. Chlorophyll (a and b) and carotenoid concentrations were determined in fresh leaf tissue while N, P and K were determined in dry leaf matter. In addition N, P and K contents in the leaf and the root were determined.

Influence of nitrogen, potassium and variety on N, P and K and pigments in wheat leaves (mg/dm^2)

Property	N		K		Variety*		significance**						
	25	100	25	100	1.	2.	A	B	C	AB	AC	BC	ABC
DM g	1.49	1.62	1.49	1.62	1.65	1.46	++	++	++	−−	−−	−−	−−
N %	3.50	3.66	3.82	3.35	3.90	3.27	−−	++	++	−−	++	−−	+
P %	1.10	0.97	1.08	0.99	1.07	1.00	−+	++	++	−−	−−	+	−−
K %	4.21	3.88	3.06	5.03	3.99	4.10	+−	++	−−	−−	−−	++	−−
Chloro. a	1.54	1.78	1.67	1.65	1.77	1.55	++	−−	++	−−	−−	++	−−
Chloro. b	0.47	0.57	0.51	0.53	0.60	0.43	−−	−−	++	−−	−−	−−	−−
Chloro. a+b	2.01	2.35	2.18	2.18	2.37	1.98	++	−−	++	−−	−−	++	−−
Carotenoid	0.82	0.78	0.84	0.76	0.89	0.71	−−	−−	++	−−	−−	−−	−−

* 1. Žitarka, 2. Slavonija; ** ++ = 0.01 sig., + = 0.05 sig.

A correlative analysis showed significant positive correlations between the chlorophyll (a+b) and dry matter contents. Also, a high, positive, correlation was found between N concentration and the pigment contents tested and a negative correlation was found between the N and K contents.

High varietal differences were expressed among leaf dry matter, N and P contents, N concentration, chlorophyll a+b, as well as carotenoid contents.

A STUDY OF THE EFFECT OF MINERAL NUTRITION IN SUGARBEET

B. JOCIĆ
(Institute of Field and Vegetable Crops, Faculty of Agriculture Novi Sad)

The aim of these experiments was to examine the effect of mineral nutrition on sugarbeet yield and quality after a perod of application of particular nutritive elements. The experiment was established in 1965 with four plant species in the following order: wheat, sugarbeet, maize and sunflower. The experiment was carried out on chernozem soil containing 0.19% of the total nitrogen, 20 mg P_2O_5 and 34 mg K_2O in 100 g of soil. The variants of nutrition were: 1. 0 (without irrigation), 2. N_2, 3. P_2, 4. K_2, 5. N_2P_2, 6. N_2K_2, 7. P_2K_2, 8. $N_1P_1K_1$, 9. $N_1P_2K_1$, 10. $N_1P_2K_2$, 11. $N_2P_1K_1$, 12. $N_2P_2K_1$, 13. $N_2P_2K_2$, 14. $N_2P_3K_1$, 15. $N_2P_3K_3$, 16. $N_3P_1K_1$, 17. $N_3P_2K_1$, 18. $N_3P_2K_2$, 19. $N_3P_3K_2$ and 20. $N_3P_3K_3$. Indices are used to mark 50, 2—100, and 3—150 kg/ha N, P_2O_5 and K_2O, respectively. The experiments lasted 20 years (1966—1985) and the results were analyzed in groups of five years.

In the analyzed period. the effect of application of nutritive mineral elements was different. The maximum root yield increase with the highest doses of fertilizers was 22%, 40%, 78% and 86% for the first, second, third and last five years, respectively.

Nitrogen had the highest effect on root yield of all element. The yield increase compared with that of the control was 11%, 22%, 33%

and 29% in the first, second, third and last five years (1981—1985). Fertilization with phosphorous did not affect the root yield in the first ten years. A significant increase of 15% was obtained from 11 to 15 year and an increase of 24% from 16 to 20 year; potassium alone did not affect the root yield.

When different doses of the three elements were applied at the same ratios, the highest root yield was obtained with the highest doses of fertilizers, however, the only significant differences were of yield obtained with lowest and with highest and medium doses.

The application of nutritive elements differently affected the yield of biological sugar. The applicaton of three elements brought high and constant sugarbeet yields. This assumption can be supported by the results obtained from 1981 to 1985: ferilization with N_2, N_2K_2 N_2P_2 and $N_2P_2K_2$ increased sugarbeet yield 25%, 38%, 68% and 83% respectively, compared to the control. We should emphasize that the highest doses of fertilizer rendered the same sugar yield as medium, or even lower yield.

From the results obtained, a conclusion may be drawn that nitrogen has the highest effect on sugarbeet root yield. However, favourable effects of phosphorus and potassium on sugarbeet root yield, the percentage and content of harmful nitrogen are very important.

EFFECTS OF FERTILIZING DOSES ON VEGETATIVE AND GENERATIVE POTENTIAL OF WILLAMETTE RASPBERRY

M. VELIČKOVIĆ and M. JOVANOVIĆ
(Faculty of Agriculture, Zemun)

Willamette is economically among the most important, leading raspberry cultivars in Yugoslavia and worldwide. Its high producing cultivation depends among other things on the application of modern methods of field cultivation as well as on adequate fruit growing technology.

With the aim achieving high and regular fertility different combinations and concentrations of mineral substances were applied and their effects were estimated on vegetative and generative potentials of Willamette raspberry in the course of two years.

Most favourable effects were achieved in most biophysical and chemical indicators as well as in the total yield of the K_2 — variant.

INFLUENCE OF NITROGEN NUTRITION ON THE QUANTITY AND QUALITY OF CARROTS

M. ČERNE and O. BRECELJ
(Kmetijski inštitut Slovenije, Ljubljana, and Fructal Ajdovščina)

During four year investigations conducted on heavy soil in Jable significantly higher carrot yields were harvested on soil fertilized with nitrogen (56 to 59 t/ha) in comparison with nonfertilized soil (45 t/ha). There was no essential difference between the yield quantities obtained regardless of whether it was fertilized with 100, 150 or 200 kg/ha N.

Investigations continued for three years showed that yields were not essentially higher if higher doses of nitrogen fertilizers were used. Even on lighter soil in Groblje, 100 kg/ha N was enough for the yield to be increased.

High carrot yields were reported in the years 1984 and 1986 (68—74 t/ha) when mean daily temperatures during growth were lower 24.00 to 27.00°C), the number of sunshine hours was smaller (980 to 1050), while the quantity of precipitation was higher (600 to 900 mm) than in the years 1983 and 1985 (33—43 t/ha) when mean daily temperatures were higher (28.00 to 30.00°C), the number of sunshine hours was greater (1300—1340) and the quantity of precipitation was lower (500 to 700 mm).

The quantities of dry matter, total acids and cellulose were the same with fertilized and nonfertilized carrots while the carotene content increased from 200 to 400 kg/ha if fertilized with nitrogen. The quantity of nitrites and nitrates also increased with higher quantity of nitrogen fertilizers, the differences in the quantity of nitrites and nitrates being very high between single years as a result of changing conditions before harvesting.

CONCENTRATION OF SOME ELEMENTS IN ROOT AND ABOVEGROUND PART OF DIFFERENT WHEAT CULTIVARS

M. R. SARIC and V. MOMČILOVIĆ

(Institute of Biology, Faculty of Sciences, Novi Sad)

The 15 wheat cultivars (1. Novosadska rana 2; 2) Partizanka; 3) Balkan; 4) Osiječanka; 5) Super Zlatna; 6) Kragujevačka — 56; 7) Skopljanka; 8) Jugoslavija; 9) Posavska-2; 10) Zvezda; 11) Mačvanka; 12) Baranjka; 13) Zelengora; 14) Nizija and 15) Žitnica) were grown under optimum greenhouse conditions. Plant material was harvested at certain ontogenetic phases of plant development. Experiments were performed in six replications during 1984—1985 and 1985—1986.

This paper describes the two-year results of concentrations of N, P, K, Ca, and Mg in root, stem, blade, and spike at earing.

The results obtained show that a remarkable varietal specificity of the element concentration was obtained. Also, varietal specificity was observed in plant organs analyzed. The elements investigated showed specific variation ranges between the highest and the lowest concentration values. It is worth to be noted that the smallest variatons were recorded ni N concentraton. Its concentration ranged from 12 to 27%, depending on the plant organ. P concentration varied from 23 to 60%, K from 35 to 78%, Mg from 33% to 90%, and Ca from 40 to 230%. It was not simple to simple to ascertain that definite wheat cultivars had the highest or the lowest concentrations of all elements. In addition, all plant organs analyzed were not characterized by maximum or minimum concentrations of the elements considered.

On the basis of the above mentioned, it may be said about the concentration average of different elements per plant but varietal specificity of concentration of a certain element, at the organ level, was also evident. Therefore, this phenomenon has to be considered in solving problems of genetic aspects of plant mineral nutrition.

FIRST INVESTIGATION RESULTS OF GENETIC SPECIFICITY OF *QUERCUS* GENUS MINERAL NUTRITION

N. KOMLENOVIC, J. GRACAN and P. RASTOVSKI

(Forest Research Institute Jastrebarsko)

The growth and nutrition of peduncultura (*Quercus robur* L.), sessile (*Quercus petraea* Liebl.) pubescent (*Quercus pubescens Willd.*) and evergreen (*Quercus ilex L.*) oak were investigated. Different provenances of the pedunculata oak were also studied. The experiment included the following treatments: substratum without fertilizers (A), substratum with fertilizers (B) and natural soil (pseudogley). The special substrata (A and B) prepared from Bosansko Grahovo peat and quartz sand were used to fill paper-pots (Fh 815). Differences related to the growth, concentration and nutrients content in dry matter as well as response to the applied fertilization were established for the species and provenance levels. The greatest heights, diameters and dry matter production were observed in pedunculata oak plants. In connection with this, the hightest contents of almost all the analyzed elements was established in these plants. As a rule, pedunculata oak is characterized by the highest N-concentrations in leaves. All the analyzed organs of pubescent and evergreen oak proved to contain the lowest N-concentration.

Sessile oak is characterized by nigh P and Mg concentrations in root. The P-content in root is as high as that of the pedunculata oak. Besides, P dry matter of pedunculata oak was found to have relatively low contents of magnesium, manganese and zinc.

The root dry matter was predominant in the total amount of dry matter with all the species investigated, except evergreen oak. Evergreen oak is characterised by high dry matter content in leaves.

The fertilisation applied had a considerable influence on the relations mentioned as well as on the concentration and content of nutrients in the plant. The dry matter content of the above ground part significantly increased whereas the root growth increased slightly or was even slowed down.

VARIETAL SPECIFICITY OF MINERAL NUTRITION WITH NITROGEN IN WHEAT

V. TRPOVSKI, M. IVANOVSKI and T. AVRAMOVSKI

(Faculty of Agriculture, Skopje)

A two-year experiment with a few varieties of wheat (Skopjanka, Orovchanka and Prilepchanka) was performed in 1985/86, investigating, the dynamics of nitrogen in the root, stem, leaf, spike and grain.

These were preliminary investigations whose aim was to establish the requirements for differential nitrogen nutrition of those varieties of wheat. Productive characteristics of wheat were studied in nine parameters at the same time.

The results are interesting both from a theoretical and a practical standpoint.

Under equal agrochemical and other conditions depending on the variety of wheat, differences were noticed in nitrogen content in the same parts of wheat and at the same stages of development.

Thus, for instance, in 1986, nitrogen content in the root at the stage of tillering, was 1.75% in Skopjanka and 2.19% in Orovchanka, which is 25.1% more than the former. At the same stage, nitrogen content in the leaf amounted to 4.71% n Skopjanka and 4.92% in Orovchanka, i.e. 45% more. Thus the Orovchanka variety showed higher absorption of nitrogen from the soil, but inadequate distribution over the leaf. At the stages of grain formation and milk maturity, nitrogen content n the leaf was by 23.33% higher in Skopjanka in comparison to Orovchanka.

Until milk- and full maturity in both years, nitrogen in the spike amounted to 0.73% in Skopjanka and 1.19% in Orovchanka, or 63% over the former. This means that the distribution of nitrogen is higher in other parts of the plant than in the spike of Skopjanka variety.

Average yields were highest in Skopjanka, with an amount of 5,205 kg per hectare, followed by Prilepchanka with 4,818 kg and Orovchanka with 4,520 kg/ha.

The reduction of physiologically available nitrogen in the soil after harvest was the lowest in Prilepchanka and the highest in Skopjanka.

NITROGEN CONTENT AND DRY WEIGHT OF PEAS IN DEPEDENCE OF DIFFERENT STRAINS OF *RHIZOBIUM LEGUMINOSARUM*

M. JARAK and Z. SARIĆ

(Facylty of Agriculture, Novi Sad)

Rhizobium leguminosarum is a bacteria widely distributed in the chernozem soil, but its ability of nitrogen fixation is not always high enough. Therefore, we isolated several strains of this bacteria from the soil and examined its efficiency on the pea.

Pea seed was sterilized by 0,1% $HgCl_2$, ethyl alchohol and rinsed several times with distilled water. Seed was sown on sterile sand and each seed was inoculated with 1 ml suspension 10^9 cells of *R. leguminosarum*. Plants were grown in greenhouse and watered with nutritious solution which did not contain nitrogen. Control plants were not inoculated.

At the stage of flowering, plants were analyzed. Shoot and root biomass and nitrogen content were determined.

Dry weight was from 175,48 to 210,49 mg in the shoot per plant and root weight per plant ranged from 91.87 to 173.47 mg per plant.

Nitrogen content in the shoot ranged from 5.47 to 6.84 mg and nitrogen contant in root ranged from 2.12 to 3.58 mg per plant.

