



















IVANOVA, J., KAPČINA, V. and DINOVA, M.

### HORMONAL REGULATION OF DEVELOPMENT AND PROPAGATION OF ORNAMENTAL PLANTS

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Hormonal regulation of ornamental plants has not been adequately studied, although they seem to be suitable model – plants for study of many phenomena in plant physiology, and also they represent the most commercial branch of agronomy, in which growth regulators have been widely used.

A differential relationship between stem growth and flowering was established in experiments with chrysanthemum, cyclamen, hyacinth and tulip. The effect of GA<sub>3</sub> on stem growth and flowering in bulbs depends in different degree on the low temperature action. A correlation between the content of gibberellin – like substances, growth and flowering of hyacinth was established.

The rooting of carnation cuttings is related to the changing balance of auxins and inhibitors. The activity of rooting preparations is related to their inhibitory action on IAA oxidase. Proline enhances auxin – stimulated rooting.

Propagation of hyacinth and tulip is correlated with the content of auxin, with the peroxidase and IAA oxidase activity, with the intensity of photosynthesis and pigment content. After auxin treatment, more daughter bulbs are produced in hyacinth and tulip. It is assumed that auxins have an important role in controlling propagation of these plants. The treatment of hyacinth and tulip with retardant and etrel also results in a higher number of daughter bulbs.

KRAJNČIČ, B.

### THE CHEMICAL CONTROL OF FLOWERING A STATUS REPORT

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As a base of the present communication, I have used the identically titled report of C.F. Cleland (1982). In the second part of this communication, the results of research concerning mainly *Lemnaceae* will be explained in more details (Kandeler, 1984; Krajnčič 1982, 1983, 1985).

The paper deals with the phenomena of photoperiodic induction and evocation of flowering. Data concerning the role of macromolecules and the effect of nutrients, salicylic acid, EDDHA and plant extracts have been presented. Hormonal control of flowering is discussed in details, particularly based on flowering in long-day clones of *Lemna minor*, day-neutral clones of *Spirodela polyrrhiza* and long-short-day clones of *Wolffia arrhiza*.

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BAVRINA, T.V., and PHYNOGINA, N.P.

**THE CHANGE IN THE CONTENT OF SOME PROTEIN ANTIGENS  
IN TRAPESOND TOBACCO PLANTS IN VIEW OF THE REGULATION  
OF VEGETATIVE AND REPRODUCTIVE MORPHOGENESIS**

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The paper reveals the qualitative and quantitative differences in proteins in the organs and tissues of Trapesond tobacco plants in view of vegetative and reproductive morphogenesis *in vivo* and *in vitro*. Protein study was conducted using the immunochemical analysis of precipitation in agar on immunochemical test systems. Antigen I, common for all the tissues and organs, and Antigen II, specific for leaf tissues, were detected in the seedlings. Antigen III, common for all the organs and tissues, and antigen VI, specific for reproductive organs, were elicited from reproductive organs. Leaves and stems of vegetative plants in comparison with the components of flower, are shown to contain more antigen I and less antigen III.

We supposed that vegetative buds, in comparison with the flowering ones, contain more antigen I and less antigen III. Antigen II is present, while antigen VI is absent in vegetative buds. In flower buds, when comparing them with the vegetative ones, antigen II is absent, antigen VI is present, and the content of antigen I is smaller, while that of antigen III is larger.

The analysis of these antigens was made in the primary 10 days old stem calluses, i.e. at the early stages of cytodifferentiation. The differences in the content of elicited antigens between the calluses with vegetative and reproductive morphogenesis were found similar as in the ones between vegetative and flower buds.

GERIĆ, I.

**ISOZYMES AND GENETIC CONTROL OF PHYSIOLOGICAL AND  
BIOCHEMICAL PROCESSES IN PLANTS**

Faculty of Agriculture, Institute of Field and Vegetabel Crops, Novi Sad

Isozymes are multiple forms of a given enzymic system. The forming of enzymes -- isozymes is controlled by structural genes and it explains why isozymes are also called gene markers. Enzymes -- isozymes catalyze certain physiological--biochemical reactions, enforcing in that way the genetic control of physiological--biochemical processes. Isozymes differ in physical and chemical characters. Another specific trait of these multiple forms is the mode of their formation in the course of the ontogenetic development of an organism, which is related with the expression of certain genes.

Generally, the most widely studied isozymes are those in corn, and we have studied the polymorphism of 12 enzymic systems: acid phosphatase, alcohol dehydrogenase, malate dehydrogenase, isocitrate dehydrogenase, phosphogluconate dehydrogenase, esterase, catalase, phosphoglucomutase, phosphohexose isomerase, endopeptidase, and glutamate -- oxalacetate transaminase.

We analysed a sizable group of corn populations. All of the analysed enzymic systems were found to be polymorphic, forming more or less genetically determined variants. The majority of gene loci in these systems were polymorphic, they were monomorphic quite rarely. The frequency of individual isozymes in the corn populations and population groups was different, indicating their genetic differences and metabolic distinctions.

It is generally considered that the enzymic polymorphism is related to the adaptability of an organism to the environment according to the principle: higher adaptability. We considered specific physical – chemical traits of certain isozymes (thermic stability, photosensitivity, affinity to substrate, etc.) as well as physiological – biochemical significance of these traits.

KRSNIK–RASOL, M.

### INVESTIGATION OF CROWN GALL TUMOUR INDUCTION AND DEVELOPMENT

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Wound infection with *A. tumefaciens* causes crown gall tumours in some gymnosperms and most dicotyledonous plants. Crown gall had first been studied by plant pathologists and only later by physiologists because of considerable loss on certain crops it occasionally causes. Crown gall has now become a subject of intensive investigation by molecular geneticists. All virulent strains of *A. tumefaciens* include a tumour-inducing plasmid (Ti-plasmid). The bacterium inserts a part of it (T-region) into the plant genom, so that transfer, integration and expression of tumour DNA make the molecular basis of plant cell transformation. The Ti-plasmid is a useful natural vector for introducing foreign genes into plant cells.

The crown gall investigation in our laboratory took three directions.

By means of ultrastructural studies we found that chloroplasts clearly show dedifferentiation processes which occur during tumour transformation in differentiated leaf cells.

Bearing in mind the possibility of gene regulation by means of protein, we tested the biological activity of mistletoe protein extract, using potato crown gall tumours as a bioassay. By estimating the number and weight of tumours we found that crude mistletoe extract considerably inhibits tumour induction and growth. The refined protein fraction, however, was even more efficient. The mistletoe proteins also showed stimulative effects on rhizogenesis.

Metabolic differences between normal and tumorous tissue also have been reflected in the electrophoretic pattern of soluble proteins and isoperoxidases, as well as in a total peroxidase activity.

KONSTANTINOV, K., DENIĆ, M., ŠUKALOVIĆ, V., VIDAKOVIĆ, M., ZARIĆ, Lj. and TADIĆ, B.

### CYTOPLASMIC MALE STERILITY – INTERREACTION OF NUCLEAR AND MITOCHONDRIAL GENOME

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Male sterility is a distinctive trait very frequent in higher plants. Common for all sterile plants is the absence of fertile pollen grains. Results published by many authors indicate that this trait is under the genetic control of mitochondrial DNA. In some plant species there are data relating this trait to the chloroplast genome control or some virus infection.

Relatively low complexity of mitochondrial genome makes possible the identification of four different types of cytoplasm in maize at the level of DNA. These types are: normal, T, C and S cytoplasm which are distinctive according to unique DNA fragments. At the same time the heterogeneity inside the fertile genotype is not linked to the male sterility. The differences have been obtained at the same types of cytoplasm at the level of the mitochondrial proteins. These differences are very clear in the proteins both isolated from intact mitochondria or synthesized on the intact mitochondria as template in cell free protein synthesizing system. When proteins isolated from immature anther tissue have been compared, obtained differences were dependent both on the genetic background and type of the cytoplasm (T, C or S).

When the level of the restoration of male fertility was investigated the difference was obtained between different genetic background which has been converted to the sterile type. Also difference was obtained in the same type of sterility but in different inbred lines. Results obtained after hormone content determination, enzymes (included in nitrogen metabolism) activity and MDH polymorphism indicated that this trait could be used as the system for two genomes interdependence.

## PHOTOSYNTHESIS, METABOLISM

VELJOVIĆ, S., SAFTIĆ, D. and PLESNIČAR, M.

**THE EFFECT OF METHYL VIOLOGEN ON SECONDARY  
FLUORESCENCE KINETICS AND CHANGES IN ATP CONTENT  
DURING PHOTOSYNTHESIS IN ISOLATED CHLOROPLASTS**

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Methyl viologen in catalytic amounts ( $10^{-7} - 10^{-6}$  M) induces in isolated intact chloroplasts a very pronounced secondary peak in chlorophyll *a* fluorescence, which is associated with the onset of photosynthetic carbon assimilation (Plesničar & Cerović, in press). The appearance of a transitory increase on the fluorescence induction curve in the presence of methyl viologen (MV) was studied in relation to the corresponding changes in ATP content.

Intact chloroplasts of high intactness (90–94%) and photosynthetic capacity ( $100-130 \mu\text{mol O}_2 \text{ mg}^{-1} \text{ Chl h}^{-1}$ ) were isolated from young pea shoots (Cerović & Plesničar, 1984). Measurements of  $\text{O}_2$  evolution, chlorophyll *a* fluorescence and ATP content were made as indicated (Saftić et al., this issue).

Methyl viologen under aerobic conditions serves as an electron sink via oxygen as terminal acceptor, allowing oxidation of Q through linear electron flow and causing a  $\Delta$  pH to be established. Both  $q_Q$  and  $q_e$  quenching occur on illumination leading to the observed rapid decline in fluorescence yield. The secondary maximum of chlorophyll *a* fluorescence in MV treated chloroplasts depends on MV concentration and could be correlated to the length of the lag phase during photosynthetic induction.

The change in ATP content is most intensive during the first minute of illumination and, in the absence of exogenous ATP, it represents a mirror image of chlorophyll *a* fluorescence trace. The increase in ATP concentration corresponds to the decrease in chlorophyll *a* fluorescence. The decrease in ATP concentration in MV-treated chloroplasts corresponds to the relaxation of  $q_e$  due to the increased ATP consumption which made ADP available to discharge the proton gradient. Further increase in the rate of carbon assimilation causes the increase in  $q_Q$  and  $q_e$  and a decrease of fluorescence.

Experiments indicate that the increase in the capacity of the electron transport chain to provide ATP in relation to its capacity to provide NADPH could influence the generation of secondary fluorescence kinetics in chloroplasts.

SAFTIĆ, D., VELJOVIĆ, S. and PLESNIČAR, M.

### CHANGES IN CHLOROPHYLL *a* FLUORESCENCE AND ATP CONTENT DURING PHOTOSYNTHESIS IN ISOLATED CHLOROPLASTS

University of Belgrade, INEP, Department of Pesticides, Zemun

Upon illumination of isolated intact chloroplasts the yield of chlorophyll *a* fluorescence decreases following a slow secondary kinetics which is associated with the onset of photosynthetic carbon assimilation (Cerović et al., 1984). The decline in fluorescence yield is influenced by the rate of linear electron flow and redox state of primary acceptor Q ( $q_Q$  quenching) and by the change in established  $\Delta$  pH which is closely related to production and consumption of ATP ( $q_e$  quenching).

Intact chloroplasts of high intactness (90–95%) and photosynthetic capacity (100–130  $\mu\text{mol O}_2 \text{ mg}^{-1} \text{Chl h}^{-1}$ ) were isolated from pea shoots. Measurements of oxygen evolution and chlorophyll *a* fluorescence were made with a Hansatech  $\text{O}_2$  electrode that had been modified to allow simultaneous assay of chlorophyll *a* fluorescence. The concentration of ATP was determined by the luciferin–luciferase assay using small samples withdrawn at timed intervals from the oxygen–electrode vessel.

Exogenous ATP strongly stimulates photosynthetic carbon assimilation by isolated intact pea chloroplasts. The ATP requirement is higher with younger plants ( $K_m$  (11 d) = 0.1 mM) than older ones ( $K_m$  (17 d) = 0.05 mM). Changes in secondary fluorescence kinetics are related to changes of photosynthetic activity in the presence of exogenous ATP. The increase in the rate and extent of quenching from the primary fluorescence peak, as associated with the decrease in the lag period (in  $\text{O}_2$  evolution) when pH of the suspending medium was increased, suggests that a large  $q_e$  component exists.

The changes in ATP content show two transitory increases during the first minute of the induction phase of photosynthesis. The extent of ATP changes is higher when higher concentration of exogenous ATP is applied, indicating its participation in the chloroplast reactions. This participation seems to be higher with younger plants. The change in ATP content in the presence of 2 mM 3-phosphoglycerate as substrate is a mirror image of chlorophyll *a* fluorescence trace, indicating the effect of  $q_e$  component in the formation of transitory fluorescence increase. However, similar change in ATP content produced by the addition of 1 mM oxaloacetate to chloroplasts shows that besides the RPP cycle some other reactions might be taking part in ATP consumption in chloroplasts.