NITROGEN NUTRITION SPECIFICITY IN DIFFERENT SOYBEAN CULTIVARS AS DEPENDENT ON ITS SOURCES

M. R. SARIĆ and B. RELIĆ

(Institute of Biology, Faculty of Sciences, Novi Sad)

Five soybean cultivars were inoculated with five different strains of *Rh. japonicum* and grown under greenhouse conditions by using the method of sand cultures, for 50 days. Plants were watered a nitrogen free Reid-York nutrient solution. Two groups of noninoculated plants served as control. One control group was watered with a nitrogen free Reid-York nutrient solution, whereas the other was watered with a full nutrient solution.

Dry mass and nitrogen concentration and content in the whole plant and its individual organs varied in relation to soybean cultivar and *Rh. japonicum* strain employed. In most parameters analyzed a statistically significant increase was recorded as the result of inoculation. Also, in a number of cases, parameter values were greater in inoculated plants grown without mineral nitrogen, in the medium (i. e. molecular nitrogen fixation) than in noninoculated plants having available mineral nitrogen.

The results obtained show presence of a remarkable genetic specificity of both members of the symbiotic system and also a significant influence of an interraction between the two nutrition modes, i. e. by mineral nitrogen and molecular nitrogen fixation, on the parameters analyzed

OCCURENCE OF BLUE-GREEN ALGAE AND FREE NITROGEN FIXING BACTERIA IN SUGAR BEET RHIZOSPHERE

N. MILOŠEVIĆ, Z. SARIĆ and M. GOVEDARICA

(Faculty of Agriculture, Novi Sad)

The occurrence of azotobacter and oligonitrophilic bacteria in rhizosphere depends not only on the plant species (Sarić, Rašović 1963, Sarić 1968, Sarić, Ristić 1982) but also on the quantity and combnations of mineral fertilizers (Sarić 1978).

The aim of the experiment was to examine the occurrence of azotobacter, oligonitrophilic bacteria and blue-green algae in rhizosphere of sugar beet in dependence of the phase of plant development, quantity and combination of mineral fertlizers.

Ten variants were examined with particular fertilizers (N_{100}, P_{100}, K_{100}) in combination of two (N_{100} P_{100}, N_{100} K_{100}, K_{100} P_{100}), NPK combinations ($N_{50}P_{50}K_{50}$, $N_{100}P_{100}K_{100}$, $N_{150}P_{150}K_{150}$) and control. The samples were taken three times in the course of vegetation of sugar beet, from the soil, rhizosphere and root.

Mineral fertilizers differently affected the occurrence of the analyzed group of nitrogen fixing basteria. The largest number of blue-green algae was obtained in the rhizosphere of sugar beet at the stage of intensive growth in P_{100} variant and with N_{100} P_{100} combination. The number of algae on root was low and depended on the stage of plant development and combination of fertilizers.

The occurrence of azotobacter depended on the zone of experiments and stage of plant development. The highest number of azotobacter was obtained in the rhizosphere at the stage of intensive plant growth. In the course of sugar beet vegetation, azotobacter were not found in the root.

The highest occurrence of olgonitrophilic bacteria was obtained in all variants in the rhizosphere and sugar beet root, at the stage of intensive plant growth at the end of vegetation.

DYNAMICS OF DEVELOPMENT OF NODULES IN SOME SOYBEAN VARIETIES IN DEPENDENCE OF *R. japonicum* STRAIN

Z. SARIĆ, N. MRKOVAČKI and V. MILIĆ

(Faculty of Agriculture, Novi Sad)

It is well known that the number of nodules varies in the course of vegetation season. The intensive increase of nodular weight and number continues until the end of soybean flowering and then begins to decrease (Z. Sarić, M. Sarić 1959). It has also been found that the number of nodules varies in different soybean varieties in dependence of the applied strain of R. japonicum in the course of thirty days.

In this study we examined the interaction of soybean genotypes and particular highly efficient strains of *R. japonicum* during thirteen weeks of soybean growing. We used five soybean varieties in the experiment, which are widely applied in production and 8 highly efficient strans of *R. japonicum*. The experiments were conducted in the glasshouse, in soil cultures in the course of three years. Nitrogen fixation was monitored simultaneously with the dynamics of nodular formation by determining the total nitrogen and nitrogenase activity.

The results have shown a difference in the dynamics of formation and the total number of nodules. None of the analyzed strains showed the same dynamics of nodular formation. The number of nodules varied from 9 to 40. In NS-10 variety, the average number of nodules was lowest, while inNS-6 it was highest. This proved that the interacton of genotypes of analyzed soybean varieties and R. japonicum strains determined the variations in the nodular formation and nitrogenase activity, as well as the quantity of fixed nitrogen.

The level of efficiency of particular strains of R. japonicum depended not only on their ability to fix nitrogen, but also on the interaction of the strains with the host variety, i. e. the efficiency of nitrogen fixationis due to the interaction of soybean genotypes and *R. japonicum* strains.

However, these studies did not reveal mechanisms which produce the differences in the interaction of particular soybean genotypes and *R. japonicum* strains. We may assume that the synthesis of particular growth materials of *R. japonicum* strains may accelerate the penetration of bacteria cells and nodular formation. Taking in consideration this character of strains, we may naturally expect differences in the interaction of the strains and particular soybean varieties.

THE EFFECT OF DIFFERENT CONCENTRATIONS OF Cu, Zn, Mn AND Fe ON SUPEROXDE DISMUTASE ACTIVITY IN SUGAR BEET

[1]*D. ŠTRBAC,* [1]*R. KASTORI and* [2]*D. ŠTAJNER*

(Faculty of Agriculture,[1] Novi Sad and
Faculty of Sciences[2], Novi Sad)

Superoxide dismutase (SOD) EC 1. 15. 1. 1. is an enzyme from the group of oxidoreductases which catalyzes the dismutation of the superoxide radical (O_2^-) to hydrogen peroxide (H_2O_2). SOD isolated from some plant species differs in prostetic groups, which may be: Cu and Zn or Mn or Fe. The aim of the experiments was to examine the effect of different concentrations of these metals in nutrient solution on SOD activity in young sugar beet leaves. because it is not yet clearly understood which of these metals forms the prostetic group of this enzyme.

The plants were grown in water cultures under controlled conditions on a nutrient solution with different concentrations of Cu (0.05—500 $\mu g \cdot dm^{-3}$), Zn (0.1—1000 $\mu g \cdot dm^{-3}$), Mn (0.001—10 $mg \cdot dm^{-3}$) and Fe (0.004—40 $mg \cdot dm^{-3}$). SOD activity was determined in leaves by photochemical inhibition of autooxidation of adrenaline after the method of Beauchamp and Fridovich. The weight and portion of dry matter were determinated as well as the concentrations of chloroplast pigments and Cu, Zn, Mn and Fe contents in dry matter.

The results show that high and low concentrations of Cu, Zn, Mn and Fe significantly decreased the concentrations of chlorophyl a, b and carotenoids. They also increased the content of dry matter. An increase in the concentrations of these metals in nutrient solution increased their content in dry matter. The effect of the metals investigated on SOD activity depended on their concentrations in plants. The SOD activity was the lowest in plants grown on nutrient solution with low concentrations of Cu

Session 3.

WATER RELATIONS, RESISTANCE

A COMPARATIVE INVESTIGATION OF THE TRANSPIRATION RATE AND PATTERN IN SOME TOBACCO HYBRIDS OF THE VIRGINIA TYPE

K. DUBRAVEC, K. DEVČIĆ and I. PRIMORAC
(Institute for Plant Protection, Faculty of Agricultural Sciences, University of Zagreb and Tobacco Institute, Zagreb)

Comparative research into the transpiration of tobaccos of the Virginia type (hybrids Drava, Podravina and Zagreb) leads to the following results:

1. The greatest transpiration was observed in the hybrid with the greatest number of stomata, i. e. in the hybrid Drava which had mean daily Tr values of 4.48—12.56 mg/g/min (Božjakovina) and 3.58—10.51 mg/g/min (Pitomača), followed by Zagreb with Tr values of 3.91—9.26 mg/g/min (Božjakovina) and 3.41—11.78 mg/g/min (Pitomača), and by Podravina with Tr values of 4.60—10.03 mg/g/min (Božjakovina) and 3.37—8.80 mg/g/min (Pitomača).
2. The highest mean Tr values were observed in the middle leaves (with the exception of Drava in the Pitomača location), and the lowest in the technologically mature bottom leaves and sometimes in the leaves just below the blossom (Zagreb at the Pitomača location and Podravina at both locations).
3. The most intensive transpiration in the hybrids under investigation was observed from the beginning of flowering up to full bloom. Sufficient quantities of water should therefore be supplied in these phases of plant development in order to achieve high yields.
4. The hybrids Podravina and Zagreb, considering their transpiration capacity, are suitable for cultivation in relatively dry habitats.

INFLUENCE OF DROUGHT ON THE AMOUNT OF OSMOTICALLY ACTIVE COMPOUNDS IN THE LEAVES OF TWO MAIZE LINES DIFFERING IN DROUGHT RESISTANCE

R. STIKIĆ, B. ZARKOVIĆ and M. STAJKOVIĆ
(Faculty of Agriculture — Zemun)

Osmotic adjustment is an adaptive mechanism facilitating turgor maintenance in the leaves at low water potential values, by an active accumlation of osmotically active componds in cells such as sugars, amino and organic acids and ions.

The paper reports the results of investigations into drought effects on the amounts of osmotically active compounds in the leaves of two maize lines differing in drought resistance: ZPBL-1304 (resistant) and ZPL-389 (susceptible). Plants were grown under controlled conditions up to the fifth leaf stage and. then, exposed to drought by witholding water for 7days. In the conditions of mild stress (leaf water potential around -0.8 MPa) and severe stress (leaf potential around -1.0 MPa), samples were taken for estimation of the following compounds: sugars (by HPLC method): cations (K, Na. Ca and Mg by method of atomic absorption spectrophotometry), Cl (by method of potentiometry) and proline (by ninhydrin method).

Results show that leaves of both maize lines accumulate osmotically active compounds, primary sugars, what points out to their capa-

bility of osmotic adjustment. It is, also, shown that participation of these compounds is less expressed in severe stress conditions indicating smaller capacity of both lines to adjust osmotically to these conditions. Differences in the participation of osmotically active compounds in osmotic potential between two lines, in both stress treatments, indicate greater capacity for osmotic adjustment in the resistant line.

INFLUENCE OF DROUGHT ON STOMATAL REACTION IN TWO MAIZE LINES DIFFERING IN DROUGHT RESISTANCE

R. STIKIĆ, LJ. JOVANOVAC and D. JOVANOVIĆ*

(Faculty of Agriculture — Zemun, University of Belgrade and
*INEP — Institute of Pesticides and Enviromental Protection — Zemun)

Stomatal action on moderating, regulating and optimizing exchange of CO_2 and H_2O in plants is very important for plants survival and production in stress conditions.

The paper reports the results of investigation of drought effects on stomata of two maize lines differing in drought resistance: ZPBL--1304 (resistant) and ZPL-389 (susceptible). Plants were grown in controlled conditions up to the fifth leaf stage and, then, exposed to drought by withholding water for 7 days. Changes in stomatal conductivity (measured by method of diffusion porometry) and water potential components (measured by method of psychrometry) were followed during stress treatment.

The results show different responses of stomata in two lines during stress development. The drought resistant line showed lower sensitivity of stomata to water potential changes up to the threshold value and conductivity decreasing significantly after this value, while stomatal conductivity in the susceptible line decreased at the same rate during stress treatment.

These results indicate a more efficient stomatal control of water loss and CO_2 uptake during stress in resistant genotypes by providing CO_2 uptake in mild stress conditions and preventing excessive water loss in the severe phase of stress.

EFFECT OF DROUGHT AND HIGH TEMPERATURE ON MORPHOLOGICAL AND PHYSIOLOGICAL CHANGES IN SOME MAIZE GENOTYPES

B. KEREČKI, LJ. ZARIĆ, S. PEKIĆ and M. PENČIĆ*

(Maize Research Institute, Zemun Polje, Belgrade—Zemun and
Faculty of Agriculture, Belgrade-Zemun)

Plants of different maize inbred lines were grown in field and under controlled conditions. The field trial was conducted in 1985 (an exceptionally dry year) with two treatments and without irrigation. Organogenesis stages of reproductive organs and biological yield were observed in the field trial. Activity of the GDH enzyme (glutamate dehydrogenase) was determined in plants grown in controlled conditions.

The results of determining the organogenesis stages in all inbreds tested indicated no differences in the duration of stages of tassel organogenesis between plants grown in irrigation conditions and without irrigation, whereas in medium and early maturity genotypes the grain filling stage in irrigation conditions was longer. Measurements of total biological yield and its components showed a considerable increase in the irrigated field in medium and early maturity inbreds (more pronounced in genotypes susceptible to drought), whereas in late inbreds there were no significant differences between plants grown in irrigation conditions and those grown without irrigation. Drought index was determined on the basis of grain yield. The index value higher than 1 indicates a resistant genotype, lower than 1 a susceptible one. Hence, drought index in the genotypes tested ranged as following: ZP 1304 — 1.12; ZPBL 389 — 0.94; L. 155 — 1.12; B 432 — 0.75; Polj. 17 — — 1.1; F2 — 0.65. GDH activity at high temperatures was increased in resistant in comparison to susceptible genotypes. All these indicators should be used for a better and more objective evaluation of resistance to drought and high temperatures in different maize genotypes.

INFLUENCE OF DROUGHT ON WATER STATUS PARAMETERS AND ABA CONTENT IN THE LEAVES OF MAIZE LINES DIFFERING IN DROUGHT RESISTANCE IN FIELD CONDITIONS

S. PEKIĆ, M. KOJIĆ* and LJ. JOVANOVIĆ***

(*Faculty of Agriculture, Zemun and **INEP, Zemun)

Seasonal changes of water status parameters (water potential — Ψ_L, osmotic potential — Ψ_S, turgor potential — Ψ_T, and relative water content — RWC) and ABA content in the leaves of six inbred maize lines were followed in irrigated and nonirrigated field plots during 1985. Three pairs of lines, differing within each pair in drought resistance (estimated, previously, by several drought tests) were compared. The three pairs represented early, medium and late maturity grouping. Measurements were taken during July when soil water content decreased below the level of available water. The same leaf was used (third leaf from the tassel) for all parameters. Ψ_L and Ψ_S were estimated by psychrometry, RWC by gravimetric method and ABA content by RIA test. Samples were taken at 10 h.