STANKOVIĆ, Ž.S.

### THE EFFECT OF RED AND BLUE LIGHT ON PHOTOSYNTHESIS OF ISOLATED PROTOPLASTS AND CHLOROPLASTS

Institute of Biology, Faculty of Natural Sciences, Novi Sad

The effect of red (674 nm) and blue light (450 nm) on the photosynthetic activity of isolated protoplasts and chloroplasts of wheat, barley, peas and spinach was

investigated. Photosynthetic activity was determined via O<sub>2</sub> evolution using an oxygen electrode.

The obtained results showed that the rate of O<sub>2</sub> evolution by isolated protoplasts increased with the increasing of light intensity up to 50 W · m<sup>-2</sup> in both red and blue lights. By further increasing of light intensity O<sub>2</sub> evolution was diminished and plateau was reached. Regarding to protoplasts the maximal rates of O<sub>2</sub> evolution by isolated chloroplasts were achieved at higher light intensities, i.e. above 100 W · m<sup>-2</sup>.

The quantum requirements (number of quanta per O<sub>2</sub> evolved) were different; for protoplasts from 8 to 10 in red and from 12 to 14 in blue light, while for isolated chloroplasts q/O<sub>2</sub> they were approximatively 11 in red and from 20 to 22 in blue light.

It can be concluded that the differences in O<sub>2</sub> evolution between red and blue light of the same intensity below 50 W · m<sup>-2</sup> are the result of different quantum requirements while in higher light intensities they disappear and could be attributed to the specific effect of light quality on photosynthesis process or processes occurring in photosynthetic cells and chloroplasts.

Specific differences between the plant species tested were not established, except in the maximal rates of O<sub>2</sub> evolution.

VUČINIĆ, Ž., STANKOVIĆ, Ž.\*

#### **GLUCOLATE SYNTHESIS IN ISOLATED SPINACH CHLOROPLASTS AND ITS DEPENDENCE ON THE RATE OF OXYGEN EVOLUTION**

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Bicarbonate dependent oxygen evolution was studied in an isolated spinach (*Spinacea oleracea* L.) chloroplast suspension, using oxygen polarography and 14C—labelling techniques. Our results show that the amount of glycolate produced is independent of the rate of oxygen evolution by photosynthesizing chloroplasts.

RADENOVIĆ, Č., JEREMIĆ, M.,\* MARKOVIĆ, D.,\*\* VUČINIĆ, Ž.

#### **CHANGE OF ACTIVATION ENERGY — A MEASURE OF RESISTANCE OF MAIZE PHOTOSYNTHETIC APPARATUS TO TEMPERATURE**

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It is commonly assumed that delayed light emission is represented with two curves: induction and thermal. The present investigation deals with the DLE induction curve and changes in the DLE rhythms, as affected by high and higher temperatures in the function of three different heating rates of the investigated objects (leaf segment of the selected maize genotypes) and time of establishment of the thermal curve.

Thermal DLE function, which is obtained experimentally, has parameters similar to the function:  $Y = e^{-kx^2}$  where  $k > 0$ . Their analysis and especially the analysis of Arrhenius temperature correlation enables more complex investigation of the resistance

of photosynthetic structure of different maize genotypes to temperature. The value of activation energy ( $E_a$ ) is especially considered. Activation energy has commonly been used for characterization of temperature correlation with water permeability. The cells, plant and animal, which are water impermeable have higher  $E_a$ , whereas cells which are water permeable have lower  $E_a$ . This means that higher  $E_a$  value reflects a more pronounced interaction between water and membrane and a high energetic barrier for its transport.  $E_a$  for water diffusion through the lipid membrane amounts to 44.1–52.1 kJ/mol (literature data).

The results of our investigation show that  $E_a$  amounts to: a) for inbred line ZPL 1304, which was rated as resistant to high temperatures and drought: 37.40–59.90 kJ/mol, b) for inbred line 14A, which was rated susceptible to high temperatures and drought: 65.50–116.40 kJ/mol. Other  $E_a$  results obtained in a different treatment are given. However, the answer to the question asked in the title of this paper is yet to be reached in the discussion.

MARTINČIČ, A., GABERŠČIK, A.

#### PHOTOSYNTHETIC ACTIVITY OF *PHYLLITIS SCOLOPENDRIUM* (L.) NEWM. IN CAVE CONDITIONS

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Institute of Biology, Ljubljana

*Phyllitis scolopendrium* grows in the cavatic environment on areas with very different light regimes: from nearly full daylight at the cave entrance, to 1% of full daylight growth limit zone inside the cave. Differences in light result in great changes in leaf structure. With decreasing light level the specific leaf area increased, the leaf thickness and the specific leaf weight decreased. There are also great differences in photosynthetic activity. We had measured net photosynthetic activity of plants from the natural habitat (1%, 2,5% and 6% of full daylight) and of plants growing in the greenhouse under full daylight. The differences between measured values of light compensation points are insignificant. All achieved values below  $5 \mu\text{E m}^{-2} \cdot \text{s}^{-1}$ . Differences become significant in values of the saturation point: 50 (1%) – 100 (2,5%) – 300 (6%) – 500  $\mu\text{E m}^{-2} \cdot \text{s}^{-1}$  (100% of daylight).

The intensity of net photosynthesis per unit of leaf area increases with the increasing light levels. The same relation is expressed per unit of chlorophyll although the differences are very small. But the values of photosynthetic activity per unit of dry weight are just opposite. Such statements are corresponding with differences in leaf structure in the light profil of the cave and they represent the compensation of light-limiting conditions.

The experiments with plants grown in the greenhouse under full daylight (100%) showed that differences in morphology, anatomy, chlorophyll content and photosynthesis are phenotypic.

However, it is surprising that there are small differences in the values of photorespiration, which at maximum quantum flux density amount to 15–20% of net photosynthetic rate.



MILIVOJEVIĆ, D. and MARKOVIĆ, D.

**DEVELOPMENT OF ULTRASTRUCTURE AND PHOTOCHEMICAL  
ACTIVITY OF BLACK PINE CHLOROPLASTS UNDER THE  
EFFECT OF LIGHT OF DIFFERENT SPECTRAL COMPOSITION**

Institute for the Application of Nuclear Energy in  
Agriculture, Veterinary Medicine and Forestry, Zemun

It is known that gymnosperm seedlings synthesize chlorophyll in complete darkness. Their etiochloroplasts have a developed PS I activity but do not contain the functionally organized oxidizing side of PS II. Light is necessary in order for PS II to be functional. Due to these reasons gymnosperms are good for studies of the formation of PS II activity regarding the spectral composition of light. In our work we also followed the development of chloroplasts ultrastructure and the PS II reducing ability on chloroplasts which had been isolated from black pine seedlings grown under four different spectral bands which correspond to the visible spectrum. The results were compared with seedlings which had developed simultaneously under white light and in the complete dark. The PS II activity which was measured through the photoreduction of DCIP was the lowest under the effect of blue and orange light, whose chloroplasts had a well developed grana system. In the case of red and purple light, apart from the grana, prolamellar corpuscles were also observed, and the PS II activity was similar to that of controls.

WRISCHER, M. and MODRUŠAN, Z.

**THE EFFECT OF LOW TEMPERATURES ON THE CHLOROPLASTS  
IN BLACKBERRY (*RUBUS FRUTICOSUS* L. s.l.) LEAVES**

„Ruđer Bošković” Institute, Zagreb

Blackberry bush (*Rubus fruticosus* L. s.l.) retains some of its leaves throughout the winter months. These leaves remain green and are photosynthetically active, in spite of freezing and thawing periods.

We have studied fine structure of frozen and thawed blackberry leaves by some fixation methods at temperatures below zero.

Mesophyll cells in frozen leaves did not show significant differences in their fine structure when compared to those from thawed leaves. In both leaves – besides large vacuoles – there were numerous small vesicles showing spongy appearance of the cytoplasm. Mitochondria had normal fine structures, and chloroplasts contained the abundant stroma and very large grana.

Respiration and photosynthetic activity followed the thawing and warming of the leaves. Both processes could be detected 15 minutes after the leaves had been brought to room temperature. Photosynthetic activity gradually increased and after several hours the maximum was reached\* The length of this adaptation period was the result of the temperature to which the leaf had been frozen.

\*A. Hloušek · Radojčić et al., poster presented at this meeting

HLOUŠEK–RADOJČIĆ, A., WRISCHER, M. and LJUBEŠIĆ, N.

SEASONAL CHANGES IN BLACKBERRY (*RUBUS FRUTICOSUS* L. s.l.) LEAVES

„Ruđer Bošković” Institute, Zagreb

The winter leaves of blackberry can endure very low temperatures and remain functionally active\*. In spring when the blackberry puts forth leaves the winter ones gradually fall off.

The fine structure of chloroplasts, their photosynthetic activity and the content of pigments and lipids were studied in spring, summer and winter leaves.

Chloroplasts from overwintering leaves had an abundant thylakoid system arranged into large grana, while those from spring and summer leaves contained much smaller grana. The contents of total chlorophylls and carotenoids were high in winter leaves, although their photosynthetic activity reached only about 50% of that in summer leaves. There were also evident differences in the content of some carotenoids and lipids between the old (overwintering) and young (spring and summer) leaves.

The observed seasonal differences are the consequence of ripening and senescing processes of chloroplasts and leaves. In overwintering leaves some phenomena seem to point out the adaptation to low temperatures.

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\*M. Wrischer, Z. Modrušan, poster presented at this meeting

HLOUŠEK–RADOJČIĆ, A., LJUBEŠIĆ, N.

PIGMENT AND LIPID COMPOSITION OF THE CHROMOPLASTS  
FROM THE DANDELION (*TARAXACUM OFFICINALE* WEB.) PETALS

„Ruđer Bošković” Institute, Zagreb

Chromoplasts from the developed dandelion petals contain characteristic globulo-reticulous structures. The low osmiophilic plastoglobuli are concentrated in the peripheral stroma beneath the inner envelope. They are irregular in shape and wrapped by a clearly visible membrane. The centre of the chromoplast is filled with reticulum.

Isolation procedure resulted in a small number of intact chromoplasts, because of numerous large plastoglobuli. The qualitative and quantitative analysis of the pigments extracted from the isolated chromoplasts showed that more than 90% of all pigments were two carotenes,  $\beta$ -carotene and  $\beta$ -carotene 5,6,5'6' diepoxide. The rest of the pigments (about 8%) was lutein. The lipid fraction of the isolated chromoplasts, which was performed by standard methods\*,\*\* , included galactolipids, sulpholipids, phospholipids, neutral lipids and four unidentified lipids.

By comparative investigations of the chromoplast ultrastructure and pigment and lipid composition we tried to find out the chemical composition of chromoplast structures.

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\*Williams, J.P., P.A. Meerilees, *Lipids* 5 (69) 367–370

\*\*Kramer, J.K.G., et al., *Lipids* 18 (83) 896–899

VUJIČIĆ, R. and BOJOVIĆ—CVETIĆ, D.

### DIFFERENTIATION OF VINE LEAF MESOPHYLL CELLS

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Institute for Biological Research, „S. Stanković”, Beograd

The anatomy of differentiated leaves of various plant species is already well known. Recent research, however, in the field of tissue culture indicates that leaf tissue of some species is able to give diverse morphogenetic responses to the external hormone stimulus, which implies that all mesophyll cells are not equally able to respond.

The aim of this work was to analyze ultrastructural features of the cells in very young vine leaves and to follow the changes in the course of differentiation of various leaf tissues.

The leaf structure of several vine varieties was studied by electron and light microscopy. In all analyzed varieties the very young leaf (3rd from the top) was made of three well defined cell layers between upper and lower epidermis. The palisade and spongy parenchyma cells are differentiated and their vacuoles contain large tannin inclusions. The third, medial layer, which is continuous between vascular bundles was composed of cells which are still dividing and had characteristics of meristematic cells; they have large nuclei, the cytoplasm rich in organelles, small chloroplasts without starch reserves, and vacuoles which did not contain tannin inclusions.

In the cross sections of an older leaf (8th leaf from the top) only two layers of cells can be found – the palisade and spongy layer while the medial layer has vanished.

The origin and the function of the medial layer which is so conspicuous only in young leaves, is still not clear and further analysis of macromolecular contents of all cell types is in progress.

MATIČIĆ A., DOBERŠEK—URBANC, S., ROZMAN, L.

### THE STUDY OF LEAF STOMATA IN CONNECTION TO HETEROSIS OF CORN (*ZEA MAYS* L.)

Biotechnical Faculty, Agricultural Department  
61001 Ljubljana, p.p. 486

In order to get closer to biologic phenomenon of heterosis and to find out indirect selection parameters for the detection of combining ability of inbreed lines, leaf stomata for different corn genotypes have been studied.