The results show that values of Ψ_L, Ψ_S, Ψ_T, and RWC were lower, and ABA level higher in the leaves of plants grown in nonirrigated field plots. The changes in all parameters were more expressed in the leaves of nonirrigated plants. The difference between resistant and susceptible lines of each pair was more expressed in the nonirrigated field. Comparisons between lines of the same maturity groups by the slopes calculated from: a) linear regressions Ψ_L vs Ψ_T and Ψ_L vs RWC indicate a higher capacity for osmotic adjustment in the leaves of resistant lines and b) linear regression Ψ_L vs ABA is indicative of a higher capacity of ABA accumulation in leaves of resistant lines stressed in the field.

DELAYED LIGHT EMISSION METHOD FOR EVALUATION OF HIGH TEMPERATURE RESISTANCE IN MAIZE INBRED LINES

C. RADENOVIC, D. FIDLER, R. STOJSIN and I. HUSIC

(Zemun Polje, Maize Research Institute Belgrade—Zemun, Yugoslavia)

Our papers (1—4) present the results of physiological *in vivo* studies of the photosynthetic process for leaf segments of maize inbred lines and hybrids. Data are also given on the functioning of the maize leaf photosynthetic apparatus. Methodological procedures, techniques and processing of some bioluminescent measurements are described.

The present investigation provided the following results:

The method of delayed light emission (DLE) of the maize leaf for evaluation of resistance to high, and elevated temperatures and drought is presented. The standardization conditions during measurements of the objects investigated are expounded and data processing with the analysis of the DLE thermal curve described.

It has been proved that using this method at least two critical temperatures (T_1 and T_2), whose values were different for hybrids and inbred lines and at least three activation energies, namely for the response before the first critical temperature (E_1), for the response between the critical temperatures (E_2) and for the responses after the second critical temperature (E_3).

The results of comprehensive studies of two maize inbreds (ZPL1304 and ZPBL389) are given. The former was evaluated as resistant to the investigated high and elevated temperatures and drought and had the following parameters: $T_1 = 31.4°C$; $T_2 = 41.6°C$; $E_1 = 14.1$ kJ/mol; $E_2 = 36.2$ kg/mol; $E_3 = 71.9$ kJ/mol.

The latter was evaluated as susceptible to high elevated temperatures and drought and had the following parameters: $T_1 = 30.9°C$; $T_2 = 39.5°C$; $E_1 = 12.0$ kJ/mol; $E_2 = 36.4$ kJ/mol; $E_3 = 69.3$ kJ/mol.

Data on evaluation of resistance of the inbreds studied to high and elevated temperatures and drought are presented. Exceptional resistance was found in inbred ZPL11/5 (evaluated as very resistant ($T_1 = 31.4°C$, $T_2 = 48.0°C$). Satisfactory resistance was established in three inbreds: LA672 and ZPL13/2.

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Session 4.

GROWTH REGULATORS, PHYSIOLOGY OF DEVELOPMENT, SEED GERMINATION

IS CYTOCHROME P-450 INVOLVED IN PHOTOTROPIC REACTION?

R. KONJEVIĆ and D. GRUBIŠIĆ

(Institute of Botany, Fac. Sci. and S. Stankovic Institute for Biological Research, University of Beograd)

The effect of growth retardants, phenylacetic acid and ascorbic acid, on the phototropic reaction of *Vigna radiata* seedlings was investigated. Tetcyclacis, ancymidol, paclobutrazol, phenylacetic and ascorbic acids inhibit phototropic reaction, when applied 2 or 12 h prior to the onset of unilateral blue light, while AMO 1618 and CCC are ineffective under the same experimental conditions. The specific effect of former substances on phototropic reaction was tested by comparison with their effect on geotropic reaction and growth rate. Neither of these processes was affected by the substances applied in the same experimental period. It is known that tetcyclacis, ancymidol and paclobutrazol act by inhibiting »mixed oxidases« function, whose component is cytochrome P-450. Since cytochrome P-450 is involved in the oxidation of ent-kaurene to ent-kaurenoic acid, and is believed to be involved in the transmission of the phototropic stimulus, there are two possibilities for explaining the action of the retardant. Preference is given to the second possibility, for 3 reasons: (a) the inhibitory effect of tetcyclacis, ancymidol and paclobutrazol is displayed shortly after application, when the changes in GA content have probably not become significant; (b) ascorbic acid and phenylacetic acid which probably affect the redox state of cytochrome P-450, have the same inhibitory action; (c) AMO 1618 and CCC, which inhibit GA biosynthesis, but do not affect cytochrome P-450, are ineffective in phototropic reaction.

EFFECTS OF COPPER COMPLEXES ON PHYTOCHROME-MEDIATED BETACYANIN FORMATION IN *Amaranthus* SEEDLINGS

S. OBRENOVIC

(Siniša Stankovic Institute for Biological Research, Beograd)

In order to check the involvement of superoxide anions in phytochrome phototransduction, a number of copper chelates, the respective chelators and copper ions were tested in light-induced betacyanin formation, by using etiolated, three-day-old *Amaranthus caudatus* L. half-seedlings. No effect was observed with moderately lipid soluble copper chelators, whereas their copper complexes and coper ions ($CuSO_4$) suppressed betacyanin accumulation in safelight control (0.019% of active phytochrome), at a range 100—500 μM. Lower concentrations (20—50 μM) of cupric aspirinate and Cu(II)D-penicillamine suppressed the inductive effect of brief far-red illumination (2.3% of active phytochrome) and postponed the light-signal coupling during continuous red illumination to the post-illumination period, without chaning the net inductive effect of red light (87% of active phytochrome). The same effect was achieved with 100 μM $CuSO_4$ and Cu(I)D-penicillamine, in agreement with the superoxide dismutase activity of Cu(II) form. These results support the involvement of superoxide anions in the rotenone-resistant electron transport of plant mitochondria and the deci-

sive role of pyridine redox charge in phytochrome phototransduction (Obrenović 1968, Physiol. Plant. 67: 626). Its relationship to the regulators block of cytokinins is discussed in terms of CAM phenomenon related to mitochondrial function only.

PLANT TUMOR INHIBITION BY HUMAN LEUCOCYTE INTERFERON

M. KRSNIK-RASOL[1], *LJ. MIŠIC*[2] *and D. ŠERMAN*[2]

([1]Department of Botany, Faculty of Science and
[2]Department of Biology, Medical Faculty, University of Zagreb)

Plants can produce some substances which are similar to animal interferon in regard to their molecular weight as well as in their antiviral activity. Human interferon may therefore be supposed to exibit a biological activity in plant cells too.

We were interested in whether the human leucocyte interferon (hIFN alpha) could protect plant cells from tumor transformation as it happens in the case of virus infection. We have tested its influence on the crown-gall tumor induction and growth on potato (*Solanum tuberosum* L.) according to Anand and Heberlein (1) and on kalanchoe (*Kalanchoe daigremontiana* R. Hamet et K. Perr dela Bath.) according to Beiderbeck (2).

Our results showed that interferon in a range from 10^3—10^5 units per mililiter inhibited tumor induction on potato tuber tissue. Tumor supression was observed in both cases, when hIFN had been applied at the moment of infection or 48 hours following bacterial inoculation, i.e. after the T-DNA had been integrated into plant cell genome.

Tumor induction on kalanchoe leaves, as shown by Beiderbeck's »Igel« test (2) was also inhibited by interferon. Tumors which developed despite hIFN treatment were mainly organised shooty or rooty tumors.

(1) *Anand, V. K., G. T. Heberlein,* 1977: Amer. J. Bot. 64, (2), 153.
(2) *Beiderbeck, R.,* 1970: Z. Naturforsch. 25, 407.

BIOLOGICAL EFFECTS OF AGROSTEMINE ON *LEMNACEAE*

B. KRAJNČIČ

(College of Agriculture, University of Maribor)

Investigations under standard axenic experimental conditions (Krajnčič 1985) produced the following results:

a) In the photoperiodically neutral species *Spirodela polyrrhiza* under long-day conditions, an agrostemine preparation in a near to optimal concentration (20 mg/l) increased the percentage of dry weight significantly, compared to the control plants, by 29%, in a period of 60 days.

In the same plants, under short-day conditions, the agrostemine preparation in all concentrations applied (1 — 20 mg/l) raised the contribution of dry weight up to 4%, the percentage of flowering plants from 10% (control) up to 15%.

b) In the short-day species *Lemna aequinoctialis* under short-day conditions agrostemine in its optimal concentration (10 mg/l) raised the contribution of dry weight by 3% compared to the control plants, the percentage of flowering plants increased from 58% (control) to 67% in a period of 50 days.

c) In the long-day species *Lemna minor*, under long-day conditions, agrostemine in its optimal concentration (1 — 20 mg/l) lowered the contribution of dry weight by 2% compared to the control plants, the percentage of flowering plants decreased from 26% to 1 — 3% in a period of 60 days.

Krajnčič, B., 1985: Biol. vestn. (Ljubljana) *33*, 39—52.

* Agrostemine is a bioregulative preparation produced by Bioprodukt Beograd from *Agrostemma githago* plants.

EFFECT OF ETHREL AND CCC BY INDIVIDUAL AND SIMULTANEOUS APPLICATION UPON STRAW LENGTH AND CARBOHYDRATE, PHOSPHORUS AND POTASSIUM CONTENTS IN GRAIN IN WINTER RYE »MAKEDONIJA«

I. JUSUFI and M. GORGIEV

(Department of Botany, Faculty of Science and Mathematics, Skopje)

In two-year pot experiments with winter rye Makedonija it was demonstrated that the spraying of the plants with 5 mg ethrel, 20 mg CCC, 5 mg ethrel and 20 mg CCC and 2.5 mg ethrel and 10 mg CCC per pot (6 plants) reduced the straw length and grain yield of this cereal. Straw lenght declined by the application: during tillering by 9.15%, 0.00%, 18.00% and 5.15%; during earing by 15.32%, 5.58%, 24.59% and 12.52%. The strong reduction of straw length by simultaneous application of ethrel and CCC probably is a consequence of additive and synergetic effects.

In addition to that potassium content decreased and carbohydrate and phosphorus contents (phosphorus content only by application during tillering stages) in grain increased.

EFFECT OF 2-CHLOROETHYLPHOSPHONIC ACID ON CAPSULE YIELD AND MORPHINE CONTENT OF *PAPAVER SOMNIFERUM* L. IN FIELD EXPERIMENTS

M. GORGIEV and K. STEFANOVSKA

(Department of Botany, Faculty of Science and Mathematics, Skopje)

The spraying with different concentrations of 2-chloroethylphosphonic acid (Ethrel) during rosette plant, stem elongation and flowering with capsule formation stages of *Papaver somniferum* showed different effects. At 250, 500, 750 and 1000 ppm this chemical decreased capsule yield when applied during rosette plant, and increased it when applied during stem elongation and flowering periods (the only exception during flowering stage were the plants treated with 250 ppm where capsule yield was smaller). Furthermore, at 250 and 750 ppm, it stimulated,

while at 500 and1000 ppm it retarded morphine accumulation in the capsule when applied during all three periods (especially in the plants treated with 1000 ppm during flowering where morphine content was three times as low as in the control plants). The results suggest than capsule formation and alkaloid accumulation in *P. somniferum* are hormone controlled, possibily by ethylene, which is generated by exogenous 2-chloroethylphosphonic acid.

ISOLATION AND ATTEMPTED IDENTIFICATION OF ESTERS OF INDOLEETHANOL AND UNSATURATED FATTY ACIDS

V. MAGNUS, G. LAĆAN and S. ISKRIĆ
(Ruđer Bošković Institute, Zagreb)

Indoleethanol is a native plant constituent formed from the same metabolic precursors as the growth hormone, indoleacetic acid. Indoleethanol formation is reversible, and its oxidation into indoleacetic acid is catalyzed by enzymes regulated by the preexisting amount of the growth hormone. That alcohol is, therefore, likely part of a buffer mechanism employed by plants to smooth fluctuations in the rates of auxin biosynthesis and auxin utilization. In accord with this hypothesis, indoleethanol supplied externally is generally not metabolized into substantial amounts of indoleacetic acid, but mostly transformed into conjugates: esters and glycosides. Extremely non-polar esters of similar chromatographic properties have been found as major indoleethanol metabolites in bacteria, thallophytes and cormophytes. From the basidiomycete *Craterellus cornucopioides* (L.) ex Pers. (1 kg) incubated with tryptophol, about 100 mg of those conjugates were isolated. Separation by column chromatography on silica gel and Sephadex LH-20 has so far yielded three individual esters, most likely the oleate, linoleate, and dehydrocrepenynate (octadeca-*cis*-9, *cis*-14-dien-12-ynoate). The compounds were synthesized and their NMR and mass spectra were compared with those of the plant metabolites. According to the data available so far, the data available so far, the synthesized compounds are identical to the plant esters. However, it should be remembered that an enormous variety of unsaturated fatty acids of very similar physical and chemical properties can occur in plants. Additional evidence is therefore neeeded to achieve certainty about the structures of the indoleethanol esters from *Craterellus*.

PRESENCE OF SEROTONIN IN THE SEEDS OF NETTLE (*URTICA DIOICA*) AND CLOVER (*TRIFOLIUM REPENS*)

I. REGULA and Đ. MASKALAN
(Department of Botany, Faculty of Science, University of Zagreb)

Much attention has recently been devoted to the investigation of tryptamines in plants. Although some indole compounds are frequently distributed among plants, indole alkylamines are rare. This experiment investigated the content of serotonin in the vegetative organs and embryo of clover and the embryo of nettle. Seeeds were homogenized and extracted with methanol. Concentrated extracts were passed through a columun Amberlite CG-50 ion exchanger.