Eight inbreed lines having various combining abilities were studied. From them and seven of their hybrids with different fertility, 420 samples for microscopic measurement were taken using „glu-tape method”. Samples were taken from the middle part of the lower middle leaf surface in two successive growing periods. Fisher's analysis of variance for the length and the number of leaf stomata per 0.32 sq mm was performed. Since genotypes showed statistical significance, the groups according to the Tuckey's test method could be disclosed. The number and the leaf stomata size of inbreed lines, was significantly different. Lines with good combining ability had dense and smaller leaf stomata; those with bad combining ability had rare and longer leaf stomata. In comparison to the average of parent lines their fertile hybrids had smaller number of longer leaf stomata; hybrids having a small heterotic effect on yield had above average

number of leaf stomata which were the same size as the mean size of parent lines. These results show, that especially the number of leaf stomata could represent one of the important criterion for a quicker evaluation of the combining ability of inbred lines. Further studies are in progress.

DOBERŠEK—URBANC, S., MATIČIČ, A., ROZMAN, L.

**SAMPLING VARIATION STUDIES ON COUNTS OF LEAF STOMATA  
ON DIFFERENT LOCATIONS OF CORN LEAVES (*ZEA MAYS* L.)**

Biotechnical Faculty, Department of Agriculture, 61001 Ljubljana, p.p.486

The early studies of the counts and length of leaf stomata indicated a large variability of those qualities. Numerous published data also confirm considerable variation of the number and length of leaf stomata from leaf to leaf and from one sampling point to other. It was difficult to determine from published data at which sampling point the counts of stomata are most stable. Therefore we decided to investigate the effect of different sampling positions on the number of leaf stomata. We searched for a position on the corn leaf at which the differences of stomata properties attributed to genetic cause could be most efficiently detected.

Samples were taken at the top, in the middle and at the base of each chosen leaf. The upper and the downside epiderm was sampled. One leaf was chosen at the top of the plant, one at the middle and one at the bottom. Samples for a microscopic examination (1620 in total) were taken by the microrelief method of Pazourek (1963).

The coefficient of variation for the number of stomata per area was lower for all sampling points at the downside epiderm compared to corresponding sampling points at the upper epiderm. Samples taken at the middle of a leaf yielded the lowest coefficients of variation. From the statistical point of view the sampling at the top of a leaf could be satisfactory, but was discarded because of common reason. The top of the leaf is often damaged and sometimes stomata are covered by trichomes which obstruct counting.

Upon statistical analysis and upon other relevant observations we concluded that for investigations on leaf stomata in maize the most suitable sampling position is the middle part of the lower epidermis of the middle leaf. At this position we also got the best microscopic photographs.

OBRENOVIĆ, S.

**EFFECT OF RED LIGHT ON OXYGEN UPTAKE IN ETIOLATED  
*AMARANTHUS CAUDATUS* L. SEEDLINGS**

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Oxygen uptake was monitored in etiolated, three-day-old *Amaranthus caudatus* L. seedlings (using Warburg apparatus) in the dark, under 4 h red illumination and during the post-illumination period of 1 h. It was found that in darkness about 17% oxygen uptake is sensitive to 5 mM salicylhydroxamic acid (SHAM), whereas the rest was inhibited with 5 mM KCN. The incomplete inhibition by 1 mM KCN is partly reverted by 4  $\mu$ M lumiflavin which alone does not significantly stimulate the respiration in darkness. During illumination the participation of the SHAM-sensitive respiration increases to about 35%, while in the presence of both 5 mM KCN and 5 mM SHAM some net oxygen production is observed at the onset of illumination. The sensitivity to SHAM is potentiated with 4  $\mu$ M lumiflavin, especially between 3 and 4 h illumination. The

post-illumination outburst of oxygen uptake is regularly detectable. It is sensitive to respiratory inhibitors and stimulated by lumiflavin. During this period a component of respiration, which is resistant to inhibitors, can be observed.

Increasing the number of plants per sample causes the suppression of oxygen uptake, especially during the post-illumination period, which resembles the inhibition obtained with SHAM.

These results demonstrate that during red illumination of the etiolated *Amaranthus* seedlings photosynthesis does take place. They also suggest a relationship between photorespiration and alternative respiration. Lumiflavin seems to be useful for further studies of these phenomena because it seems to stimulate photosynthesis and to cause the switch between different respiratory pathways.

PETROVIĆ, M., SARIĆ, M.\*

### THE CONTENT OF SOME PHOSPHORUS COMPOUNDS IN LEAF OF DIFFERENT COLOUR IN PLANTS OF THE *VARIEGATA* TYPE

Faculty of Agriculture, \*Faculty of Natural Sciences, Novi Sad

In an earlier study, differences were found in N, P, K, Ca, and Mg contents in green and whitish-yellow leaves of *Hedera helix* and *Acer negundo* (Sarić, 1979). Continuing to work on physiological and biochemical processes in leaves of variegata type plants, we undertook this study to find contents of certain groups of phosphorus compounds in these plants.

Phosphorus compounds were extracted and fractionated from dry leaves by the methods presented in the paper of Michalik (1971) and Petrović (1977).

The obtained results show that in both plant species (*Hedera helix* and *Acer negundo*) there existed differences in the content of phosphorus compounds which depended on leaf colour. The contents of inorganic, acid-soluble organic, and lipid phosphorus were higher in whitish-yellow than in green leaves. The difference in the contents of inorganic phosphorus was exceedingly large. The smallest differences were found in the contents of nucleic and protein phosphorus, in favour of green leaves.

The contents of all phosphorus fractions, irrespectively of leaf colour, were higher in *Acer negundo* than in *Hedera helix*. Comparing separately green and whitish-yellow leaves of the two plant species, smaller differences in the phosphorus fractions were found in green than in whitish-yellow leaves.

VÖRÖSBARANYI, I. and AĆIMOVIĆ M.

### RATIO OF FATTY ACIDS IN OIL OF HEALTHY SUNFLOWERS AND THOSE INFECTED BY *PHOMOPSIS*

Faculty of Agriculture, Institute of Field and Vegetable Crops, Novi Sad

Sunflower plant infected by *Phomopsis* in the course of the growing season suffer drastic yield reductions. There have been no reports on possible changes in oil quality caused by the disease. To follow as precisely as possible the changes in quality, the seeds

1. Sarić, R.M., *Physiol. Plant.*, 45: 301-304 (1979).
2. Michalik, I., *Acta Phytotechnica*, 22: 27-35 (1971).
3. Petrović, M., *Proc. Nat. Sci., Matica srpska*, 53: 71-116 (1977).

from each analysed head were separated in four pneumatic fractions: 1. healthy seeds, 2. incompletely filled seeds, 3. incompletely formed seeds, and 4. empty seeds.

Oil extraction from the first three fractions was conducted without applying heat to the seeds. Oil was esterified and fatty acids were determined by gas-liquid chromatography.

The content of linoleic acid was higher in seed fractions closer to the center of the head, with healthy and infected plants alike. In 1982, the content of linoleic acid was significantly higher with the infected plants of both tested hybrids, in all seed fractions. In 1983, however, the infected plants had lower contents of linoleic acid than the healthy ones. Since there exists a high negative correlation between the contents of oleic and linoleic, the results for oleic acid show the reverse tendencies, in both years and both hybrids.

The content of palmitic acid was significantly increased in the infected plants, with both hybrids and in both years.

ČUPINA, T., VÖRÖSBARANYI, I.

### SYNTHESIS OF FATTY ACIDS IN THE COURSE OF SEED FORMING AND FILLING IN SOME SUNFLOWER RESTORERS

Faculty of Agriculture, Institute of Field and Vegetable Crops, Novi Sad

1. Six sunflower restors were analysed for the ratio of essential fatty acids (palmitic, stearic, oleic, linoleic, and behenic) in the course of seed forming and filling.

2. The contents of these fatty acids in oil depended on the restorer, stage of seed development, and fatty acid. The analysed fatty acids may be arranged in the following order according to their contents: linoleic, oleic, palmitic, stearic, behenic, etc.

3. Linoleic and oleic acids were functionally dependent in the course of seed forming and filling in the analysed restorers. The portion of oleic acid was high at the beginning of seed filling but it kept decreasing to its final value at the stage of physiological maturity of seed. The portion of linoleic acid was low at the beginning but it went up and levelled at the stage of physiological maturity. These patterns indicate that oleic acid serves as a precursor to the synthesis of linoleic acid.

4. The transformation of oleic into linoleic acid was conditioned by corresponding polyenzymic systems. The biosynthesis of saturated and unsaturated fatty acids depended on temperature, time of day and night, water schedule, respiration intensity, etc.

5. Biosynthesis of unsaturated and saturated fatty acids in seed is inherited; in sunflowers, the role of the locus controlling the transformation of oleic into linoleic acid may be substituted by different alleles.

KOVAČ, M.

### PROTEINASES IN SILVER FIR SEEDS (*ABIES ALBA* MILL.)

Institute of Biology, E. Kardelj University of Ljubljana

The reserve proteins are important for germination of silver fir seeds although the lipids are the main reserve substance. We followed the proteinase activities before and

during visible germination. Proteolytic activities were measured: (a) in well germinating seeds from the Mašun region, fructification in the year 1982, (b) in seeds which did not germinate at room temperature, fructification in the year 1980.

In the silver fir seeds we found only acid proteinases with a sharp optimum at pH 3 using haemoglobin as substrate. It was difficult to get the pH optimum for hydrolysis of casein because of substrate denaturation at pH 3 to 5.

Proteolytic activities were assayed during germination at pH 3 on haemoglobin and at pH 5.6 on casein. In germinating seeds the highest activities were present in the main protein storage tissue, the endosperm, and relatively high activities were found in the resting embryo, which also contains protein reserves, while there was no activities in the growing seedling tissue. In nongerminated seeds, fructification in the year 1980, proteinase activities decreased throughout the experiment, in both the endosperm and in the embryo.

The results suggest that reserve proteins in the embryo are critical for the induction of germination and that the reserve proteins in the endosperm aid the seedling establishment after radicle protrusion. Proteolytic activities in the resting seeds do not indicate germination ability of silver fir seeds.

The effects of a few selected inhibitors on the endosperm and embryo proteinases were tested. Paracloromercuribenzoate (PCMB) and leupeptin (inhibitors of sulphhydryl proteinases) had no effect on the hydrolysis of haemoglobin at pH 3. The endosperm contains at least three acid proteinases: one sensitive to pepstatin, another inhibited by PMSF – phenylmethylsulfonyl fluoride, and the third not affected by either inhibitor. Pepstatin, but not PMSF, inhibited the haemoglobin hydrolysing activity of embryo extracts.

KRIVOKAPIC, K., GRBELJA, J., DANON, Z., ERIC, Ž.

#### THE INFLUENCE OF THE BELLADONNA MOTTLE VIRUS ON SOME PHYSIOLOGICAL CHANGES IN *ATROPA BELLADONNA* L.

We identified the virus in plant material using the reaction of hosts range, serological double diffusion tests in agar gel and electron microscopy.

We investigated the changes in content of photosynthetic pigments (chlorophylls and carotenoids), total soluble proteins and tropan alkaloids in the leaves and in the fruits of belladonna plants infected by belladonna mottle virus (BdMV). We found a lower content of chlorophylls and carotenoids, total proteins and tropan alkaloids in the leaves of the infected plants. However, the fruits of the infected plants were found to have a higher content of total proteins and much lower content of alkaloids.

TADIĆ, B.

#### ISOENZYME POLYMORPHISM OF MALATE DEHYDROGENASE IN SOME LOCAL MAIZE POPULATIONS

Maize Research Institute „Zemun Polje”, Beograd

Isoenzyme polymorphism of malate dehydrogenase was investigated in ten local maize populations. Two groups of populations were studied: eight-row soft dents and

long-ear flints. Analyses were conducted on the coleoptilar sections of 5 day old seedlings according to *Cardy et al.* (1980). Eleven different alleles were found in five gene loci (*Mdh1*, *Mdh2*, *Mdh3*, *Mdh4* and *Mdh5*). Three alleles were detected on the first locus (*Mdh1-1*, *Mdh1-6* and *Mdh1-10.5*) as well as on the second (*Mdh2-3*, *Mdh2-3.5* and *Mdh2-6*). On the third locus we found two alleles (*Mdh3-16* and *Mdh3-18*) and on the fourth only one allele (*Mdh4-12*). Two alleles were identified on the fifth locus (*Mdh5-12* and *Mdh5-15*). Isoenzyme banding patterns of ten investigated populations had nineteen phenotype variants in which band number varies from four to eleven. Populations from the dent group had more polymorphic loci and more alleles than populations from the flint group.

ZLOKOLICA, M., GERIĆ, C. and GERIĆ I.

### DIFFERENTIAL EXPRESSION OF CATALASE AND ALCOHOL-DEHYDROGENASE GENES IN THE COURSE OF ONTOGENETIC DEVELOPMENT OF CORN

Faculty of Agriculture, Institute of Field and Vegetable Crops, Novi Sad

The expression of genes controlling catalase (CAT) and alcoholdehydrogenase (ADH) in corn depends on the stage of plant development. There exists a certain dynamics in the expressing of alleles of individual loci which control the corresponding enzymic systems.

We analysed some corn inbred lines for CAT and ADH in the scutelum several times during seed germination. The enzymes were separated by horizontal electrophoresis on starch.

The obtained isozymic profiles showed that the analysed inbreds differed regarding the dynamics of occurrence and disappearance of some alleles of CAT and ADH. On the first day, the activity of catalase isozymes, controlled by the alleles from both loci, was balanced in some inbred lines. Later on, the activity of CAT 1 isozyme dropped rapidly while the activity of CAT 2 isozyme increased proportionally. That finding is in disagreement with literature data on the dynamics of the alleles of the two loci for catalase in corn.

The analysed inbreds had three ADH isozymes in the scutelum of seeds germinating for 24 hours. In the course of germination, less active isozymes (ADH 3 and ADH 4) tended to disappear from the ADH profile. ADH 2 isozyme was most persistent but its persistence varied from line to line. In some lines, ADH 2 was present in the scutelum of germinating seeds for six days. One line had all three isozymes to the fourth day of germination, conversely, three isozymes were hardly active in some lines already on the third day. No ADH isozymes were found in a single line at the end of the observed period of germination.

According to some researchers, the absence or reduced synthesis of ADH subunits occurring concurrently with plant aging is not the consequence of the terminated synthesis of ADH but rather the consequence of the intensified synthesis of the endogenous inhibitor for that enzyme. A slow or fast disappearance of ADH isozymes, i.e., the synthesis of the inhibitor may be defined as genotypic character of certain corn inbred lines.



HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, V.

**ACTIVITY OF NITROGEN METABOLISM ENZYMES DURING  
KERNEL DEVELOPMENT IN MAIZE**

Maize Research Institute, Zemun Polje, Yugoslavia

We attempted to make a contribution to the knowledge of nitrogen metabolism processes in the kernel by studying the activity of nitrogen metabolism enzymes in the embryo and endosperm of the developing maize kernel.

Therefore, we investigated the activity of the following enzymes: glutamine synthetase (GS), glutamate dehydrogenase (GDH), alanine and aspartate aminotransferase (GOT and GPT), as well as soluble protein content in the embryo and endosperm of inbred lines Oh 43 and Oh 43 o<sub>2</sub> over the period of 15 to 45 days after pollination.

Results of these studies show that the kernel protein synthesis capacity, which after pollination is mainly located in the endosperm, decreases and about 40 days after pollination protein synthesis is taken over by the embryo.

Comparative data on the activity of the investigated enzymes in the embryo and endosperm show that the glutamine and glutamate synthesis in the embryo tissue is more intensive, whereas transamination processes at the initial stages of embryo development are less intensive than their counterparts in the endosperm. The roles of the embryo and endosperm subsequently interchange.

Biosynthetic processes of soluble precursors for protein synthesis in the embryo and endosperm of the developing kernel are mutually coordinated. Changes due to the presence of the o<sub>2</sub> gene may be considered to be an indicator of higher or lower correlation between the investigated embryo and endosperm processes.

## MINERAL NUTRITION, POLLUTANTS

POHLEVEN, F.\* , TOMŠIČ, D.\* , GOGALA, N.\* , ŠENTJURC, M.\*\* , SCHARA, M.\*\*

### THE ROLE OF Z AND ZR IN TRANSPORT OF IONS AND MEMBRANE FLUIDITY IN CULTIVATED MYCELIUM OF MYCORRHIZAL FUNGUS *SUILLUS VARIEGATUS*

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Natural cytokinins from root exudate of a pine *Pinus sylvestris* L. influence the absorption of ions by mycelium. During the growth the ion content of the culture is changing.

The transport of ions (K, P, Ca nad Na) was investigated in threefolded petri dishes. Into one compartment a normal MMN medium was poured in and inoculated with the mycelium culture of *Suillus variegatus*. In other two compartments was a minerals depleted medium. To one compartment of the latter zeatine (Z) or zeatine-riboside (ZR) was added in various concentrations ( $10^{-5}$  –  $10^{-5}$  mol/l). From the 6th to 27th day of growth concentration of single ions in mycelium and in nutrient medium from each compartment was measured.

During growth in the medium and in the mycelium the concentrations of ions are changing quickly. Absorption, transport and releasing of ions in mycelium is changing periodically under the influence of Z. Main role in those processes has concentration of cytokinins. For the transport of K, P and Na the optimal concentration of Z is  $10^{-6}$  mol/l, for the transport of Ca higher concentrations are more efficient. Z and ZR influence in certain (physiological) concentrations the transport of ions along the hyphae of cultivated fungus.

The observed differences in ion transport, mediated by the mycelium between the compartments, caused by the presence of Z can be explained by the hormone induced fluidity changes of the hyphal membrane with respect to the control.

The EPR data on molecular mobility changes of spin probes in the model lipid membranes show that there is a nonspecific increase of membrane fluidity of the lipid bilayers in presence of the hormone, but these changes are much larger in the membranes of the hypha, due to effects triggered by the low concentration specific binding of Z.

STOJANOVIĆ, D., DJURDJEVIĆ, M., VUČKOVIĆ, M.

**POTASSIUM, MANGANESE AND ZINC CONTENTS IN WHOLE  
KERNEL AND ITS COMPONENTS DURING GROWTH  
OF HYBRID MAIZE SK-67-O<sub>2</sub>**

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Veterinary Medicine and Forestry (INEP), Zemun

Curves of characteristic shape were obtained by following changes in potassium, manganese and zinc contents in whole kernels of hybrid maize SK-67-O<sub>2</sub> and its components – the pericarp, endosperm and germ – during cultivation in field conditions.

Potassium and zinc contents in the kernel decrease during the milk phase, while they remain constant during physiological maturity. Manganese content in the kernel of this hybrid mostly remains constant during development.

The application of compound fertilizers (NPK-15 : 15 : 15) into the soil which is moderately supplied with potassium, and rich in zinc and manganese, has no significant effect on changes in the contents of these metals in the kernel.

Regarding changes in the distribution of metals in certain components of the kernel, the following has been established: potassium content in the pericarp of hybrid SK-67-O<sub>2</sub> decreases during development from the milk phase to physiological maturity, while manganese and zinc contents first increase during the milk phase and then suddenly drop at the time of physiological maturity.

Potassium and zinc contents in the endosperm decrease during the milk phase and remain constant at the time of physiological maturity. Manganese content in the endosperm generally remains constant during development.

Changes in the contents of the studied metals in the germ have a characteristic course. Potassium content first increases and then decreases, while manganese and zinc contents decrease during the milk phase and increase during physiological maturity.

On the basis of the obtained curves depicting the dependence of certain metals in the function of time, it seems that the milk phase of kernel development can be differentiated from the phase of physiological maturity.

KRSTIĆ, B., GEBAUER, G.\* , and SARIĆ, M.

**SPECIFIC RESPONSE OF SUGAR BEET CULTIVARS TO DIFFERENT  
NITROGEN FORMS**

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Six cultivars of sugar beet (Maribo monova CR and Carpo – high sugar content is characteristic for them; Monopur and KW pura – normal sugar content and yield; and Al mono cultivar and Monofort – high yield and low sugar content) were grown using water culture method on nutrient solution Reid-York for 30 days. Nitrogen in the forms of

$\text{NH}_4\text{NO}_3$ ,  $\text{NH}_4\text{Cl}$  and  $\text{Ca}(\text{NO}_3)_2$  was added, and also three salt concentrations were used: 1/2 of the whole nutrient solution (105 mg N), the whole nutrient solution (210 mg N) and twice increased nitrogen concentration (420 mg N).

The obtained results showed that the dry weight, as well as the leaf area depended on the cultivar, nitrogen form and its concentration in the nutrient solution. The smallest matter of shoot and the smallest leaf area were obtained when ammonium as the nitrogen source was used.

The concentration of the total nitrate and ammonium nitrogen in the shoot depended on the cultivar and the concentration of the same in the nutrient solution.

The concentration of oxalic acid varied depending on experimental parameters, and the highest concentration was obtained when the plants were grown on nitrates as the nitrogen source. No synthesis of oxalic acid was noticed in the plants grown on ammonium form of nitrogen.

VASILEVSKI, G.

### THE EFFECT OF NITROGEN FERTILIZATION AND AGROCLIMATE CONDITIONS ON THE YIELD OF BARLEY STRAW

Institute of Agriculture and Horticulture, Skopje

From the past up to the present time barley has been one of the most important crop plants. However, since the straw, as a byproduct, can be utilized in livestock feeding, it is also considered to be a forage plant. Barley straw possesses a higher nutritive value than wheat and rye straw, but a lower value than oat straw. Its nutritive value can be improved if elaborated by alcohol, NaOH or  $\text{NH}_3$ . In general, since the ancient Egypt, the straw has been considered to be a main reserve of animal feed.

A shortage of qualitative animal feed during last years in SR Macedonia, has stimulated a higher straw consumption. As the straw represents a cheaper animal feed, it also improved the accumulation of producing farms.

Besides the yield of the grain, the yield of the straw can also be influenced by genetic properties of the varieties, as well as by agrotechniques and climate of the regions. Therefore, the investigations on the effect of some factors on the increase or decrease of straw yield, particularly fertilization, may help in directing the agrotechnique measures to favorize development of the desired plant parts.

We carried out two years investigations on barley cv. Union, in three different regions of SR Macedonia: Ovče Polje, Župa and Debarca, using six variants of nitrogen nutrition.

The obtained results show that the straw yield is more affected by the climate conditions than by nitrogen nutrition. The grain/straw ratio was as follows: 1 : 2,95 in Debarca, 1 : 1,81 in Ovče Polje and 1 : 1,47 in Župa. The highest yield of straw was recorded in Debarca (10865 kg/h) and the lowest in Župa (7087 kg/h). Variation of straw yield as affected by fertilization was as follows: 8083 kg/h (variant  $\text{N}_{30}$ ) to 9965 kg/h (variant  $\text{N}_0$ ).

PETROVIĆ, N., MILOŠEVIĆ, R. and UBAVIĆ, M.

### EFFECT OF DIFFERENT NITROGEN DOSES ON THE ACTIVITY OF NITRATE REDUCTASE IN SOME SUGARBEET VARIETIES

Faculty of Agriculture, Novi Sad

Nitrate reductase is an adaptive enzyme which is synthesized only if nitrates are present in the cytoplasm. Since the synthesis of nitrate reductase is activated by the presence of nitrates, it is assumed that the activity is a reliable indicator of the level of plant provision with nitrogen.

Having in mind the above assumption, as well as the importance of nitrate reductase for nitrogen reduction, we decided to investigate the effect of different doses of nitrogen on the activity of nitrate reductase in some sugarbeet varieties.

We investigated the effect of four nitrogen doses on nitrate reductase activity in normal and high-yielding types of sugarbeet. The following conclusions were drawn.

Increases in nitrogen doses brought corresponding increases in the activity of nitrate reductase. The rate of increase differed from one variety to another.

With the high-yielding varieties there was a positive correlation between nitrogen dose and nitrate reductase activity. With the normal varieties, the highest nitrogen dose tended to depress the activity of nitrate reductase.

RICHTER, R.\* , PETROVIĆ, N.\*\* and KASTORI, R.\*\*

### EFFECT OF HEAVY METALS ON NITRATE REDUCTASE ACTIVITY

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Heavy metals are regularly found in soil and plants. Some of them are indispensable for vital activities of higher plants. At high concentrations, however, they may become toxic on account of their pronounced affinity for acids, thiol groups of proteins, and nucleotides. They may cause considerable changes in the activity of a number of enzymes if they accumulate in plants.

Considering the importance of nitrate reductase in nitrogen assimilation and the increasing presence of heavy metals in the biosphere, we decided to study the effect of different concentrations of several potentially toxic heavy metals, Pb, Cd, Hg, and essential Fe, on the activity of nitrate reductase.

We studied disks of sugarbeet leaves treated with different concentrations of the heavy metals mentioned before. The effect of Fe excess and deficiency on nitrate reductase activity was examined in sugarbeet plants grown in a water culture, withholding or adding different Fe concentrations to the nutritive solution.

The following conclusions were drawn:

Increased concentrations of the heavy metals depressed the activity of nitrate reductase, but not uniformly. The highest depression was caused by Hg, then by Cd and Fe.

Fe-deficiency depressed the activity of nitrate reductase in sugarbeet leaves but stimulated the activity in roots. In the case of Fe-excess, the activity of nitrate reductase was depressed in both plant parts.

TRPESKI, V.

### CHLOROSIS IN VINEYARDS IN THE TIKVEŠ REGION AND PREVENTIVE MEASURES

Faculty of Agriculture, Skopje

Chlorosis (jaundice), or reduction of chlorophyll in the leaves of vines and other plants, is a serious problem which has a negative effect both on the growth and development of the plants, as well as on the quality of the fruit and the economics of produce. This problem is of great concern to scientists, experts and economists in Yugoslavia and abroad. So far no universal or economic means of definitively preventing chlorosis as a physiological disease has been discovered to our knowledge. The basic reason for the complexity of this problem is that the causes of this phenomenon are numerous and varied and there are probably some which are not known to science or praxis. Several types of chlorosis have been established (iron, manganese, magnesium, boron, etc.).