Chromatographic and spectrophotometric investigation of extracts or efluents from columns indicate that the vegetative part of clover does not contain indolylalkilamine but it was found to be present in the embryo in a quantity of 0.008$\mu g \cdot g^{-1}$ and in the embryo of nettle in a quantity of 0.4 $\mu g \cdot g^{-1}$ fresh weight.

Positive histochemical reaction with 6% p-dimethylaminobenzaldehyde in conc. HCl and fluorescence with ninhydrin acetic acid and fluorescence under U.V. light confirms the presence of serotonin in embryo's tissue.

ENDOGENOUS GIBBERELLINS IN MALE AND FEMALE *RUMEX ACETOSELLA* CLONES GROWN UNDER DIFFERENT PHOTOPERIODS

B. KOZOMARA, V. TEŠEVIĆ and LJ. ĆULAFIĆ*

(Institute for Biological Research, Belgrade and *Faculty of Chemistry, University of Belgrade)

Endogenous gibberellins have been examined in male and female *R. acetosella* clones in relation to photoperiodic treatments. Clones were obtained by *in vitro* propagation, and subjected to an inductive long day treatment (16h period of light) and noninductive short day treatment (8 h period of light).

Extracts were made from leaves of male and female plants, grown in long or short days. Leaves were collected in both groups when stem elongation in induced plants was in progress. Methanolic extracts were partitioned against ethyl acetate at pH 2.8. Gibberellins were further purified by using a DEAE-Sephadex A-25 column and analyzed by barley endosperm bioassay. Fractions with biological activity weree combined and franctionated by preparative high-performance liquid chromatography (HPLC), and their biological activity was tested by bioassay.

Preliminary experiments showed that there was a significant increase in the gibberellin level under long days, especially in male clones, such increase coinciding with stem elongation and the formation of inflorescencences. Male clones had generally a higher gibberellin activity than female.

The use of analytical HPLC showed the presence of gibberellinlike substances that co-chromatographed with GA_1/GA_3.

REGULATION OF FLORAL INDUCATION AND DEVELOPMENT OF FLOWERS WITH IAA, 6-BENZILADENINE AND EDDHA

B. KRAJNČIČ

(College of Agriculture, University of Maribor)

Lemnaceae from Yugoslavia were used as experimental plants (Krajnčič and Devidé, 1980; Krajnčič, 1983, 1985).

The effects of IAA, 6-benziladenine and EDDHA combinations at various concentrations on floral induction and floral development under long- and short-day conditions were investigated.

The experiments were conducted in two growth chambers under controlled conditions. Sterilized isolated plants were grown axenically in a modified Pirson-Seidel nutrient medium, illuminated by 3000±100 lx. and under a constant day and night temperature of 28±1°C.

It was found for the first time that the combination (EDDHA 7.02 mg/l, IAA 50 μg/l and 6-benziladenine 50 μg/l) enhanced floral induction under long-day conditions in the long-day species *Lemna minor* and in the photoperiodically predominantly neutral species *Spirodela polyrrhiza.*

By the dissection of plants and a comparison of histologic sections it was furthermore established that under inductive conditions the same combination in a near-to-optimal concentration also enhanced post-inductional stages of flower development in the long-day species *Lemna minor.* Under photoperiodically non-inductive conditions, however, IAA, 6-benziladenine, EDDHA and their combinations did not induce flowering in this species at any of the applied concentrations.

The results of the research represent a contribution to the programming of flowering and the development of flowers.

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THE INFLUENCE OF N^6- Δ^2- ISOPENTENYLADENOSINE ON THE GROWTH AND ION TRANSPORT IN MYCELIUM OF *FUNGUS SUILLUS* VARIEGATUS

F. POHLEVEN

(Biotehnical Faculty, Department of Biology, E. Kardelj University, 61000 Ljubljana

N^6- Δ^2- isopentenyladenosine is a natural cytokinin influencing growth and ion transport in mycorrhizal fungi. Its most pronounced effects compared to other cytokinins (KIN, zeatin, zeatinriboside) are regulatory changes in the ion transport between mycelium and nutrient medium.

Higher concentrations, clearly inhibitory to mycelial growth, stimulate the transport of K, Ca and especially P. from mycelium into the medium. No significant changes. however, could be observed in Na transport.

The apparent selectivity and specificity of 2iP concentration in fungal growth and ion transport could be the major role of cytokinin mediators in establishing and regulating the dynamics of symbiotic interactions.

EFFECTS OF NATURAL CYTOKININS ZEATIN AND ZEATIN RIBOSIDE ON CHLOROPHYLL SYNTHESIS IN THE BLACK PINE SEEDLINGS

G. JELIĆ and M. BOGDANOVIĆ
(INEP, Department for Biotechnological Research, 11080 Zemun)

Isolated pine embryos can synthesize chlorophyll if grown under the light, while intact seedlings are capable of carrying out the process also in the darkness.

Effects of the natural cytokinins, zeatin (io^6Ade) and zeatin riboside (io^6A) on the chlorophyll synthesis in isolated (*Pinus nigra* Arnold) black pine embryos were evaluated for the initial stages of their ontogenesis.

The embryos were isolated 24 hours upon imbibition and were grown under controlled conditions with added cytokinins. The cytokinins stimulated chlorophyll synthesis in cotyledons of the embryos grown under the light. The rate of chlorophyll accumulation depended on the hormone concentrations and duration of treatment.

Both cytokinins that were applied allowed the synthesis of chlorophyll in the embryos grown in the darkness. In this case, too, the rate of accumulation of the pigments depended on the hormone concentrations and duration of treatment.

The effect of zeatin was stronger than that of zeatin riboside. The patterns of chlorophyll changes within the first 10 days of ontogenesis were similar in all the seedlings treated with zeatin, zeatin riboside or BAP, both under the light or in the darkness.

The results indicate the significance of cytokinins for the induction of chlorophyll synthesis. The differences found for cytokinin activity of the embryo extracts and megagametophytes are also in favour of the suggestion that cytokinin may be one of the essential megagametophytic factors which provide for the development of photosynthetic apparatus.

ANTAGONISM BETWEEN ABSCISIC ACID AND CYTOKININ ON CHLOROPHYLL SYNTHESIS IN PINE SEEDLINGS

M. BOGDANOVIĆ and G. JELIĆ
(INEP, Department for Biotechnological Research, Zemun)

Effects of abscisic acid and cytokinin on chlorophyll synthesis were observed in whole seedlings and isolated black pine (*Pinus nigra* Arnold) embryos grown in the light and in darkness.

Abscisic acid inhibited germination of the seeds depending on its concentration, while cytokinin (6-BAP, 10^{-5}M) was not effective. The intact seedlings synthesized chlorophyll both in the light and in the dark. The process is, therefore, stimulated by cytokinin and delayed by abscisic acid.

The isolated embryos synthesized chlorophyll only in the light if grown on a mineral solution. The synthesis of chlorophyll in the isolated embryos in the light was also stimulated by cytokinin and inhibited by abscisic acid. The isolated embryos grown in darkness did not synthesize chlorophyll in the absence of cytokinins. Under a simulta-

neous treatment with 6-BAP and ABA occurrence of the chlorophyll synthesis will depend on the ratio of their concentrations.

On the basis of references and our previous investigations it can be assumed that the effects of the applied growth regulators are rather indirect (affecting the processes of protein synthesis) than direct on the biochemical pathway of chlorophyll synthesis.

EFFECT OF CALMODULIN ANTAGONISTS ON HORMONE-INDUCED GROWTH RATE

G. NAUNOVIĆ, M. NEŠKOVIĆ and J. JOVANOVIĆ
(Institute of Botany, Faculty of Science, and Institute for Biological Research, University of Belgrade)

In measuring the rate of elongation of etiolated pea stem induced by hormones (IAA and GA_3) during the first 140 min, it was established that the responses to both hormones were biphasic. The calmodulin antagonist chlorpromazine (10 mM) in the presence of IAA (10 μg) prevented the first stimulation of growth and did not affect the second one. When GA_3 was applied, chlorpromazine prevented the second GA_3-inducible acceleration, whereas the first proceeded unimpaired. These results point to different mechanisms of growth stimulation involved in rapid responses to IAA and GA_3. In continuation of this work, experiments were repeated with other inhibitors of the Ca^{2+}-calmodulin complex, and the results are presented herein.

PHYTOHORMONE ACTIVITY IN *AZOTOBACTER* CULTURE

B. RELIĆ, M. GOVEDARICA and M. NEŠKOVIĆ***
(Institute of Biology, Faculty of Science, Novi Sad,
*Institute of Field and Vegetable Crops, Faculty of Agriculture and
**Siniša Stanković Institute for Biological Investigations, Beograd)

It has been stated that *Azotobacter* affects plant nutrition not only by biological fixation of nitrogen, but, also, by producing substances acting as hormones. Occurrence of substances of auxin, gibberellin, and cytokinin types in liquid medium in which *Azotobacter* was cultured (strain No. 16 isolated from maize rhisosphere) was investigated to foster an understanding of a specific relationship between bacteria and plants.

Active substances were extracted from a liquid medium of a three-day old culture by using organic solvents and separated chromatographically while their activity was determined by standard bioassays. Substances of auxin type were also determined spectrophotofluorimetrically.

The bioassay results showed the occurrence of a considerable amount of a substance characterized by an auxin-like activity. Chromatographic and spectrophotofluorimetric analyses showed that this active substance was possibly identical with indol-acetic acid. Also, a substance exhibiting an extremely inhibitory effect in auxin bioassay was isolated from the medium.

Two additional substances were isolated from the medium. One of these two substances shows activity in bioassay for gibberellins, whereas the other is active in bioassay for cytokinins.

THE EFFECT OF HORMONES ON THE CONTENT OF TOTAL AND SOLUBLE SUGARS IN POPY (*PAPAVER SOMNIFERUM* L.)

M. MULEV, and M. SPASENOSKI

(Institute of Biology, Faculty of Science, Skopje)

Poppy (*Papaver somniferum* L.) was grown in Micherlih pots in a greenhouse. Dry soil of 5 kg was put in each pot. During the vegetation the humidity of soil was kept at 70% from retention capacity.

The effect of different hormones on the acumulation of total and soluble carbohydrates was investigated. The plants of poppy were treated with different hormones: IAA, IAA+GA_3, IAA+GA_3+BAP and GA_3 at a concentration of 300 mg l^{-1}. As control, plants were sprayed with distilled water of the same volume. Carbohydrates were measured in the stage of rosette, blossom and at the end of vegetation.

The results show that the plants which were treated with hormones differ in the contents of soluble and total carbohydrates. Higher contents of carbohydrates — total as well as soluble — were found in the plants which were sprayed with IAA in all phases of growth and in all organs (root, stem, leaf). A lower value of carbohydrates was recorded in plants treated with hormones in combination with IAA+GA_3, showing lower values of total as well as soluble carbohydrates in all phases of growing and in all organs in comparison with the control plants. However, the values of soluble and total carbohydrates were higher in plants treated only by GA_3 than in combination with IAA+GA_3.

THE INFLUENCE OF PROMETRYNE AND SIMAZINE ON DUCKWEED *(LEMNA MINOR), TOBACCO AND SOYBEAN CALLUS TISSUE CULTURES*

K. KRIVOKAPIĆ and Ž. ERIĆ

(Faculty of Science, University of Sarajevo)

We investigated the influence of prometryne and simazine on duckweed plants, tobacco and soybean callus tissue cultures grown *in vitro*. These substances inhibited the production of chlorophylls and carotenoids in duckweed plants. The inhibition of the production of photosinthetic pigments was in correlation to the reduction of the membrane system of the chloroplast. Prometryne had an inhibitory effect on the neoformation and proliferation of tobacco and soybean callus tissue, while simazine showed an inhibitory effect only at a higher simazine concentration, and at a lower concentration it even had a stimulative effect. Electron-microscopic analyses of the tobacco and soybean callus tissue cultures grown in the presence of the substances tested showed some changes in the ultrastructure.

Our results suggest the possibility of the application of duckweed, tobacco and soybean callus tissue cultures for investigations of phytotoxic effects of different xenobiotics.

INFLUENCE OF HERBICIDES ON MYCORRHIZAL FUNGI

N. GOGALA and R. RUŽIĆ

(Biotehnical Faculty, Department of Biology, E. Kardelj University, 61000 Ljubljana, Aškerčeva 12, Yugoslavia)

Research into the effects of herbicides and fungicides used in nurseries on the growth of symbiotic microorganisms has become increasingly important with the tendency to produce mycorrhizal plants in agriculture and forestry. We tested the effects of the herbicides Roundap, Gramoxone, Basamid and the fungicides Benomil, Captan and Cupra-blau on the growth of mycorrhizal fungi *Suillus variegatis* and *Boletus bovinus*. Both species showed specific reactions to fungicides and herbicides tested. Among fungicides only Captan did not have pronounced inhibitory effects.

All herbicides strongly inhibited the growth of the mycelium. Regular application of these chemicals not only influence parasitic fungi and weed plants but also the growth of symbiotic microorganisms and thus lower the biological properties of cultivated trees.

ELECTRICAL STIMULATION: ITS INFLUENCE ON FUNGAL GROWTH

D. KRIŽAJ, F. POHLEVEN, N. GOGALA and L. VODOVNIK

(Biocybernetics Lab, Faculty for Electrical Engineering, Trzaska 25, 61000 Ljubljana, Yugoslavia and
VTO za biologijo in Institut za biologijo Univerze Edvard Kardelj, Aškerčeva 12, 61000 Ljubljana)

Endogenous electrical phenomena are an integral part of the functional organization of biological systems. Voltage gradients and weak electric currents accompany cellular and tissue regeneration, polarized growth and differentiation in algae, higher plants and fungi; all these processes are also prone to be influenced by electrical stimulation (1—3). It has been shown (4), that plant roots drive weak electric currents through themselves, therefore it has been asked, whether and how would these currents influence the growth and ion accumulation in the root symbiont, mycorrhizal fungus *Suillus variegatus*.