Our research into the problem consisted of experiments set up in the region of Kavadarci on the vineyards of the Tikveš Farming Collective, which cover an area of over 2,000 hectares. For four years we kept track of the following elements: the emergence of chlorosis and its degree of intensity; the dynamics of chlorosity before and after treatment of the vines; the chemical composition of the leaves ( $N_1 P_2 O_5$ ,  $K_2 O$ , Fe, Mn, Cu and Zn), whether chlorotic or healthy; agrochemical and chemical features of the soil; etc.

We employed the following treatments for chlorosis: via the leaves (vucsala antichlorosa, iron helate and gresite) and via the soil (powdered recsene, recsene pelate and sequestrene).

The results of the research showed that there is an insufficient balance among the elements in the vine leaves. This is particularly true of the relation between iron and manganese. In the majority of analyses manganese was present to a greater degree than iron. The measures applied against chlorosis had differing effects.

BLAGOJEVIĆ, S., PETROVIĆ, M. and ŽARKOVIĆ, B.

### THE INFLUENCE OF FERTILIZATION AND SOIL SUPPLY ON THE UPTAKE OF PHOSPHORUS BY OAT PLANTS

Faculty of Agricultural Sciences, Zemun

The purpose of this investigation was to determine the influence of the available phosphorus content and of the fertilization with increasing amounts of nitrogen, phosphorus and potassium on the uptake of phosphorus by oat plants.

The investigation was carried out on four pseudogley soils differing in the content of available macro- and micronutrients. The content of available phosphorus, determined by the AL-method of Egner and Riehm, was in the range of 5.2 to 61.0 mg  $P_2 O_5$  per 100 g of soil. The following doses of fertilizers were applied in this experiment: 120, 180 and 240 kg N/ha; 80, 120 and 160 kg  $P_2 O_5(K_2 O)$ /ha. The plants were harvested 2.5 months after sowing, and the content of phosphorus in the plant material was determined

colorimetrically. The obtained results were then subjected to statistical analysis by means of „t-test”.

The following important conclusions could be drawn:

1. The uptake of phosphorus by oat plants depends on the content of available phosphorus in the soil.
2. Fertilization with nitrogen, phosphorus and potassium causes a significant increase in the uptake of phosphorus by oat plants in comparison with the unfertilized variant of the experiment.
3. Higher doses of phosphorus did not exert statistically significant influence on the uptake of this element by oat plants.
4. The situation is very similar with nitrogen and potassium fertilizers although in some cases a higher dose of N or K causes a significant decrease or increase in the uptake of phosphorus.

ČMELIK, Z. and MIČIĆ, N.

### COPPER CONCENTRATION IN TISSUES OF DIFFERENT VARIETIES OF DRUPES

Faculty of Agriculture, Sarajevo

Copper was determined in the whole mature fruit and in its parts pericarp, endocarp and seed in the following species: *Prunus domestica*, *Prunus instititia*, *Juglans regia*, *Crataegus monogyna*, *Cornus mas* and *Olea europaea*. In *Pirus communis* it was determined in pericarp and sclereides.

From the obtained results it can be concluded:

- the lower concentration of Cu, in the examined species, except for pear, was recorded in pericarp, slightly higher in seed and significantly higher in endocarp.
- copper distribution in the fruit of pear is different compared to the other examined species. Its concentration is lower in sclereides, anatomical analogues of endocarp, than in fruit flesh.
- copper concentrations of the whole fruits differ in different species which is partly caused by relative abundance of the particular tissue and partly by different Cu concentration in botanically same tissue of the fruit.

ČERNE, M.

### THE QUALITY OF RED BEET AND CARROTS AS A FUNCTION OF POTASSIUM NUTRITION

Kmetijski inštitut Slovenije, Ljubljana

In the four year average no essential difference was observed between the red beet yields fertilized with 100 to 400 kg/ha  $K_2O$ . In the year when the crop was damaged by hail the yield was lower by one half while the dry matter increased from 5,3 (control) to 7,7% (fertilization with 400 kg/ha  $K_2O$ ). The colour intensity measured with the Perkin Elmer photometer 124 at  $\lambda$  535 nm wave length and pH 3 amounted to 1,170 when fertilized with 260 kg/ha  $K_2O$  and to 1,279 when fertilized with 320 kg/ha  $K_2O$ .

In the tubers with 3 to 5 cm diameter considerably more coloured matter was found than in thicker tubers. After storage the quantity of coloured matter decreased by 35%.

In carrots the fertilization with different quantities of potassium on humus soil did not change essentially either the yield or the dry matter (9,0 to 9,5%), the total acids (0,12 to 0,18%) and the crude cellulose (1,1 to 1,3%), but it caused increase of total carotenoids and of  $\beta$ -carotene. When fertilized with 200 kg/ha  $K_2O$  the quantity of total carotenes was by 10% and that of  $\beta$ -carotene by 12% higher, and when fertilized with 340 kg/ha  $K_2O$  the quantity of total carotenes was by 15% and that of  $\beta$ -carotene by 16% higher than when the carrots were not fertilized with potassium. If carrots with 2 to 4 cm diameter were fertilized with 200 kg/ha  $K_2O$  the quantity of total carotenes increased by 30% and that of  $\beta$ -carotenes by 29% if compared with the control. When fertilized with 340 kg/ha  $K_2O$  in the carrots with more than 4 cm diameter the quantity of total carotenes increased by 36% and that of  $\beta$ -carotene by 31% if compared with nonfertilized carrots. On heavy soil the higher quantity of potassium (from 100 to 450 kg/ha) caused increase of dry matter from 6,5 to 7,2, of total carotenes from 4,1 to 5,8 mg/100 g and of  $\beta$ -carotene from 3,8 to 5,7 mg/100 g.

KOMLENOVIĆ, N., RISTOVSKI, P.

### THE EFFECT OF PHOSPHORUS ON THE GROWTH AND NUTRITION OF SOME CONIFERS

Forestry Research Institute, Jastrebarsko

The influence of phosphorus on the growth and chemical composition of plant matter of three conifer species (*Picea abies* Karst., *Pinus sylvestris* L. and *Pinus nigra* Arn.) was investigated.

We found a positive effect of the applied phosphorus on the growth of forest tree species under study. P-treatment resulted in an increased phosphorus content in needles, but a lower content of nitrogen, calcium, zinc, and in pines also of iron, was observed.

The applied phosphorus had the greatest influence on the Norway spruce growth and the poorest influence on the Black pine growth.

SARIĆ, M.R.\* , SARIĆ, Z. and GOVEDARICA, M.\*\*

### SPECIFIC RELATIONS BETWEEN SOME STRAINS OF DIAZOTROPHS AND CORN HYBRIDS

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In our studies carried out so far, particular attention was paid to relations between *Azotobacter* strains and corn and wheat genotypes. However, the studies were later extended to include not only *Azotobacter* but also other diazotrophs. The main objective of these studies was to investigate a number of strains of different genera on different genotypes of cultivated plants, in order to find the best combinations of strains and genotypes with the greatest efficiency of atmospheric nitrogen fixation.



The studies included 21 *Azotobacter* strains, 18 *Klebsiella* strains, 12 *Escherichia* strains, 7 *Dexia* strains, 4 *Azospirillum* strains, and 4 *Bajerinckia* strains isolated from certain zones of the corn rhizosphere. Inoculation of seed of four corn hybrids, (NSSC-606, NSSC-78, NSSC-530 and NSSC-425) was performed simultaneously with all strains of diazotrophs and hybrids at planting time. The trials were carried out in the greenhouse using sand culture, and the plants were grown for 30 days without nutrient solution, i.e. only distilled water was added. We analyzed several parameters in the course of the studies, but this paper deals only with the nitrogen concentration in the roots and above-ground parts of the experimental plants.

The results obtained showed that the concentration of nitrogen in roots and above-ground parts varied in dependence of diazotrophic genera and strains, as well as of corn hybrids. The best efficiency was displayed by genera *Klebsiella* and *Azotobacter*, while the hybrids ranked in the following order regarding their reaction: NSSC-78, NSSC-425, NSSC-606 and NSSC-530. All genera included both highly efficient and inefficient strains. However, as was the case with previous studies with *Azotobacter*, a high specificity of azotrophic strains and corn hybrids was found.

DENIĆ, M., KONSTANTIVNOV, K., HALDA, L., KEREČKI, B., STOJNIĆ, O., KAPOR, S.,\* and SAJDL, V.\*

#### STUDY OF ASSOCIATIVE NITROGEN FIXATION BETWEEN BACTERIA AND MAIZE ROOTS

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\*INEP, Belgrade-Zemun

Increase in biological fixation of atmospheric nitrogen at the level providing acceptable yields should lead to the reduction of N-fertilizers broadcasting or improvement of yields in regions without N-application. The results obtained show the presence of bacteria „inside” the maize roots. On the basis of acetylene reduction activity (ARA) these bacteria exhibited nitrogen fixation in root homogenate, excised and intact roots. Differences in ARA were found between maize genotypes and between bacterial isolates. Biochemical studies showed large ratio between disaccharides and monosaccharides in the roots of S-20 maize genotype where bacteria were not found in the primary roots of the 34-day plants. Characteristics of total lipids in plasma membranes from roots of the same genotype are the lowest content of palmitic and the highest of stearic acid, with respect to protein part in plasma membranes the S-20 genotype exhibited the lowest amount of nonpolar amino acids. In this group of amino acids proline exhibited the lowest amount in S-20 genotype and the highest portion in W64A-N genotype in which bacteria were regularly found inside the roots.

BAŠOVIĆ, M., PRICA, V., ČMELIK, Z.

### PRESENCE OF Pb IN LETTUCE GROWN NEAR MOTOR ROADS

Faculty of Agriculture, Sarajevo

The investigation of contents of Pb in washed and unwashed lettuce, grown 4 to 120 m. from the Stup Loop – Rajlovac section of the Sarajevo – Zenica Highway, done in the course of three vegetation years (1981, 1982 and 1983), has shown:

– frequency of traffic on the checked section causes an increase of Pb in salad leaves;

– the greatest contents of Pb in salad leaves is at the distance up to 4 meters and, in the average for three generations, it amounts up to 32,23  $\mu\text{g/g}$  of dry mass of leaves;

– with greater distance, the concentration of Pb is reduced. The trend of reduction of Pb in unwashed salad leaves goes up to 50 m. of distance, and then the concentration holds at the level of 8,45 to 9,22  $\mu\text{g/g}$  of dry mass of leaves, which is significantly above the level of Pb concentration in standard salad sample (3,37 and 2,00  $\mu\text{g/g}$ );

– washing the salad in cold running and distilled water, the average of reduction of Pb is 24,75 to 76,13%, but the reduction of Pb by washing is greater if the contents of Pb in unwashed salad was greater. The effect of washing depends on a great number of factors, which should be examined in detail.

HOXHA, Y., JABLANOVIĆ, M., ABDULLAI, K. and FILIPOVIĆ, R.

### CATALASE ACTIVITY IN PLANTS POLLUTED BY HEAVY METALS

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Some data (predominantly obtained in experiments with animal tissues) concerning catalase activity point at the changes in that activity during intoxication by heavy metals.

On the basis of such results, as well as some of our earlier results concerning amylase activity, we started investigating catalase activity in plant tissues (seedlings of maize) after intoxication by heavy metals.

Catalase activity was determined by measuring oxygen liberated from the enzymatic reaction after three minutes. Treatment of plants by Pb–acetate caused an increase of catalase activity, probably as a result of increased production of  $\text{H}_2\text{O}_2$ .

Presence of  $\text{H}_2\text{O}_2$  in the medium also caused an increase of catalase activity (substrate induction).

In the plants permanently exposed to heavy metals pollution we found no increase of catalase activity.

On the basis of our results as well as the up to date knowledge, catalase (increasing) is probably involved in protective mechanism against heavy metals toxicity.

**WATER RELATIONS, STRESS PHYSIOLOGY**

ZARIĆ, Lj., KEREČKI, B., PEKIĆ, S. \*, PENČIĆ, M., RATKOVIĆ, S.

**SOME PARAMETERS OF RESISTANCE TO LOW TEMPERATURES  
IN MAIZE INBRED LINES**

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\*Faculty of Agriculture, Zemun

We studied resistance of some maize inbred lines to low positive temperatures (1°C, 8°C and 10°C) as well as the effect of frost (-5°C) on the above-ground part of the plant while the root system was exposed to 5°C.

Plants were grown in controlled conditions. Survival of plants at -5°C and germination at 8°C were recorded. Subsequent measurements were made of the length and weight of the above-ground part of the plants which were exposed to 10°C for 20 days, as well as of utilization of reserve materials of the kernel endosperm.