Fungal mycelium was inoculated onto a modified MMN nutrient medium and placed between the electrodes. We used an adapted version of Ag/AgCl wick electrodes and also, in some experiments, stainless steel electrodes. After 14 days the wet and dry weight of the mycelia was determined with potassium, sodium and calcium contents measured by flame photometry and phosphate content spectrophotometrically by the method of Sletten and Bach.

Significant increases in wet weight (225%) and dry weight (180%, control = 100%) of the stimulated mycelia were observed together with significant increases in potassium and calcium and decreases in sodium and phosphate contents. Similar results were, interestingly, obtained after treating mycelia with IAA (5), which could perhaps indicate the existence of a more complex electrochemical contact between the plant and its symbiont.

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RATE OF FRUIT GROWTH IN SOME ECONOMICALLY IMPORTANT PLUM CULTIVARS

M. VELIČKOVIČ and M. JOVANOVIČ

(Faculty of Agriculture, Zemun)

The rate of fruit growth is a biological feature of a given cultivar, although it my also be partly in the function of certain agroecological conditions.

Investigations have shown that developing fruits have two emphasizing periods of growth which are in correlation to other developing stages in the annual cycle of fruit trunks. The results of investigations point to the possibility of selecting modern methods of field cultivation and fruit growing technology at the right moment.

The rate of fruit growth was investigated in Stanley and Požegača plum cultivars, which are considered to be of great economic importance to this contry.

DYNAMICS OF DRY MATTER OF SOME ORGANS IN DIFFERENT WHEAT CULTIVARS

B. KRSTIČ, M. R. SARIČ, Ž. STANKOVIČ

(Institute of Biology, Faculty of Sciences, Novi Sad)

The dynamics of dry matter of individual organs and yield were analyzed in different wheat genotypes characterized by the same growing season, at earing, milk-waxy maturity, and full maturity. The 15 wheat cultivars (1) Novosadska rana 2, 2) Partizanka, 3) Balkan, 4) Osječanka, 5) Super zlatna, 6) Kragujevčanka, 7) Skopljanka, 8) Jugoslavija, 9) Posavka, 10) Zvezda, 11) Mačvanka, 12) Baranjka, 13) Zelengora, 14) Nizija and 15) Žitnica) were grown under field conditions where optimum cultivation measures were applied. Dry mass of internodes, leaf sheaths, blades, and spikes was evaluated.

Considerable differences in dry mass of internodes between the cultivars under investigation were observed. An extremely high dry mass of internodes was obtained with cvs. 8 and 1 at earing while with cv. 6 at milk-waxy maturity. In certain cultivars, e.g. cv. 12, the differences between the developmental phases were small.

Dry mass of leaf sheaths showed considerably smaller variations not only between cultivars, but also between developmental phases analyzed. The greatest variations of dry mass of leaf sheaths was recorded at earing, and the smallest at full maturity.

Dry mass of leaves may be grouped into 5 groups when a level of significance of 1% is employed. The highest dry mass was obtained in cv. 8, then in cv. 10, whereas the lowest in cv. 2. Dry mass variations at ring, milk-waxy maturity, and full maturity were 178, 181, and 192% respectively.

Certain other parameters such as number of kernels per spike, kernel mass per spike, and a 1,000 kernel mass also confirmed the differences between cultivars. For example, cv. 12 had the greatest number of kernels per spike (32), whereas cv. 9 was characterized by the smallest kernel number (17). The highest kernel mass per spike was obtained with cvs. 1 and 8.

AUTOVEGETATIVE PROPAGATION OF DAWN REDWOOD (*Metasequoia glyptostroboides* Hu et Cheng)

M. JURKOVIĆ and B. JURKOVIĆ-BEVILACQUA***

(*Faculty of Science and **Faculty of Forestry, University of Zagreb)

The possibilities of the rooting of winter and summer cuttings of dawn redwood have been examined. One-year old cuttings were used, which had not been treated with synthetic growth substances usually recommended for conifers. The cuttings ere taken from two ortet dawn redwood trees of various ages, one 10 years old and the other 25. The experiment showed significant differences (26.17% and 22.50% respectively of rooted cuttings) in favour of the younger tree, and the significance test of proportion differences showed significance at a level of 0.01%.

One of the aims of the study was to determine the number and arrangement of sclerenchymatous elements in the primary bark and in the primary phloem. In order to obtain comprehensive data, comparisons were made of cross-sections of the base, the middle and top parts of single cuttings of the species investigated. The examinations have shown that the differences among individual cuttings are insignificant and that they lie within the natural variability among the shoots of the same plant and among those of different trees.

THE EFFECT OF KNO_3 ON LIGHT SENSITIVITY OF *Paulownia tomentosa* SEEDS

D. GRUBIŠIĆ and R. KONJEVIĆ

(S. Stanković Institute for Biological Research and Institute of Botany, Fac. Sci., University of Beograd)

The irradiation time needed to induce maximum germination of *Paulownia tomentosa* seeds varies from a few minutes to several days, which is usually ascribed to seed maturation conditions. In seeds whose

light requirement for germination is saturated by short term irradiation, light sensitivity is decreased by prolonged imbibition in darkness. They resemble the seeds with natural low light sensitivity. Potassium nitrate (10 mM) restores the high light sensitivity in both seed groups, provided the seed are fed with KNO_3 24 h prior to irradiation.

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THE EFFECT OF DEUTERIUM OXIDE ON GA_3- INDUCED GERMINATION OF *Paulownia tomentosa* SEEDS

V. ORBOVIĆ and D. GRUBIŠIĆ

(S. Stanković Institute for Biological Research, University of Beograd)

The germination of positive photoblastic seeds of *Paulownia tomentosa* can be induced in darkness by GA_3. Maximum germination occurred when the seeds were imbibed in water for 3 days, followed by addition of 1 mM GA_3 for 12 h. When the water is replaced by D_2O, germination is retarded. The rate of germination depends on the phase of germination, the concentration of D_2O and duration of treatment. If the seeds are imbibed in D_2O and then induced to germinate by GA_3, their germination is slowed by 12—24 h depending on the concentration. If heavy water is added to the seeds after incubation in GA_3 these seeds will germinate 1—14 days later than in the control. If the transfer of seeds from GA_3 to heavy water is delayed for 12 h or longer, the retardation effect decreases.

SEED LONGLIVEDNESS OF DIFFERENT MAIZE GENOTYPES (*ZEA MAYS* L.)

A. MATIČIĆ, L. ROZMAN and M. VIDRIH

(Biotechnical Faculty, Department for Agronomy, 61000 Ljubljana, p.p. 486)

Seed longlivedness, lasting until individual seeds of certain genotypes are still in a process of germination, plays an important role in plant breeding. Selection material from corn germ-plasm bank was, for this reason, studied at Biotechnical Faculty in Ljubljana.

Seeds of mostly autochtone varieties and Lj-corn lines of differently inbred stages were raised in controlled pollination between 1971 and 1979 at a testing field in a pre-Alpine cold and humid climatic area (301 m above the sea level), $\bar{x}$ year temperature 8.5—9.4°C, 1300—1700 mm of rain per year). The seeds were dried to 14% of humidity and stored under usual storage conditions. To keep the gene fond under controlled climatic conditions (in refrigerator), the seeds of many corn genotypes, 6 to 13 years old, were renewed in the years 1984, 1985, 1986 at the same location where they had been raised ($\bar{x}$ minimal day T in time of sowing 5 cm above ground was 0.1—1.5°C, 5 cm deep 4.1—7.0°C). The results of plant emergence of individual genotypes vary considerably in germination ability of variously aged seeds. The six and seven year old seeds germinated still well at all 35 sowed varieties, as well as all 35 sowed lines of various origin. The 11 year old seeds of varieties ger-

minated only in 68.5%; they were mostly autochtone flints. Only 25% of seeds of lines of the same age, mostly of their own selection, germinated well. Twenty-two Lj-lines germinated even after 13 years; three o fthem even in more than 80%. Forty-seven lines, i.l. 68% ceased to germinate after 13 years.

The results show that most domestic flint varieties and a fair number of lines represent a perfect selection material for the breeding of hybrids to germinate under unfavourable growing conditions of Slovenia in the period of sowing and emergence.

STARCH DEGRADATION AND AMYLOLYTIC ACTIVITY IN SILVER FIR *(ABIES ALBA Mill.)* SEEDS DURING GERMINATION

M. KOVAČ and I. KREGAR

(Institute of Biology, University of Ljubljana, Aškerčeva 12, 61000 Ljubljana, Yugoslavia and Jožef Štefan, Institute, Ljubljana)

Starch degradation and amylolytic activity were observed in weakly and deeply dormant silver fir seeds. The germination of deeply dormant seeds was induced by 1% H_2O_2 solution or by removal of the seed coat. Weakly dormant seeds germinated at room temperature. The amount of starch and amylolytic activity were determinated separately in the embryo and in the endosperm, before and during visible germination.

Starch is a minor food reserve in silver fir seed, the greater part of it is found in the endosperm. Degradation of starch in the endosperm of weakly dormant seeds was observed just before radicle protrusion. In the embryo, the amount of starch increased greatly during visible germination.

Amylolytic activity, too, was higher in the endosperm than in the embryo. Considerable activity was found in the extract of the dry seeds endosperms from both deeply and weakly dormant seeds. In weakly dormant seeds maximum activity was reached after 10 days of germination, followed by a gradual decrease. The amylolytic activity of the endosperm of deeply dormant seeds showed a constant decrease during the germination period studied. In the embryo of weakly dormat silver fir seeds the amylolytic activity decreased during the experiment while in the embryo of deeply dormant seeds it remained constant.

The results suggest that the amylolytic activity of the dry seeds is not an indicator of silver fir seed dormancy. The hydrolysis of starch in the endosperm aids seedling establishment after radicle protrusion.

DYNAMICS OF FATTY ACIDS CONTENT DURING DEVELOPMENT OF SUNFLOWER SEED OF SOME SUNFLOWER LINES AND HYBRIDS

I. VEREŠBARANJI and T. V. ČUPINA

(Faculty of Agriculture Institute of Field and Vegetable Crops Novi Sad)

The aim of this study was to determine the dynamics of synthesis of fatty acids in the course of ontogenetic development of grain of some Novi Sad hybrids and their parent components.

In the course of ontogenetic grain development every seven days after pollination, whole sunflower heads were harvested, threshed and kept at a temperature of 40°C with 12% of humidity.

The treatment was done with two hybrids NS-H-26-RM and NS-H-43, whose parent components were CMS-HA-W-9831-3-4 mother and RHA--58 father component and OCMS-21 mother and RHA-SNRF father component, respectively.

In this study nine different fatty acids were determined by gas chromatography and four of them, which were the most widely distributed, are presented in diagrams at different stages of plant development. In mature seed of hybrids and lines, the most widely distributed fatty acid was linoleic (18 : 2) with approximately 60%. Oleic acid was the next (18 : 1) with approximately 20%. then palmitic (16 : 0) with approximately 8% and stearic acid with approximately 6%.

In the course of ontogenetic development the most widely distributed fatty acids, linoleic and oleic acids, showed significant variations, while the distribution of palmitic and stearic acids did not vary. The literature and our experience, recently published, indicate that in the course of ontogenetic development, oleic acid is used as a component for the synthesis of linoleic acid, which is presented on the poster diagrams. It is interesting that this transformation of oleic acid to linoleic is more or less expressed in the analyzed genotypes at different stages of seed development, especially in OCMS-21 mother component of NS-H-43 hybrid which affected the dynamics of the synthesis in this hybrid combination.

PROLINE METABOLISM IN THE GERMINATING MAIZE KERNEL

V. HADŽI-TAŠKOVIĆ ŠUKALOVIĆ and D. JELENIĆ

(Maize Research Institute, Zemun Polje, Beograd—Zemun)

Knowledge of proline metabolism in different tissues of the maize plant is important for further studies of the physiological basis of resistance to environmental factors and studies of maize seed physiology.

Proline metabolism was observed in the endosperm, scutellum and seedlings of ZPSC704 hybrid maize germinated on water measuring changes in free proline content and activity of the proline dehydrogenase enzyme.

An increased content of free proline was found in all the tissues investigated during germination and proline oxidation by means of proline dehydrogenase in the scutellum and seedling in the initial period of germination until 72 hours of imbibition. Proline oxidation in the endosperm does not occur with this enzyme.

This shows that proline oxidation through proline dehydrogenase functions at the earliest stage of plant development, when proline conversion to glutamic acid is intensive for increasing the pool of glutamic acid, which is an important amino acid donor for the development of the young plant.

LASER TREATMENT EFFECTS ON ALFALFA SEEDS

G. VASILEVSKI, F. FIDANOVSKI and R. GJURIČ

(Faculty of Agriculture, Skopje)

In optimal conditions of temperature, humidity and light, plant seeds germinate. At the stage of partially differentiated embryo, development begins after a certain period of dormancy. This period of dormancy presumes the process of supplemental maturity. Retardation in germination, stop or non-appearance of germ often occur as a consequence of some deficiency factors or of the dormant state of the seeds. There are various factors and methods for seed stimulation and reduction dormancy period. Laser application for this purpose is a novelty in the world and in this country.

We examined laser treatment effects on three varieties of alfalfa (M-2, OS-66 and Debarska) from two regions, Skopje and Ovče Pole, in the SR of Macedonia.

Germination energy, germination, the length of root and hypocotyls were investigated in the laboratory.