In plants exposed to 1°C (12 hours) and 10°C (20 days) chlorogenic acid content in the leaves was measured by the method of Zucker and Ahrens. RIA test was used to measure the abscisic acid content in the leaves of the plants kept at 1°C for 12 hours.

Gibberellic acid and some cytokinins were analyzed by bioassays in the plants in which the above-ground part was exposed to -5°C and the root to 5°C.

Differences were found in all parameters both between different inbred lines and different temperatures. The role of ABA at low temperatures is evident and can be considered to be an important parameter of resistance. Results show that resistance cannot be treated through individual indicators but in a complex manner.

FURTULA, V., VUČINIĆ, Ž., \* RADENOVIĆ, Č.\*

**TEMPERATURE DEPENDENCE OF THE ACTIVITY OF THE PLASMA  
MEMBRANE BOUND K-Mg ATPase ISOLATED FROM PRIMARY MAIZE  
(ZEA MAYS L.) ROOTS**

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Institute of Physical Chemistry, Beograd

\*Maize Research Institute, Department of plant physiology, Beograd

Potassium-stimulated, magnesium-activated ATPase (E.C.3.6.1.3), located on plasma membranes of higher plants, is an enzyme that plays an important role in ion uptake and regulation of intracellular ionic concentration. Chilling temperatures (<

285°K) disable growth and survival of plants like maize, and according to the hypothesis of Lyons and Raison, the primary cause for such a phenomenon is the occurrence of phase transitions in the membrane lipids, causing the inactivation of some of the enzymes embedded in the membranes. Since this ATPase is a membrane-bound enzyme, it could be that it is one of the primary targets of the chilling temperatures. Studies by a number of laboratories have shown that its activity shows changes in the activation energy at certain critical temperatures.

We have studied the activity of the enzyme as a function of temperature with the aim to determine what are its characteristics in maize, and also whether there are differences between genotypes exhibiting different degree of resistance to chilling temperatures. Plasma membranes were isolated from primary maize roots on a discontinuous sucrose gradient, and enzyme activity determined by the amount of phosphorous released, in the temperature interval from 275 to 310°K.

Our results demonstrate that the enzyme activity can be approximated in the Arrhenius plot by a straight line, and we could not observe changes in the slope. No significant differences could be noticed in the enzyme activity on plasma membranes isolated from different genotypes. However, significant displacement of the activity from the linearity could be detected in shorter temperature intervals at certain critical temperatures (284, 292–298 and 302–307°K). These critical points coincide with those at which we determined changes in the membrane fluidity, as supported by measurements using differential scanning calorimetry and ESR spectroscopy.

STIKIĆ, R., JOVANOVIĆ, D. and JOVANOVIĆ Lj.

### HYSTOCHEMICAL INVESTIGATIONS OF MAIZE STOMATA

Faculty of Agriculture, Zemun

Mechanism of stomatal movements is related to active ion transport. Histochemical investigations provide 1) an establishment of ions and starch localisation in the stomatal complex and their relative amounts and 2) simple examination of the influence of various factors on stomata.

The purpose of this work was to investigate the influence of ABA on stomata of two maize lines 1304 (drought resistant) and 389 (drought susceptible) on the basis of histochemical tests for  $K^+$  and starch.

For the experiments segments of fifth leaves were incubated in two ABA solutions ( $5.6 \times 10^{-4}$  M and  $10^{-3}$  M) in light and  $CO_2$ -free air for 3h. Epidermal strips were, then, used for histochemical establishment of  $K^+$  and starch localisation.  $K^+$  was determined by staining with  $Na_3 Co(NO_2)_6$  and precipitation with  $(NH_4)_2 S$ , and starch with  $J_2 / KJ$ .

Histochemical tests show that ABA induced the decrease of potassium and the increase of starch content in the guard cells of both investigated lines. Lower ABA concentration ( $5.6 \times 10^{-4}$  M) had smaller effect in both lines than the higher one ( $10^{-3}$  M). The sensitivity of stomata to lower ABA concentration was less expressed in line 389 than in 1304. This could be attributed to the differences in the degree of drought resistance between these two lines which has been previously confirmed.

JOVANOVIĆ, Lj., JEVTIĆ, D. and STIKIĆ, R.

### INFLUENCE OF DROUGHT ON WATER REGIME IN MAIZE

Faculty of Agriculture, Zemun

The well known fact is that drought conditions after various physiological processes in plants and that these changes depend of their capability to adapt on such conditions, i.e. of the degree of their drought resistance. The purpose of this work was to investigate if drought conditions cause the differences in water regime parameters between two maize lines differing in drought susceptibility, and, thus, point out to new methods of evaluation of drought resistance in maize.

The investigations were done on two maize lines: 1304 previously determined as drought resistant and 389 previously determined as drought susceptible. Plants were exposed to soil water shortage in the phase of fifth leaves for 4 and 7 days, when plants were rewatered. During the course of drought daily measurements of water regime parameters were done. Parameters measured in the leaves were: 1) total water potential –  $\psi$  (by method of psychrometry); 2) osmotic potential –  $\psi_s$  (by method of freezing and thawing and, then by psychrometry); 3) turgor potential –  $\psi_p$  (by calculating the difference of the values  $\psi$  and  $\psi_s$ ); 4) relative water content – RWC (by method of gravimetry) and 5) stomatal conductance (by porometer).

The obtained results show that during the whole drought period the values of  $\psi$  and  $\psi_s$  were lower and  $\psi_t$  and RWC were higher in 1304 than in 389. At the mild stress (up to 4 days) the stomatal closure was faster in the line 389, while in the conditions of severe stress (after 7 days) stomata of both lines reached the threshold value for closure. According to the data of all parameters, recovery after severe stress was faster in the resistant line 1304, than in the susceptible 389.

The results show that the differences in drought reactions of these two investigated lines could be, partly, attributed to the differences in osmotic adjustment. Line 1304, due to higher decrease of  $\psi_s$  maintains  $\psi_p$  allowing, so, normal leaf physiology in spite of the lower  $\psi$ . This should be proved by further investigations using method of P–V curves.

STIKIĆ, R., MARTINOVIĆ, B.,\* JOVANOVIĆ, Lj. and VUČKOVIĆ, M.\*

### ISOLATION OF GUARD CELL PROTOPLASTS IN SUGAR BEET

Faculty of Agriculture, Zemun

\*INEP, Zemun

The purpose of this work was the identification of guard cell protoplasts (GCP) in beet and their separation from epidermal (ECP) and mesophyll protoplasts (MCP). For minimizing the contamination of other tissues epidermal strips were used.

The isolation procedure consisted of the following steps: 1) collection of intact epidermal peels in the solution of 0,23 M mannitol + 1 mM  $\text{CaCl}_2$  in 5 mM MES (pH 5.5) + 2% PVP; 2) incubation (60 min.) at 30°C in 4% cellulysin in 0.4 M mannitol+1 mM  $\text{CaCl}_2$ ; 3) washing twice with 0.4 M mannitol and centrifugation; 4) washing the supernatant containing epidermal peels with intact stomata with 0.4 M mannitol; 5) incubation (90 min.) of epidermal strips in 4% cellulysin in 0.5 M mannitol + 1 mM  $\text{CaCl}_2$  /5 mM MES (pH 5.5) and centrifugation; 6) washing the pellet consisting of GCP

0.5 M mannitol + 1 mM CaCl<sub>2</sub> and centrifugation; 7) taking pellet in 0.5 M mannitol + 1 mM CaCl<sub>2</sub>; 8) counting isolated and stabilized GCP. Counted no. of GCP/mm<sup>-2</sup> was 150; no. of counted GCP based on epidermal areas of 30.300 mm<sup>2</sup> was 2.02 x 10<sup>6</sup>; potential yield of GCP from the same epidermal areas was 4.55 x 10<sup>6</sup>; diameter of GCP was 8.0–11.2–12.5 μm and efficiency of isolation procedure was 44%.

The obtained results show that the efficiency of isolation procedure was not satisfactory enough for the investigations of stomatal physiology and its improvement will be the task of our further investigations.

PEKIĆ, S. and QUARRIE, S.

### ABA ACCUMULATION CAPACITY IN DIFFERENT MAIZE GENOTYPES

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Plant Breeding Institute, Cambridge, UK

There is good evidence that abscisic acid (ABA) is involved in mediating responses to drought. There is genetic variation in wheat, rice and pearl millet in drought – induced capacity for ABA accumulation. In wheat and millet, this capacity is highly heritable (1).

The purpose of this work was to investigate genetic variability of ABA accumulation capacity in different maize genotypes.

A detached leaf test (DLT), the method usually used in these experiments (2), was used to investigate the capacity to accumulate ABA in four pairs of inbred lines (Nos 3–10) of maize (previously determined to differ in drought resistance) and their F<sub>1</sub> hybrids (Nos 11–14). Pairs differed in the duration of the vegetation period.

For the DLT third leaves were sampled at around the time of ligule emergence. Detached leaves were desiccated to 90% of their initial fresh weight (ifw) and incubated for 5 h at 20°C in the dark. ABA was measured in individual leaves using a radioimmunoassay with a monoclonal antibody to ABA.

Results show that, except for line N<sup>o</sup>5, ABA accumulation in other inbred lines was very similar (125–177 mg ABA/g ifw) and largely independent of either the drought resistance category or the maturity classification. So, the biggest difference in ABA accumulation has been found between resistant line N<sup>o</sup> 5 (363 ± 39 ng ABA/g ifw) and susceptible N<sup>o</sup> 6 (136 ± 6 ng ABA/g ifw) – pair belonging to FAO group 400. Although line N<sup>o</sup> 5 accumulated nearly 3 times as much ABA as line N<sup>o</sup> 6 did in response to stress treatment, the control levels of ABA in the two lines were the same (20 ± 1 ng ABA/g ifw). This pair of inbred lines would, so, provide a good system for investigating the nature of the inheritance of ABA accumulation capacity in maize. The fact that their F<sub>1</sub> hybrid N<sup>o</sup> 13 (6 ♀ x 5 ♂) had the same level of ABA accumulation in the DLT as its maternal parent (line N<sup>o</sup> 6) indicates the possibility of maternal inheritance of ABA accumulation capacity in maize.

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MRATINIĆ, E.

**THE EFFECT OF SUBSTRATE USED ON WATER  
REGIME OF STRAWBERRY GROWN UNDER  
CONTROLLED CONDITIONS**

Faculty of Agriculture, Belgrade

The paper deals with two strawberry cultivars—Senga—Sengana and Gorella, grown under protected space on two different substrates—soil and sand. Above conditions are for strawberry quite artificial, since this species is cultivated exclusively in open fields. However, since strawberry is a cultivated plant whose fruits ripen very early, even during May, its cultivation in protected space is more and more interesting throughout the world. By such way it could be possible to accelerate fruit maturity at least for one month, due to control of microclimate, which is economically very important.

Taking into consideration that for any cultivated plant, and not only cultivated, water utilization is essential condition for all biochemical—physiological processes, reflecting always final productivity of plants (being goal of every production), our objective was to investigate determined parameters of water regime, depending on the medium used for strawberry cultivation. On the basis of results obtained we intend to determine potential possibilities of fruiting of above mentioned cultivars grown under controlled conditions, ie. protected space.

The following parameters in the frame of water regime have been investigated: transpiration, dynamics of water content in leaves, water retention capacity of foliage—refractometric and osmotic values of sap.

Significant differences concerning all above mentioned parameters are observed, which depend from both—characteristics of cultivars and medium used for strawberry cultivation.

RISTIĆ, Z.

**ECOANATOMICAL AND ECOPHYSIOLOGICAL CHARACTERISTICS  
OF LEAVES IN WHEAT CULTIVARS OF NOVOSADSKA RANA  
1 AND NOVOSADSKA RANA 2**

Faculty of Agriculture, Zemun

The investigation of some general biological characteristics in different wheat cultivars is very important for correct selection and their cultivation in different ecological conditions. In this paper the results of investigation of some anatomical characteristics of leaves and some characteristics of water regime in two wheat cultivars, Novosadska rana 1 and Novosadska rana 2, are presented. The experiment with the wheat cultivars was carried out under the field conditions. These wheat cultivars were grown in the same agroecological conditions. The anatomical characteristics examined were: 1) the thickness of leaf, 2) the thickness of epidermal tissue on face and back side of leaf 3) the thickness of mesophyll, 4) the number and magnitude (surface) of stomata, 5) the total surface of stomata on  $\text{mm}^2$ . The characteristics of water regime examined were: 1) the intensity of transpiration, 2) the amount of water in leaves, 3) the osmotic pressure of

cell sap in leaves. In the same time with the measurements of these ecophysiological characteristics the measurements of microclimate conditions were done.

The results of investigation showed that there are the significant differences between these wheat cultivars. The wheat cultivar Novosadska rana 2 has thicker leaves and epidermal tissue and bigger total surface of stomatas on  $\text{mm}^2$ . This wheat cultivar also has higher intensity of transpiration, less amount of water and higher osmotic pressure. All these results show that the wheat cultivar Novosadska rana 2 seems to have more xeromorphic leaves than wheat cultivar Novosadska rana 1.

STEFANOVIĆ, L., HADŽI-TAŠKOVIĆ ŠUKALOVIĆ, V.

### EFFECT OF SOME HERBICIDES ON MAIZE INBRED LINES

Maize Research Institute, Zemun Polje

Herbicides are compounds used for weed control in agriculture. However, they can adversely affect the grown plant particularly if improperly used.

Studies have shown different resistance of maize genotypes to herbicides.

A problem in evaluating resistance are methods to be used for assessing the response of the investigated plants. This is especially pronounced when a large number of inbreds and herbicides have to be tested.

The objective of this investigation was to attempt to introduce rapid methods for assessing resistance of different maize inbreds to herbicides by measuring free proline content.

The method of measuring dry weight of the root and above-ground part of the plant has commonly been used as an indicator of plant response to herbicides. This method appropriately indicates the changes occurring in the plant. Therefore, the measurements of dry weight of the investigated inbred lines were compared to the results obtained with the applied method.

Effect of three herbicides (Alachlor, Atrazine and Eradicane) on ten maize inbred lines was studied. Free proline content and dry weight of the plants were measured.

Free proline content was determined by colorimetric method in the shoot and root of the 5-day old seedlings which germinated in  $10^{-4}$  mol/l. Control plants germinated in water. Results were expressed as percent of control.

Dry weight of the above-ground part and root of the maize plant was determined by drying the material at  $105^{\circ}\text{C}$  to a constant weight. In order to determine dry weight, plants were grown to the 3-leaf stage in controlled conditions in sand. Herbicide was added to the treated variants at planting only. Control and treated plants were watered.

The results show that the Atrazine, Alachlor and Eradicane herbicides affect free proline content in the shoot and root of seedlings of some of the investigated maize inbred lines.

A reduction of free proline content higher than 20% in regard to control was found in the shoot of the seedlings in six inbred lines treated with Eradicane, in the shoot of four inbreds treated with Atrazine and in the shoot of three inbreds treated with Alachlor. Increase in free proline content higher than 20% was found in the shoot of inbred TD-81.

Reduced dry weight of the above-ground part of the seedlings treated with Atrazine and Alachlor coincided with the reduced free proline content implied by high



correlation coefficients ( $r=0,73$ ) for Atrazine and ( $r=0,70$ ) for Alachlor, whereas in plants treated with Eradicane there was only a low positive correlation.

Data on the change in free proline content and above-ground mass of maize seedlings indicate that the change in free proline content in plants treated with Atrazine and Alachlor could be used for rating resistance of maize inbred lines to these herbicides instead of the common method based on measuring weight of the above-ground part.

In the root of seedlings treated with herbicides free proline content increases or decreases depending on the investigated inbred and the herbicide used. Root dry weight of some inbreds simultaneously decreases. However, there is only a low positive correlation between the change of free proline content and dry weight, so we think that free proline content in the root is not a suitable indicator of plant resistance to the investigated herbicides.

POPOVIĆ, R., JANKOVIĆ, M.M., DIMITRIJEVIĆ, J., STEFANOVIĆ, K.,  
KARADŽIĆ, B.

### ECOPHYSIOLOGICAL CHARACTERISTICS OF PLANTS AND HABITATS CONDITIONS IN BIRCH FORESTS ON THE MOUNTAIN MALJEN

Institute for Biological Research „Siniša Stanković”, Beograd

This work gives results of investigations of habitat conditions, water regime and photosynthesis of the most important plant species. The investigated birch forests, in the localities of Čalački potok and Slapovi, have developed after the fire, as the pioneer communities in the pine tree and birch tree habitats. The soil has favourable physical and chemical properties and belongs to the type of brown soils on serpentine.

Investigations of plant water regime have shown that the transpiration is relatively low: the mean daily values range from 0,222 up to 11,452 mg.gr/min. The daily transpiration dynamics corresponds to the changes of the basic exterior microclimate factors and is represented by single-peak curves form. Season dynamics shows the greatest transpiration at the beginning of the vegetation period and it decreases towards the autumn. Similar dynamics has water contents in leaves, while osmotic values of cell juice increase from the beginning, towards the end of the vegetation period: the mean daily values range between 9,781 and 27,448 b. Basic characteristics of plant water regime (rather low transpiration, small quantity of water in leaves and the increased hydrature of plants) depend on the morpho-anatomic characteristics of the tested species as well as on the habitat conditions, most of all on the soil water regime.

Photosynthesis intensity analysis shows that the plants at the bottom floor have greater values (15,391–35,404 mg CO<sub>2</sub>/gr/h) than the investigated species of trees (7,107–14,101 mg/CO<sub>2</sub>/gr/h).

Investigations of plant water regime and photosynthesis have shown that the pioneer birch communities develop very successfully and that they have been formed by the species well adapted to the conditions of the severe mountain climate.

PLAVŠIĆ—GOJKOVIĆ, N., DUBRAVEC, K., MRVA, K.

**COMPARATIVE INVESTIGATION OF SOME EXOTIC  
PLANTS TRANSPIRATION**

Faculty of Agricultural Sciences, University of Zagreb, Institute for Plant  
Protection, Department for Agricultural Botany

The investigation of transpiration ( $Tr$ ) of *Catalpa bignonioides* (North America), *Davidia involucrata* (China) and *Parrotia persica* (Persia) leaves was carried out using Stock's method in natural conditions in park of Faculty of Agricultural Sciences. Measurements were performed on 14.06. and 30.07.1984. Stomata openness degree was determined by infiltration method. Microclimatic investigations were also performed.  $Tr$  was expressed in mg of water transpired per gram of leaf per minute (mg/(g min)).

On the basis of these investigations the following results were obtained:

- The biggest among the measured  $Tr$  values was 17.8 mg/(g min) and this value was determined for *Davidia involucrata* on 14.04.1984. at 14 h. Minimal  $Tr$  value, 1.17 mg/(g min) was measured on 30.07.1984. at 14 h in case of *Parrotia persica*.
- The average quantity of transpired water in grams in a day per gram of leaf was 2.97 for *Parrotia persica*, 3.90 for *Davidia involucrata* and 5.11 for *Catalpa bignonioides*.
- According to the Gračanin's classification *Parrotia persica* and *Davidia involucrata* have medium  $Tr$  intensity whereas *Catalpa bignonioides* has high  $Tr$  intensity.
- Correlation between  $Tr$ , temperature and openness of stomatas was established.

## GROWTH SUBSTANCES, DEVELOPMENTAL PHYSIOLOGY

JANDOVA, B., ČERVINKOVA, J.

### CHANGES IN ENDOGENOUS PHYTOHORMONES DURING THE REGENERATION OF CUCUMBER (*CUCUMIS SATIVUS* L.) IN CALLUS CULTURE

Faculty of Science, UJEP, Brno, ČSSR

Plant regeneration through organogenesis was obtained within 4 months, from hypocotyl fragments in culture. The regeneration process consisted in 4 phases:

- a) Callus induction  
(BDS – mineral solution, 2,4-D – 2,5 mg l<sup>-1</sup>)
- b) Callus growth and initiation of meristematic centers  
(BAP – 0,4 mg l<sup>-1</sup>)
- c) Differentiation of vascular tissue
- d) Organogenesis  
(BDS medium, hormones omitted)

In each differentiation phase the weight of cultures was measured and the content of chlorophyll and phytohormones (biological tests for auxins, gibberellins, cytokinins) was determined. Growth of callus was accompanied by a high auxin and gibberellin content, while their content declines during differentiation. Intensive chlorophyll synthesis and high cytokinin content are characteristic for the phase of organogenesis.

JELIĆ, G., ČULAFIĆ, Lj.\* KAPOR, S.

### ENDOGENOUS CYTOKININS OF THE DIOECIOUS PLANT *RUMEX ACETOSELLA* L. INEP, Zemun

\*Institute of Botany, Faculty of Science, Beograd

The sex of plants is genetically determined, but it can be modified by the effect of hormones and environmental factors. Our previous investigations, based on bioassays, have shown that male and female *Rumex acetosella* plants may differ in respect to gibberellin and cytokinin content, depending of their developmental stage.

In the present paper we report the results on the analyses of endogenous cytokinins in vegetative and reproductive organs of *R. acetosella*, using improved methods of purification, suitable for demonstrating possible qualitative and quantitative differences in plants of different sexes.

Qualitative and quantitative analysis of TMS cytokinin derivatives was performed using GLC method. Cytokinin standards zeatin ( $io^6Ade$ ), zeatin riboside ( $io^6A$ ), isopentenyl adenine ( $i^6Ade$ ) and isopentenyl adenosine ( $i^6A$ ) were used for identifications.

$i^6A$ ,  $io^6A$ ,  $io^6Ade$  and  $i^6Ade$  were detected in all the samples, more so in female than in corresponding male plants.

Zeatin glucoside was measured through zeatin which was released by enzyme hydrolysis and was detected in male plants only.

The qualitative and quantitative differences in the content of endogenous cytokinins of *R. acetosella* plants of different sexes imply the possible role of these hormones in sex determination.

BRAŠANAC, J., KOZOMARA, B., KONJEVIĆ, R., and NEŠKOVIĆ, M.

**THE INFLUENCE OF LIGHT AND HERBICIDE NORFLURAZON  
ON THE CONTENT OF GIBBERELLIN – LIKE  
SUBSTANCES IN *PISUM SATIVUM* AND *PHASEOLUS  
AUREUS* SEEDLINGS**

Institute for Biological Research „S. Stanković” and Institute  
of Botany, University of Belgrade

It has been previously shown that short red light irradiation of etiolated pea plants cv. Aleska brings about transient increase in endogenous GA – like substances, detectable by barley endosperm test. The same transient increase was recorded in white light grown plants.

It is possible to envisage that light:

- a) controls some step in GA – biosynthesis;
- b) brings about hydrolysis of conjugated forms and
- c) causes the release of gibberellins from cell compartments (plastids). In order to check the last hypothesis, plants of *Pisum sativum* cv. Alaska and *Phaseolus aureus* were raised in the presence of the herbicide norflurazon. The herbicide causes plastid destruction in light absorbed by chlorophyll. Plants were grown in darkness, continuous far-red and/or white light. In *Phaseolus aureus* plants, the estimation of GA – like substances was performed at the end of 5 days growing period. The seedlings of *Pisum sativum* were, additionally, subjected to 5 min red light irradiation prior to GA extraction which was done 10, 20, 30 and 120 min after the red pulse.

The obtained results showed that norflurazon reduced the content of GA – like substances irrespectively of light pretreatments. However, this effect was drastically pronounced in white light. In addition, the transient increase of GA – like substances, induced by red light in pea plants, was not affected by the herbicide.

BARAŠEVIĆ, B., GOVEDARICA, M.,\* SARIĆ, M.

### CONTENT OF GIBBERELLINS IN DIFFERENT *AZOTOBACTER* STRAINS

Institute of Biology and \*Institute of Field and Vegetable Crops,  
University of Novi Sad

Our previous studies on the effect of different *Azotobacter* strains on various genotypes of wheat and maize suggested that a specific relationship between individual strains and certain genotypes exists. In order to elucidate relationship bacterial strain – plant genotype, a preliminary assay was carried out.

In this experiment three different *Azotobacter* strains which had been isolated from the maize rhizosphere were investigated. The content of gibberellins (both intra- and extracellular) was determined separately. Bacterial liquid culture was centrifuged and the hormones were extracted both from the pelleted cells and the supernatant. After chromatography on the DEAE Sephadex – A 25 column, content of gibberellins was estimated by gibberellin  $\alpha$ -amylase test.

Content of gibberellins in bacterial cells was at the detection limit by this method. However, the content of extracellular phytohormones was recorded with more success and it showed variation among different *Azotobacter* strains.

GOGALA, N.

### THE INFLUENCE OF JASMONIC ACID ON GROWTH OF MYCORRHIZAL FUNGI

VTO za biologijo Biotehniške fakultete and Institut za  
bilogijo Univerze E. Kardelja, Ljubljana

Jasmonic acid (JA) and methyl-jasmonic acid (MeJA) are subjects of many recent physiological investigations due to the discovery of their functions as growth regulators. They inhibit growth and promote aging of green plants.

In our experiments we investigated the influence of both substances on growth of mycorrhizal fungi and tested by bioassay methods the presence of these substances in roots and root exudate of the pine *Pinus sylvestris*. ABA, the well known growth inhibitor of green plants does not have any similar effect on fungal tissue.

JA and MeJA was added to nutrient medium for a mycelium (MNM), inoculated by fungi *Suillus variegatus* and *Boletus bovinus*. Substances in concentrations between  $10^{-10}$  and  $10^{-6}$  g/l were tested. Both fungus species were not equally sensitive to the application of JA and MeJA. *Suillus variegatus* was at higher concentrations of these substances more inhibited than *Boletus bovinus*.