The results indicate that the germination energy increased by 2.3% in the M-2 variety, by 14.6% with the Debarska variety and up to 35.2% in OS-66. An increase in germination started at 2.0% with the M-2 variety, 15.4% in Debarska and was 34% in the OS-66 variety.

The length of hypocotyls increased by 0.18% in Debarska, by 0.30% in 0S-66 and 1.30% in the M-2 variety in comparison with the control, after ten days.

The length of the primary roots increased from 0.16 cm in Debarska, to 0.42 cm in M-2 and up to 0.67 cm in the 0S-66 variety.

LASER TREATMENT OF *CARTHAMUS TINCTORIUS* AND *SESAMUM INDICUM* SEEDS

G. VASILEVSKI, P. EGUMENOVSKI and V. SAMARDŽIOSKI

(Faculty of Agriculture, Skopje)

Red laser light was used for the treatment of seeds of the industrial cultures of saflower and sesamum, to test its stimulative effects upon the initial development of the plants.

Laboratory analyses were repeated two times. First a month after the treatment of seeds and again after 2.5 moths. Laboratory analyses were done after JUS methods.

Measuring was repeated four times on 400 seeds, and the results are shown for the average of one plant.

The results were as follows:

— The average germ energy of saflower seeds increased by 1% and in sesamum by 3.5%, under the influence of laser irradiation.

— The increase in the total germ of saflower seeds was 3.3% as compared to the control, and in sesamum it was 7.1%.

— The effect of laser light was observed in the lenght of the germ. The increase was 6.7 mm in saflower and 0,3 mm in sesamum.

— The differences in dry mass weight were 7.6 mg per plant in saflower and 0.2 mg per plant of sesamum.

— By analogy with the weight of fresh mass, the differences in dry mass broken seeds after laser irradiation were 2.7 mg per plant in saflower and 1.0 mg per plant in sesamum.

— Differences between broken and unbroken seeds were noticed in all items investigated after the treatment with laser beam.

Positive results obtained for all parameters examined testify to the stimulative effect of red laser light upon the germ at the initial stage o plant development.

EFFECT OF PRE-SOWING SEED TREATMENT WITH He-Ne LASER BEAM ON INITIAL GROWTH OF SOME PLANT SPECIES

V. BORŠOŠ-MATOVINA and R. KASTORI

(Basic Organization of Associated Labour AGROCOOP, Novi Sad and Faculty of Agriculture, Novi Sad)

Recently, a number of studies has been published, which emphasize the favorable effect of laser treatment on seed germination, growth and plant development as well as on the quality of some plant species.

Considering the mentioned data as well as the fact that some economic and marketing organizations are interested in the use of laser in agriculture, we began to analyze the effect of laser treatment on germination and initial growth of some plant species.

The laser treatment of air dry seed was preformed with a He-Ne laser beam of 20 to 50 mW and wave length of 630 nm. The treated seed of field pea, sugar beet, tomato, carrot, parsley, parsnip and cabbage showed different germinability. In most species, seed was treated once, twice and three times, while in sugar beet, it was treated once, three and five times. In laboratory conditions, the energy of germination and germinability was analyzed at different temperatures as well as the initial growth of seedlings. In addition, we analyzed the effect of laser treatment on the accumulation of nitrogen, phosphorus, potassium, calcium and magnesium in the above-ground part and root of field pea in water culture.

On the basis of the results, we may conclude that the effect of laser treatment was different and depended both on the plant species and variety and on the frequency of treatments. In some species and varieties we determined an increase of germination energy, germinability and increased initial plant growth. The seed treatment with laser beam also affected the accumulation of particular elements. Considering that these experiments are only preliminary, we cannot yet give a definite answer to the question of how the laser treatment affects the parameters analyzed. However, the results show that further investigations of this problem have to be done, although the probability of reproduction of the effects obtained is very small, which may be a limiting factor in application of laser irradiation in agricultural practice, as experience teaches.

CONTRIBUTION TO KNOWLEDGE OF JUVENILE STAGE OF SOYABEAN *GLYCINE MAX* (L.) MERILL

LJ. BARBALIĆ[1], *R. HENNEBERG*[2], *D. KODRNJA*[3], *D. PAVIŠIĆ-STRACHE*[3] *and R. VEZMAROVIĆ*[1]

([1]Institute of Pharmaceutical Botany, Faculty of Pharmacy and Biochemistry, Zagreb; [2]Department of Plant Breeding, Genetics and Methods of Reseaarch, Institute for Breeding and Production of Field Crops, Faculty of Agricultural Sciences, Zagreb; [3]Department of Chemistry, Faculty of Pharmacy and Biochemistry, Zagreb)

The juvenile stages of two cultivars, SREĆKA and ZVIJEZDA, as well as the line L 1159/65—1 of the Department of Plant Breeeding, Genetics and Methods of Research of the Institute for Breeding and Production of Field Crops, Faculty of Agricultural Sciences, Zagreb, have been investigated.

The content of iron in cotyledons of germinated soyabean seeds is important because of the alimentary significance of soya.

The stages of germination were examined in laboratory conditions and content of iron determined by the AAS method in cotyledons of dry, imbibed and germinated seeds.

By comparing the results of the investigation, it was determined that the tested genotypes had different quantity of iron in cotyledons.

It was also found that iron in cotyledons of a particular genotype was not mobile during the first 90 hours of germination.

Session 5.

CELL AND TISSUE CULTURE, MICROPROPAGATION

DIFFERENTIATION AND ORGANOGENESIS IN PROTOPLAST CULTURE OF *SOLANUM TUBEROSUM* L. cv. Bintje

J. BERLJAK[1] *and CH. H. BORNMAN*[2]

([1]Department of Botany, Faculty of Science, University of Zagreb, [2]Hilleshög AB, Landskrona, Sweden)

Plant regeneration in protoplast culture depends on several factors. Among the most important are the physiological state of plant material from which protoplasts will be isolated, and appropriately selected media for protoplast culture and plant regeneration respectively.

Protoplasts have been isolated from potato leaf mesophyll, of Bintje cultivar. As plant material for protoplast isolation, plants (3—4 weeks old) grown in sterile conditions on a MS medium with GA_3 were used. The mean number of protoplasts was 2×10^6 per gram of plant tissue. After isolation sterile and viabile protoplasts were cultured on two kinds of media: a) liquid, with cytokinin and auxin, and b) softened, using agar or agarose with cytokinin and auxin.

In darkness and at room temperature, protoplasts regenerated cell walls, started dividing and forming microcalli. Calli were subcultured with agarose on a modified MS medium without NH_4NO_3 and containing cytokinin and auxin. Shoot regeneration occurred after single calli cultivated on the medium with zeatin.

Shoot elongation, growth of regenerated plants and micrǫclonal propagation were achieved on the MS medium with GA_3.

PHOTOPERIODIC AND HORMONAL REGULATION OF FLOWERING IN MALE AND FEMALE *RUMEX ACETOSELLA* CLONES REGENERATED *IN VITRO*

LJ. ĆULAFIĆ, T. V. BAVRINA, N. LOZHNIKOVA, N. D. DUDKO and M. KH. CHAILAKHYAN

(Institute of Botany, University of Belgrade, and Institute for Biological Research, University of Belgrade (Lj. Ć.), K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow)

Clones of male and female plants were obtained by using the method of micropropagation. The primary explants were vegetative lateral buds of adult plants isolated in the flowering phase. Plant regeneration involved phase of bud multiplication (BAP 10^{-5}, IAA 10^{-6} M) and rhizogenesis (IBA). Plants adapted to nonsterile conditions were grown under two photoperiodic regimes: long-day (16-h light) and short-day (8-h light). Endogenous hormones (abscisic acid from leaves and cytokinins from root extracts) were analyzed under both photoperiodic regimes by a combination of gas chromatography and biological tests. The effect of exogenous addition of gibberellin (GA_3) and kinetin was examined in relation to photoperiodic induction. Clones of both sexes regenerated in culture had the same photoperiodic requirement and reaction to gibberellin and kinetin as the control plants grown from seeds.

ORGANOGENESIS IN SHOOT TIP CULTURES OF OAKS

B. PEVALEK-KOZLINA and S. JELASKA

(Department of Botany, Faculty of Science, Zagreb)

Experiments were carried out in order to obtain the effect of explants origin, basal medium composition and growth regulators on differentiation in culture of *Quercus robur* L. and *Q. petraea* Lieb.

Apical and axillary buds, taken from seedlings and young plants (10 days to two years old) were used as initial explants. The buds were satisfactorily disinfected by successive use of 1% Izosan-G (a chlorine product) and 1% mercuric chloride. The effect of three basal nutrient media was investigated: ACM (1), mod. De Fossard (2) and mod. WPM (3). Various combinations and concentrations of growth regulators (BA, KIN, 2iP, NAA, IBA and GA_3) were added to all media investigated.

Elongation of cultured buds was satisfactory on ACM and mod. WPM medium. There was no difference between apical and axillary bud reactions. The induction of adventitious buds on the cut surface of explants was achieved with both species investigated, but they were induced more frequently in *Quercus robur* cultures. Better adventitious bud induction was obtained on the explants of younger donor plants. Adventitious roots developed in culture of *Quercus petraea* when mod. De Fossard medium was used, but they were not functionally connected with the vascular system of shoots. Our investigations indicate that the composition of salts is as important for a successful oak tissue culture as adequately used hormones.

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THE INFLUENCE OF JASMONIC ACID ON THE GROWTH AND VIRUS CONTENT IN MERISTEM CULTURE OF POTATO cv. VESNA

M. RAVNIKAR[1] *and N. GOGALA*[1,2]

([1]Institute of Biology, University of Ljubljana, 61000 Ljubljana, Aškerčeva 12, Yugoslavia
[2]Biotehnical Faculty, Department of Biology, E. Kardelj University, 61000 Ljubljana, Aškerčeva 12)

Meristems (0.2 — 1.2 mm) were explanted into an MS medium supplemented with IAA (11.4 μM), KIN (9.2 μM), GA_3 (3.88 μM) and different concentrations of jasmonic acid (JA — endogenous plant growth regulator) 0; 0.47; 0.95; 9.5; 23.8 μM.

Germs growing from the tubers in the dark (A) were used as a source for meristems in three series of experiments. In other four series (B), the germs were exposed to light. Dormant tubers for the B series were treated with GA_3.

The potato plants were temperature treated (34—37°C) for 2, 4, 6 or 8 weeks.

JA in concentrations of 0.47—9.5 μM had a positive influence on the shoot differentiation of A meristems, while no effect was noticed with B meristems. Both were compared to a control medium without

JA. Concentrations higher than 9.5 μM retarded the whole growth of the inoculum as well as shoot differentiation.

The potato cv. Vesna in its entirety was infected with PMV (potato mosaic virus). It is possible that PMV enters the growing meristem tip because the elimination of viruses was not dependent on the size of isolated meristems. PMV was eliminated in two different ways:

1. Meristem culture, without preliminary temperature treatment, JA was added to the medium.

2. Temperature treated infected plants and isolation of meristems. The addition of JA to the medium increased the number of avirous plants.

PMV tests were done for each sample with ELISA and with electron microscopy once to four times.

VEGETATIVE MULTIPLICATION OF SUGAR BEET FROM VARIOUS EXPLANTS THROUGH *IN VITRO* CULTURE

S. MEZEI and L. KOVAČEV

(Faculty of Agriculture, Institute of Field and Vegetable Crops, M. Gorkog 30, 21000 Novi Sad)

In vitro vegetative propagation of sugar beets facilitates a long term and stable maintenance of superior genotypes. It also enables the production of identical plants which is of importance when testing lines for homozygosity and combining ability in breeding.

We made a clones originating from one seed, a part of floral stalk, petioles and cotyledons. We conducted a study using three genetically diverse sugar beet genotypes.

Explants were grown on different media. The research objectives were to determine the best homone combination for micropropagation of sugar beet. After four weeks, axillary buds appeared on the explants. Further subcultivation included the separation of buds and their monthly transfer to a fresh medium. The percent of rooting achieved in an auxin free medium was 90%.

Observing the results, we came to conclusion that the size and origin of explants played an important role in sterilization. All three genotypes differed in regeneration capacity. The highest morphogenetic potential was found in the regenerants of 73S genotype.

It should be possible to maintain genotypes in the form of shoot cultures for more than two years at 4°C. Phenotypic variability was observed. A cytological analysis showed that there were no significant changes in the number and structure of chromosomes.

VARIABILITY OF SOMATIC EMBRYOGENESIS IN DIFFERENT CLONES OF MALE AND FEMALE PLANTS OF *RUMEX ACETOSELLA* L.

S. BUDIMIR, LJ. ĆULAFIĆ and R. VUJIČIĆ

(S. Stanković Institute for Biological Reesearch, Beograd and Institute of Botany, University of Beograd)

In our previous work we defined the conditions for the induction of somatic embryogenesis in the culture of the dioecious plant *R. acetosella* L. previously maintained by micropropagation for three years. Somatic embryogenesis was induced by an increase of the osmotic concentration in the medium, and embryo maturation was enhanced by the presence of gibberellin in the secondary medium.

Since it was found that some of the clones of male and female plants possessed different embryogenic capacity, the phenomenon was further studied on newly isolated male and female clones. A comparison was made of the number of embryos (torpedo, cotyledonary stage and germinated embryos) in each individual explant of three male and three female clones. A significant variability of embryogenic potential was found in each individual clone, as well as in relation to the plants of the opposite sex.

The effect of a two-phase medium (solid-liquid) containing gibberellic acid (GA_3 10 mg l^{-1}) was studied and it was found that gibberellic acid present in the liquid medium expedited embryo maturation.

CLONAL PROPAGATION AND PLANTLET ANALYSES IN LONG--TERM EMBRYOGENIC CULTURES OF PUMPKIN

B. JURETIC, M. KRSNIK-RASOL and S. JELASKA

(Department of Botany, Faculty of Science, Zagreb)

Embryogenic pumpkin callus lines cultivated on an MS medium with auxins (IBA, IAA or 2,4-D) had retained their embryogenic potential for more than 15 years. Certain factors had been suppressing continual embryoid laturation on the maintaining callus lines medium and their development into adult plants. To enable embryoid maturation, a sequentional treatment of the cultivating callus lines was investigated on different media. High frequency of plant development (77%), was obtained on an MS medium with 11.4 μM IAA. Regenerated plants were cloned by axillary bud culture on the MS medium with 5.7 μM IAA. All the plants showed considerable seasonal growth variability, although they had been produced from callus cultures maintained under artificial conditions of a culture-room for more than 15 years.

Shoots developed first from axillary buds in the nodal segments culture. This was followed by the development of adventitious roots. Shoot rooting depended upon the clone. The plants were successfully aclimatized to conditions *in vivo;* they flowered and produced fruits. They did not show significant morphological differences in comparison with control plants developed from seeds.

Electrophoretic analyses of proteins isolated from cloned plant leaves also showed great mutual resemblance.

GENETIC STABILITY IN ANTHER-DERIVED DIHAPLOIDS OF TOBACCO HYBRIDS

D. PAPEŠ[1], *S. JELASKA*[1] *and V. KOZUMPLIK*[2]

([1]Department of Botany, Faculty of Science, University of Zagreb and

[2]Tobacco Institute, Zagreb)

Tobacco dihaploid plants were colchicine induced from anther culture haploids. Anthers used for cultures were isolated from hybrids of two parental combinations: the first lot with a parent slightly (GV—3 × DELGOLD) and the second lot with a parent strongly resistant (CH. M. × DELGOLD) to peronospora.

All analysed tobacco dihaploid plants were euploids with $2n = 4x = 48$ chromosomes. Their behaviour during meiosis was very similar. Along with the normal bivalent number $n = 24$ in 63% diakinesis, pollen mother cells also appeared with 23, 25 and 26 bivalents. Segregation irregularities revealed micronucleus frequency in prophase (16.38%) and in telophases I and II (14.75%), which did not affect high pollen fertility (80—95%). Very high recombination, revealed in the chiasma frequency per bivalent (2.42—2.66) proved absolute homozygosity of anther-derived dihaploid tobacco plants. Their seeds could therefore be used for selection.

PROTEIN PATTERN OF PUMPKIN CALLUS AND TUMOR TISSUE

T. BAKRAN-PETRICIOLI, M. KRSNIK-RASOL and S. JELASKA

(Department of Botany, Faculty of Science, University of Zagreb)

Differential synthesis of proteins can be expected at different developmental stages of cell. This led us to compare the electrophoretical patterns of soluble proteins of various pumpkin (*Cucurbita pepo* L.) callus lines and of tumor tissue in order to find characteristic markers for specific tissue culture.

We analysed soluble proteins in seven different callus lines and in tumor tissue by electrophoresis in SDS-polyacrylamide gel and we determined total peroxidase activity.

The pumpkin callus lines were grown on an MS medium different growth regulators: line TOH (2 mg l^{-1} TOH, DE (1 mg l^{-1} 2,4-D), $Ž_5b$ (1 mg l^{-1} IBA), MSS O (O mg l^{-1}IAA), MSS (1 mg l^{-1}IAA), NA_3 (1 mg l^{-1} NAA+adenine sulphate), Wc (0.3 mg l^{-1} 2,4 D+2 g l^{-1} yeast extract).

Primary tumors were induced on sterile cotyledone explants with *Agrobacterium tumefaciens*, strain B_6S_6. Explanted tumor tissue was grown on the MS medium with addition of 500 mg l^{-1} Carbenicilline and without growth regulators during three subcultures.

The electrophoretic pattern of soluble proteins was very similar in all the callus lines except for slight differences in the protein group of molecular weight below 45 000 d. Tumor tissue (grown on Carbenicilline) showed fewer polypeptide groups than callus tissue. The peroxidase activity in the tumor tissue was as three times as high as in the callus lines.

CONDITIONS FOR BUCKWHEAT AND ALFALFA TUMORAL TRANSFORMATION
I. Cell and protoplast culture

S. NINKOVIĆ, J. MILJUŠ-ĐUKIC, R. NOVAKOVIĆ and M. NEŠKOVIĆ

(Institute of Botany, Faculty of Science and S. Stanković Institute for Biological Research, University of Beograd)

Tumoral transformation of higher plants, caused by *Agrobacterium tumefaciens,* consists in the transfer of a DNA fragment from the bacterial plasmid into the higher plant genome. Isolated plant cells and protoplasts can function as recipients for T-DNA when co-cultivated with bacteria. The genetic transformation of selected species can be successful if specific methods for tissue culture are well elaborated, including cell and protoplast culture and regeneration. For this purpose, culture conditions for buckwheat *(Faqopyrum esculentum)* cv. Pennquad and Siva, and for alfalfa *(Medicago sativa)* cv. Zaječarska 83 and Regen S have been studied.

Buckwheat callus culture grows well in mineral solutions MS, B_5 and WPM, while the Heller and White media do not support tissue growth. Casein hydrolysate improves growth in all cases. Sucrose can be used in a broad range of concentrations, from 2—6%. IAA (10^{-5} M), NAA (10^{-6} M) and 2,4-D (10^{-6}M) can be used, with BAP (10^{-5} M), rather than kinetin (10^{-5} M) for optimal callus growth. Buckwheat tissue is peculiar in its reaction to GA_3 and ABA, the former being inhibitory (10^{-8} to 10^{-5} M), while the latter significantly stimulates callus growth (10^{-7} to 10^{-5} M). Suspension culture of buckwheat cells was not feasible in the liquid media with the same composition. Viable protoplasts were obtained with 4% cellulase Onozuka R10 and 0.5% macerozyme R10, after an incubation of 15 h.

Callus tissue of the local alfalfa variety, Zaječarska 83, displays a moderate regeneration capacity in culture, gives good cell suspension in the liquid medium, single cell clones, and viable protoplasts, which regenerate the cell wall. It is similar to other alfalfa varieties described in the literature.

CONDITIONS FOR BUCKWHEAT AND ALFALFA TUMORAL TRANSFORMATION
II. Tumor induction and features of transformed buckwheat tissue

M. NEŠKOVIĆ, B. HORVAT, J. MILJUŠ-ĐUKIĆ and S. NINKOVIĆ

(Institute of Botany, Faculty of Science and S. Stanković Institute for Biological Research, University of Beograd)

In a recent review (1) buckwheat *(Fagopyrum esculentum)* is not included in the list of plants susceptible to *Agrobacterium tumefaciens.* This probably means that tumors are not noted in field-grown plants, and that laboratory experiments have not been attempted. We have inoculated buckwheat plants with the following *A. tumefaciens* strains: A 281, A 348, ACH 5, A_6 and A 136. Plantlets regenerated in culture, hypocotyl fragments and cotyledons of aseptically germinated

seedlings were used for inoculation. Stems were injured by a needle, while hypocotyl and cotyledon fragments were dipped into bacterial suspension. After three days the bacteria were eliminated by carbenicillin. All bacterial strains, except the avirulent A 136, produced tumors. The tumor tissue was excised and transferred to nutrient medium for buckwheat, lacking the hormones BAP and IAA. All tissue strains have been able to grow without hormones for 6 passages so far. The tissue transformed by A 281 exhibited a particularly high proliferation capacity. Preliminary assays for endogenous growth substances, however, showed that the tumor tissue extracts had auxin-like, cytokinin-like, and gibberellin-like activities comparable to normal buckwheat tissue.

1. *DeCleene, M., Deley, J.* 1976: Bot Rev. 42, 389.

PROTOPLASTS ISOLATED FROM MYCORRHIZAL FUNGI

R. KOMEL[1], *M. DERMASTIA*[1], *J. ŽEL*[2] *and N. GOGALA*[2,3]

([1]Institute of Biochemistry, Medical Faculty, 61000 Ljubljana, [2]Institute of Biology, University of Ljubljana, 61000 Ljubljana, Aškerčeva 12, Yugoslavia, [3]Biotehnical Faculty, Department of Biology, E. Kardelj University, 61000 Ljubljana, Aškerčeva 12)

Protoplast release from various Basidiomycetes was achieved by using *Trichoderma viride* 1131 CBS 353—33 enzymes.

Lytic enzyme preparations were prepared from *Trichoderma viride* culture filtrates after induction with cell-wall fragments of *Suillus variegatus* and *Lactarius piperatus*. Regeneration of protoplasts of *Lactarius piperatus* was achieved.

CONTENT OF NICOTINE IN TOBACCO (*NICOTIANA TABACUM* L.) *IN VITRO* CULTURE

M. SPASENOSKI

(Institute of Biology, Faculty of Science, Skopje)

Tobacco tissue culture was obtained from the Prilep variety, produced in the Tobacco Institute — Prilep.

The purpose of the work was to study the nicotine content of this variety in culture.

Explants were isolated from different parts of germinated seeds and then cultivated on MS (Murashige and Skoog, 1962) mineral solution in the presence of IAA (0.5 mg $\cdot$ 1^{-1}) and kinetin (0.1 mg $\cdot$ 1^{-1}). After 30 days explants were subcultured on the same medium with IAA (0.5 mg $\cdot$ 1^{-1}) and kinetin (0.1 mg $\cdot$ 1^{-1}), where bud multiplication was obtained. The best results in bud multiplication were achieved with explants from apical buds. Induction of the adventive roots was obtained with IAA (0.5 mg $\cdot$ 1^{-1}) and kinetin (0.1 mg. 1^{-1}).

The rooted plantlets were transferred to non-sterile conditions and in less than 5 months they produced seeds. It is concluded that tissue culture methods are applicable to the Prilep variety.

The content of nicotine was determined in the plants *in vitro*, with different hormone contents, as well as in the plants transferred to non-sterile conditions. The results show that the content of nicotine in the tobacco *in vitro* on MS medium with different contents of plant hormones is different. The highest percentage of nicotine was achieved with IAA (0.5 $mg \cdot l^{-1}$) and kinetin (1 $mg \cdot l^{-1}$), while the content of nicotine in the plants transferred to non-sterile conditions was highest in the leaves of the terminal part.

SPECIFIC FEATURES OF *PICEA ABIES* L. (Karat.) VEGETATIVE BUDS DURING WINTER-SPRING PERIOD

V. CESAR and D. CESAR

(Pedagoški fakultet, Osijek and Institute for Medical Research and Occupational Health, Zagreb)

Recent studies of *P. abies* vegatative buds have shown that one part of the bud, the crown, has an influence on the transport of some substances and consequently has an effect on the growth of vegetative buds in in-vitro culture. This paper presents part of investigations into the morphological and anatomical changes of the *P. abies* vegetative bud during winter-spring period. Dormant vegetative buds of *P. abies* were taken weekly from four-year-old trees at Hilleshög AB nursery, South Sweden, for 11 weeks from the middle of February to the end of April in 1985. Buds for anatomical observations and measurement were isolated from scales and fixed in glutaraldehyde, dehydrated and fitted in thermoplastic resin. Longitudinal sections, 3 μm thick, were made and stained with toluidine blue. Data obtained by measurement were statistically processed (Pavlić 1977). Vegetative buds of *P. abies* have three parts:

(1) embryonal cells which produce spring shoot,
(2) the crown made up of small, densely congested thick wall cells and
(3) the cavity, bordered with long cells and filled with loose cells.

Transport tissue from the branch reaches the lower margin of the cavity. The area and height of these structures of two different clones were measured throughout the 11 weeks. Significance tested of the ratio difference in the areas of structures 1/2, 1/3, 3/2 showed that there was a significant difference in increase of the ratios 1/2 and 1/3 in the middle of April (spring). This difference did not exist for the ratio 3/2, which indicates the sudden growth of structure 1 area. Similar results were obtained by testing the height of the same structures. Results for both investigated clones coincide.

FLOWERING OF *SINAPIS ALBA* AND *CHENOPODIUM RUBRUM* SHOOTS GROWN *IN VITRO* IN THE PRESENCE OF THE HERBICIDE SANDOZ 9789

T. V. BAVRINA, LJ. ĆULAFIĆ, N. P. FINOGINA, V. Z. PODOL'NII and M. KH. CHAILAKHYAN

(K. A. Timiryazev Institute of Plant Physiology, Academy of Sciences of the USSR, Moscow. Institute of Botany, Faculty of Science, and Institute for Biological Research, University of Beograd)

Stem tips of *Sinapis alba* and *Chenopodium rubrum* were isolated and grown in sterile culture. The medium contained agar, Murashige-Skoog mineral solution, sucrose, vitamins, and the herbicide Sandoz 9789. The herbicide Sandoz 9789 provoked total inhibition of carotenoid synthesis and destruction of chlorophylls. The growth of *Ch. rubrum* stems was significantly affected by Sandoz 9789, while the effect on *S. alba* growth was insignificant. The photoperiodic requirement of both species was not changed in the presence of Sandoz 9789. The treated plants flowered under the inductive regime, as did the green control plants, i.e., all *S. alba* and *Ch. rubrum* plants flowered under long days or short days, respectively.

IN VITRO PROPAGATION OF *JANKEA HELDREICHII (GESNERIACEAE)*

B. HORVAT

(S. Stankovic Institute for Biological Research, Beograd)

Jankea heldreichii is an endemic and relict species of the Balkan Peninsula (Greece). Micropropagaton was performed with the aim to prevent the extinction of this endangered species, and to introduce it, if possible, into the horticultural production.

Seeds were surface sterilised in 0,8% NaOCl for 30 minutes, thoroughly washed in sterile water, and germinated on half strength WPM (Anderson, 1978) without growth substances. Cotyledons, leaves, hypocotyls, and apical buds were used as explants for induction of adventitious buds. On WPM and MS, supplemented with BA 5.0 mgl^{-1} and NAA 0.1 mgl^{-1}, all explants produced numerous shoot buds. The highest production of adventitious buds was observed from leaf and cotyledon explants. There was no significant difference between WPM and MS mineral solutions and in the later studies only MS-based media was used.

Shoot bud cultures were maintained and multiplied on MS + BA 1.0 mgl^{-1} + IBA 0.1 mgl^{-1} media.

Abundant development of adventitious buds caused cultures to appear as compact bunches, composed of numerous interconnected rosettes. On media containing BA at 1.0 mgl^{-1} shoots were short and stunted. To increase their length it was necessary to decrease the BA concentration to 0.5—0.2 mgl^{-1}.

Rooting was obtained on MS + IBA 0.5 mgl^{-1} media. Rooting was 100% independently of the size of explants. Roots were short (2.0—3.0 mm) and numerous. When these roots were removed and plants re-cultured on media without growth substances, new roots appeared and their number per rooted culture decreased, while the length of individual roots increased to 6.0—7.0 mm.

MICROPROPAGATION OF HYBRID *AMARYLLIS*

N. GOGALA and J. GOLOB-KLANČIČ

(Biotehnical Faculty, Department of Biology, E. Kardelj University, 61000 Ljubljana, Aškerčeva 12)

We were interested in the methods of micropropagation of hybrid *Amaryllis*. The most suitable starting material for this purpose proved to be bulbs after the break of dormancy. Best results (up to 100 plantlets after two transfers) were obtained by using NAA and BAP in MS medium. Further continued multiplication produced up to 8000 young plantlets from a single bulb. These plantlets can be planted efficiently in soil without any special procedure. Young *Amaryllis* plants from the in vitro cultures already have good roots and are not very sensitive to the changes in relative humidity.

MICROPROPAGATION OF GERBERA BY TISSUE CULTURE

LJ. RADOJEVIČ[2], *N. ĐORĐEVIĆ*[1] *and L. MANČEVA*[2]

([1]S. Stanković Inst. Biol. Res., Beograd and
[2]Oranžerija Cvet, Đevđelija, Yugoslavia)

Nutrient requirements for gerbera (cv. Bingo, Clementine and Merleen) micropropagation by meristem culture with 2—3 leaf primordia (size 1—2 mm) or for organogenesis *via* culture of segments of young capitulum, floral- or leafstalk (size 3—5 mm) were studied. Explants were cultured on MS mineral solution (Murashige and Skoog, 1962) supplemented with B vitamins, amino-acids, indol-acetic acid (IAA) and kinetin (Kin). In cultures of shoot apices different percentages of leaf rosettes were formed: 19% Bingo, 27% Clementine and 11% Marleen. Segments of the capitulum, floral and leafstalks showed potential for adventitous shoot formation followed by intermediary callus development and accelerated necrosis. The number of explants with morphogenetic response depended on both the origin of the explant and the cultivar.

Multiplication of the shoots originating from shoot apices was achieved in the MS + IAA + Kin medium *via* axillary buds. Best results of shoot multiplication were obtained in the 5th (cv. Bingo and Clementine) and 7th (Marleen) subcultures.

Rooting of the shoots of all three cultivars examined was achieved in MS medium supplemented with IBA. Acclimatization of the plantlets was quite satisfactory (Bingo 97%, Clementine 92% and Marleen 70%).

INFLUENCE OF LIGHT INTENSITY ON THE ELONGATION OF ADVENTITIOUS ROOTS OF *DRACAENA FRAGRANS* Ker. DURING MICROPROPAGATION

D. VINTERHALTER

(S. Stanković Institute for Biological Research, Beograd)

During the trial commercial micropropagation of *Dracaena fragrans* Ker., marked differences in the speeds of root elongation among production batches have been observed.

Investigations showed that the main factor controlling the speed of root elongation was the light intensity under which rooting was performed.

In darkness root elongation was minimal. Increase of light intensity (1, 1.3, 2.0, 3.5, W/m^2) significantly increased the speed of root elongation which was highest on the highest light intensity tested (7.2 W/m^2). In the shoots rooted at 7.2 W/m^2, roots were more than 10 times longer than in the shoots rooted in darkness.

In the shoots which were first rooted in darkness for 4—5 weeks and then transferred to light, root elongation proceeded at the speed characteristic of the light intensity employed.

Also in the shoots which were first rooted at 7.2 W/m^2 for 2—3 weeks and then transferred to darkness, the speed of root elongation decreased drastically. At the end of the first week in darkness, the speed of root elongation was the same as in the shoots rooted in darkness from the beginning.

Rooting was performed on an MS based medium supplemented with IBA 0.5 mgl^{-1}. Rooting was nearly 100% efficient in all treatments.

MICROPROPAGATION OF *RHODODENDRON SIMSII*

S. BABIČ[1,2], *M. CAMLOH*[2], *N. GOGALA*[1,2] *M. RAVNIKAR*[2] *and J. ŽEL*[2]

([1]Biotehnical Faculty, Department of Biology, E. Kardelj University and [2]Institute of Biology, University of Ljubljana, 61000 Ljubljana, Aškerčeva 12)

In this applied research we investigated the influence of various cytokinins on the initiation of buds on the shoot tips, the possibility of continuous shoot cultures, and rooting of shoots in the species *Rhododendron simsii.*

Initiation of buds was best on the Murashige-Skoog medium at 1/10 strengthth (basal medium — BM) with addition of zeatin. The number of initiated buds varied between 5 and 8.

Continuous shoot culture was achieved with secondary bud formation after elongation on hormone-free BM and subculturing, into BM with 2iP.

Rooting was best (above 60%) in the hormone-free basal medium.

Two to three months were needed for the development of rooted plants. After 4 months the plants were transferred to soil, maintained under high humidity for a few weeks, then moved to normal conditions.

VEGETATIVE PROPAGATION OF GRAPEVINE (*VITIS VINIFERA* L.) IN VITRO

D. HARTL and S. JELASKA
(Institut za jadranske kulture i melioraciju krša, Split, Botanički zavod Prirodoslovno-matematičkog fakulteta, Zagreb)

Microclonal propagation of grapevine was established by shoot tip culture and accelerated development of axillary buds of the cultivars: Alphonse Lavellee, Grenache, Muscat Adda, Palomino, Pinot white, Pinot gris, Ugni blanc, and rootstock hybrids 41B, 5BB, SO4.

Excised shoots with 2—4 leaf-primordia (0.5—2.0 mm long) were cultured on an MS medium supplemented with 4.4 μM BA and 2.9 μM IAA. Multiplication rate of multiple shoot cultures was tested during the six subcultures (6 weeks each). Depending on the cultivar, 3—15 shoots per culture were obtained. Developed shoots 1.5—3 cm high rooted on the MS medium with 2.9—11.4 μM IAA. Plantlets in axenic cultures showed juvenile characteristic — shortage of tendrils and spiral phyllotaxis.

TRANSFORMATION OF *BETA VULGARIS* BY *AGROBACTERIUM RHIZOGENES*

*V. BOŠNJAK and CH. H. BORNMAN**
(Department of Botany, Faculty of Science, University of Zagreb, and *Cell and Tissue Culture, Hilleshög Research AB, Landskrona, Sweden)

Beta vulgaris L. (sugar beet) hypocotyls from whole seedlings, hypocotyl segments and immature embryos (ovules twenty days after pollination) were transformed by A. *rhizogenes* strains 15834 and 8196.

Roots and occasional adventitious shoot buds were induced on hypocotyl segments. Control gave roots rarely but never shoots. However, adventitious shoot buds subcultured on PG_0 agar medium (De Greef and Jacobs 1979) were rooted. It is encouraging that a regeneration of adventitious shoots in *Beta vulgaris* L. with the aid of *A. rhizogenes* is possible.

VEGETATIVE *IN VITRO* PROPAGATION OF JOSTA

B. GOLOŠIN[1], *LJ. RADOJEVIĆ*[2] *and M. KORAĆ*[1]
([1]Faculty of Agriculture, University of Novi Sad, Novi Sad
[2]S. Stanković Inst. Biol. Res., Beograd, Yugoslavia)

Isolated shoot apices (size 0.5—0.7 cm) of josta (*Ribes nigrum* × × *Ribes grossularia)* were cultured on nutrient media based on MS mineral solution (Murashige and Skoog, 1962) containing 3% sucrose, 0.7% agar and (in mg l^{-1}): inositol 100, glycine 2, vitamin B_1 0.1 and B_6 0.5. The media were supplemented with different combinations of indol-butyric acid and benzylamino purine (IBA and BAP, respectively). After four weeks of culture a different number of leaf rosettes was formed, depending on the composition of the nutrient medium.

Multiplication of josta shoots was achieved on MS + BAP (0.5 mg l^{-1}) and MS + BAP (0.5 mg l^{-1}) + IBA (1.0 mg l^{-1}) media. Average number of shoots obtained from single initial shoot was 5.42 and 4.75 in the fourth subculture, depending on the composition of the nutrient medium. The shoots were multiplied *via* axillary buds. Other nutrient media checked throughout these studies were unsuitable for *in vitro* propagation of josta because necrosis of both leaf rosettes and shoots was induced. The effect of different IBA levels on rooting of josta shoots was examined as well.

PREVENTION OF APICAL NECROSIS DURING MICROPROPAGATION OF *PRUNUS TENELLA* Batsch.

N. GHALAWNJI and D. VINTERHALTER

(S. Stanković, Institute for Biological Research, Beograd and Institute of Botany, Fac. Sci. Univ. Beograd)

Prunus tenella Batsch. is a small hardy bush well adapted to dry soil conditions. It could be used as interesting starting material for breeding new dwarf *Prunus* rootstocks.

In vitro cultures were started from lateral buds. Surface sterilisation and media composition were the same as for rootstock Colt (Vinterhalter, Horvat, 1986).

Optimal shoot bud multiplcation was obtained on MS + BA 1.0 mgl^{-1} + IBA 0.1—0.2 mgl^{-1}. Shoot elongation was fast at the beginning of every subculture, but later most of the shoot tips were damaged by apical necrosis. Apical necrosis prevented shoots elongating to a length suitable for the next stage in micropropagation, i.e. rooting.

In the attempts to overcome apical necrosis, two approaches were tried, both suggested by Ph. Boxus (personal communication).

In the first, subculture duration was shortened from 4—5 to 3 weeks. In this way, shoots were subcultured before the first signs of necrosis appeared. This approach only postponed the appearance of necrosis and it was suitable only for the maintenance of stock cultures during shoot multiplication.

The second approach was to cultivate shoots on a two-phase culture medium. Culture flasks were first filled with agar solidified media, on top of which 2—3 mm deep layer of the same liquid medium lacking sucrose and inositol was poured. This approach efficiently prevented the appearance of apical necrosis, and significantly prolonged the duration of the subculture.

Vinterhalter. D., Horvat. B., 1986: Nauka u praksi 16, 41—50.

MORPHOGENESIS IN TISSUE CULTURE OF *ROBINIA PSEUDACACIA* L.

B. KOLEVSKA-PLETIKAPIĆ[1], *Z. TOMOVIC*[2] *and B. JELENČIĆ*[1]

([1]Department of Botany, Faculty af Science, University of Zagreb) [2]Poplar Research Institute of Novi Sad)

Morphogenetic studies undertaken in tissue culture of black locust (*Robinia pseudacacia* L.) have been directed toward developing the

micropropagation method. This method is supposed to be the right way for the production of clonal reproductive material needed in breeding programs of black locust.

Callus proliferation, caulogenesis and rhizogenesis were studied in black locust culture. The axillary bud meristems were exicised from one-year-old shoots collected from 4 selected trees aged more than 5 years. A high rate of callus proliferation and adventitious shoot development were induced in primary cultures grown on Aspen Culture Medium (1) supplemented with 2.2 μM BA and 0.1 μM NAA. Callus proliferation and caulogenesis on adventitious shoot explants was reproducible in all following subcultures. The number of shoots varied from 5 to 14 per explant, depending on the genotype and subculture. Rhizogenesis was induced by transferring 2—5 cm long shoots to the same medium supplemented with 2.5 μM IBA. After the root system had developed, complete plants regenerated, providing material for further transplantation.

(1) *Ahuja, M. R.*, 1983: Silvae Genet. *32*, 131.

REGENERATION OF EUROPEAN BIRCH PLANTS *IN VITRO*

V. BESENDORFER, B. KOLEVSKA-PLETIKAPIĆ and D. PAPEŠ

(Department of Botany, Faculty of Science, University of Zagreb)

The plantlet regeneration of *Betula pendula* Roth. from axillary buds and callus culture were investigated. Axillary buds from 2-year-old and 4-year-old birches were used as primary explants. The shoots were developed on BTM (1) and ACM (2) containing a low level of cytokinin 2.2 μM BA. Nodal segments used as initial explants produced new shoots within 4 weeks. The rate of multiplication depended on the genotype and nutrient media.

Internodal segments were used as initial explants for callus culture. The callus was initiated on ACM and MS (3) media containing 2.7—5.4 μM NAA and 2.2—8.9 μM BA. Adventitious buds regeneration was obtained both in the initial callus culture and on small callus pieces transferred onto ACM and MS regeneration media containing 8.9 μM BA.

Adventious and axillary buds were rooted in ACM containing a low level of auxin (1.0 μM IBA). After 2 weeks 100% of shoots formed roots.

In order to control genetic (in)stability we performed cytological analysis of birch plantlets formed *in vitro* by adventitious bud regeneration. We investigated changes in chromosomal number, occurrence of chromosomal aberrations and mitotic activity. A higher mitotic activity (4.97%) and 4.18% of chromosomal aberrations in plants obtained from adventitious buds were found.

(1) *Chalupa, V.*, 1983: Comm. Inst. Forest. *13*, 7.
(2) *Ahuja, M. R.*, 1983: Silvae Genet. *32*, 131.
(3) *Murashige, T., F. Skoog*, 1962: Physiol. Plant. *15*, 473.