Tomato hypocotyl bioassay for growth inhibitors indicates the presence of JA in the roots and the root exudate of the pine tree *Pinus sylvestris*.

KRIVOKAPIĆ, K., PLAVŠIĆ, B., ERIĆ, Ž.

### THE CHANGE OF ENDOGENOUS HORMONES LEVEL IN THE SUGER-BEET ROOT INFECTED BY BEET YELLOWS VIRUS

Institute of Biology, Faculty of Science, Sarajevo

Using different standard methods (chromatography, bioassay and spectrophotofluorimetry) we analysed the change in the level of auxins, gibberellins and cytokinins in the roots of plants infected by beet yellows virus (BYV).

Spectrophotofluorimetric (SPF) analysis of auxins from water fraction showed a lower level of these active substances in the infected plants than in the healthy ones. However, the SPF analysis and bioassay do not always give the same results for all the active substances. When the results of the SPF analysis of active substances similar to DL-tryptophan and IAA in Rf and in activation and fluorescence spectra, are compared, their inverse relationship become noticeable: the level of tryptophan is higher in healthy and that of IAA in diseased plants. The level of endogenous gibberellins is almost the same in infected and healthy plants, while the level of endogenous cytokinins is much higher in diseased than in healthy plants.

KEVREŠAN, S., KANDRAČ, J., GRUJIĆ, S., BEĆAREVIĆ, A.

### EFFECT OF AUXIN TREATMENT ON POLYRIBOSOME TRANSLATION IN CELL-FREE SYSTEM

Institute of Chemistry, Faculty of Sciences, Novi Sad  
Faculty of Technology, Novi Sad

Little is known about mechanisms by which plant hormone, auxin, influences so different processes as cell extension, division and differentiation. There is a lot of data in the literature, which are in agreement with the hypothesis that physiological effects of auxin are the result of the altered gene expression. The aim of this work was to give a contribution to elucidating the auxin effect on gene expression by translation of polyribosomes, which are isolated from untreated and auxin-treated maize mesocotyl segments, in cell-free system and by analysis of translational products.

Experiments were carried out by spraying three-day-old maize seedlings with 2,4-D solution in order to avoid the difficulties in explanation of results which might be caused by plant injury. After treatment the elongating mesocotyl segments were excised and used for polyribosome isolation. Translation of isolated polyribosomes were carried out in wheat germ cell-free system and the translation products analysis by polyacrylamide gel electrophoresis and autoradiography.

Polyribosomes isolated from auxin-treated segments show higher activity in cell-free system than polyribosomes isolated from control segments. The analysis of translational products by one-dimensional gel electrophoresis showed that there were no qualitative changes in translational products pattern as the result of hormone treatment.

Densitometric analysis of autoradiograms showed that there was an enhancement of intensity in polypeptides of higher molecular masses and a decrease of intensity in polypeptides of lower molecular masses as the result of hormone action.

These results indicate that the effect of auxin on gene expression could be also realised at the translational level.

NAUNOVIĆ, G., and NEŠKOVIĆ, M.

### POSSIBLE ROLE OF $\text{Ca}^{2+}$ IONS IN RAPID RESPONSES IN STEM GROWTH

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It was established in preliminary experiments that changes in external  $\text{Ca}^{2+}$  concentration affect the elongation of etiolated pea stem sections. Substances influencing membrane transport of  $\text{Ca}^{2+}$  ions also affected section growth. The effect of these factors was studied in rapid responses of decapitated, dark-grown Meteor pea stems. The growth rate was measured by a position sensing transducer, in darkness, or following a brief red light irradiation (3 min, max 657 nm). As expected,  $\text{CaCl}_2$  (10 mM) decreases the elongation rate both in light and darkness. EGTA (1 mM) reverses this effect.  $\text{GA}_3$  (10  $\mu\text{g}$ ) reverses the inhibition caused by  $\text{CaCl}_2$  or red light, but has no additional stimulation over the EGTA effect. The herbicide amiprophosmethyl and the ionophore A 23187 applied after EGTA, further increase the growth rate of pea stems. It is concluded that growth rate can be accelerated by factors decreasing  $\text{Ca}^{2+}$  activity in cell walls and by factors increasing  $\text{Ca}^{2+}$  accumulation in the cytoplasm.

KONJEVIĆ, R.

### RED – BLUE LIGHT INTERACTION IN THE RESPONSE OF *SINAPIS ALBA* SEEDLINGS TO THE EXOGENOUS GIBBERELLIN

Institute of Botany, Fac. Sci. and Institute for Biological  
Research "S. Stanković", University of Belgrade,

The influence of blue and red part of the spectrum on the extension of *Sinapsis alba* hypocotyl, treated with different doses of  $\text{GA}_3$ , has been examined. While the action of red light in morphogenesis is explained on the basis of well-established and characterized photosensor pigment phytochrome, the action of blue light is mediated by the cryptochrome – a pigment operationally defined by the action spectra of different blue light-induced responses, which can not be explained by phytochrome properties. The effect of light in morphogenesis has repeatedly been sought on the basis of its interaction with plant hormones (1). An action between phytochrome and exogenously added  $\text{GA}_3$ , in the control of hypocotyl extension of *Sinapsis alba* seedlings, has already been shown (2) and the results of this work point to a significant interaction between phytochrome and blue light in the control of the response of *Sinapsis alba* seedlings to different  $\text{GA}_3$  doses, in a very weak white light. The seedlings respond to  $\text{GA}_3$  as they were grown in darkness, i.e. as the active phytochrome were removed from the system. The effect of the weak white light can be mimicked by simultaneous irradiation of seedlings with blue and red light. The application of the herbicide norflurazon abolished the effect of white light.

1. Wareing, P.F., Thompson, A.G. (1976), In: Light and Plant Development, H. Smith ed., Butterworths, London, pp. 285–294.
2. Konjević, R., Schafer, E., Mohr, H. (1980), Photoreceptors and Plant Development, J. De Greef ed., Antwerpen Univ. Press, pp. 413–422.

GRUBIŠIĆ, D., STEFANOVIĆ, M.

### THE EFFECT OF RETARDANTS ON SEED GERMINATION

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and Faculty of Science, Kragujevac

The relative importance of gibberellins, in seed germination, may be studied by the application of growth retardants, known to inhibit the biosynthesis of endogenous gibberellins. The effect of AMO 1618, CCC, ancymidol and tetcyclacis (BASF 106 W.), on the germination of several species, was examined. Tetcyclacis and ancymidol, which were tested in wheat, alfalfa, rape, mung bean and mustard seeds, had no effect. Their germination is light independent. However, the retardants strongly affected germination of light dependent seeds. *Amaranthus* and tomato seeds could be inhibited only by tetcyclacis. Ancymidol and tetcyclacis inhibited germination of *Paulownia tomentosa* while all applied substances inhibited *Ramonda serbica* seeds, both known to be light requiring. The obtained results point to the role of endogenous gibberellins in light controlled seed germination.

KRSNIK-RASOL, M., and REGULA, J.

### EFFECTS OF SOME BIOGENIC AMINES AND AMIDE ON POTATO TUMOUR GROWTH

Department of Botany, Faculty of Science, University of Zagreb

Biosynthesis of cytokinin and auxin in normal plant cells is controlled by specific developmental regulation mechanisms which is not the case with tumorous cells. Tumours may therefore be a suitable system for the investigation of metabolic pathways in auxin synthesis.

Our experiments indicate that addition of IAA, glutamine, tryptamine or serotonin (5-hydroxytryptamine) to a suspension of *Agrobacterium tumefaciens*, at the moment of potato tuber infection stimulate the induction and growth of tumours.

Peroxidases activity was considerably higher in tumorous tissues than in normal ones. Addition of biogenic amines affected this activity (measurements still in progress).

Electrophoretic analysis of isoperoxidases obtained through anodic separation indicates that there are differences in isoperoxidases between normal and transformed tissues.

Investigation of the auxin content in tumour tissue indicated that tissues with added substances contained slightly higher quantities of IAA. It is likely that tryptamine, besides glutamine, acted as a donor of amino nitrogen.



KRSNIK-RASOL, M., RUBELJ, J., ŠERMAN, D.

### ELECTROPHORETICAL PROTEIN PATTERN OF NORMAL AND TUMOUROUS POTATO TISSUE

Department of Botany, Faculty of Science and  
Department of Biology, Medical Faculty, University of Zagreb

In the process of their tumour transformation a part of Ti-plasmid is integrated into the potato cell genome. It causes metabolic changes which might be reflected in electrophoretic protein pattern.

PAGE (polyacrylamide gel electrophoresis), in 7,5% gel was performed according to Davis and Ornstein (Davis, B.J., Ann. N.Y. Acad. Sci. 121, 404, 1964, Ornstein, L., Ann. N.Y. Acad. Sci. 121, 321, 1964) to obtain protein patterns of potato callus, as well as of normal and tumourous potato tuber tissues.

We found differences in protein patterns between normal and tumourous tissues. The protein bands Rm 0,1 and 0,78, which had been hardly detectable in normal tissue were abundantly present in tumours as well as in callus. Thus it seems that these proteins are products of plant genes rather than of integrated ones. In tumourous cells the most abundant storage proteins of potato tuber are synthesized in smaller quantities or not at all.

BESENDORFER, V., BOSILJEVAC, V., and PAPEŠ, D.

### INSTABILITY OF THE EUROPEAN BLACK PINE (*PINUS NIGRA*) NUCLEAR GENOME INDUCED BY SYNTHETIC AUXINS AND CYTOKININ

Department of Botany, Faculty of Science, University of Zagreb

The aim of this work was to test the effect of three synthetic growth regulators frequently used in the black pine tissue culture:  $\alpha$  - naphthalenacetic acid (NAA), indolyl-3 - butyric acid (IBA) and 6-benzylaminopurine (BA).

The test objects were roots of one week old black pine seedlings treated for 6 hours in a range of concentrations ( $5 \times 10^{-4}$  -  $5 \mu\text{M}$ ), followed by a 7 hours reparation period in water. The stability of chromosome complement was determined through a study of types and frequency of chromosome aberrations and mitotic activity.

Mitotic activity was increased along with the increase of auxin concentrations. At the highest concentration ( $5 \mu\text{M}$ ) of NAA the mitotic activity was 24,6%, and the activity of IBA was 24,9%. In the control it was 9,4%. Increase of mitotic activity was followed by an increase of chromosomal aberrations. Except polyploidy, which was predominant at  $5 \times 10^{-1} \mu\text{M}$ , other types of aberrations were noticed, like for instance chromatin clumping, chromosome fragmentation, lagging chromosomes, anaphase and telephase bridges and micronucleoli. Mitotic activity kept the same value in the reparation period, though the frequency of chromosome aberrations decreased.

Cytokinin BA showed the opposite effect. Mitotic activity was very similar to control (9,0%) at highest concentration but during the period of reparation it increased to 18,9%. Frequency of chromosome aberrations was smaller than the frequency induced by auxin treatment. Increase of chromosome aberrations was noticed in reparation period after the highest concentration of cytokinin BA.

VUJIČIĆ, R., BOJOVIĆ—CVETIĆ, D., and NEŠKOVIĆ, M.

### THE EFFECT OF HORMONES ON CYTOCHEMICAL CHANGES IN COTYLEDONS IN VITRO

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Institute for Biological Research "S. Stanković", Beograd

It has been reported (Nešković, Vujičić and Srejšević, Ann. of Bot., 1985, in press) that roots, shoots or callus could be induced in isolated buckwheat cotyledons, by changing the auxin/cytokinin ratio and their concentration.

The aim of the present study was to follow the primary changes in different types of cotyledon cells and define cytochemically their contents and the ultrastructure.

Cotyledons grown on the control B5 medium, without hormones, were composed of palisade and spongy parenchyma cells and vesicular tissue.

In the cotyledons grown on the B5 medium with high 2,4-D ( $5 \text{ mg l}^{-1}$ ) and low dose of kinetin ( $0.1 \text{ mg l}^{-1}$ ) a new medial layer was conspicuous between palisade and spongy cells. The cells of the medial layer were dividing intensively in anticlinal and periclinal plane and if the incubation was prolonged, the callus tissue was formed originating from this layer. From the cytochemical analysis it was evident that the various cell types present in the cotyledons were structurally and cytochemically different; palisade and spongy cells were large and vacuolated, the vacuoles contained reserve material, most likely proteins, while the plastids had large starch grains. In the medial layer, however, the cells were with thin cell walls, the cytoplasm rich in organelles, while the plastids did not accumulate starch reserves.

The structural differences found in the control and induced cotyledons indicated that high auxin and low kinetin dose stimulated divisions only in a particular cell type. This means that certain cotyledon cells were able to respond and act as target cells for a particular hormone. It is left to be found what is the origin of the cells induced to divide, and it is hoped that cytochemistry at the ultrastructural level might help in the study of this problem which is of wide interest.

RADENOVIĆ, Č., and JOVANIĆ, B.\*

### BIOPOTENTIAL AND GEOREACTION OF PRIMARY MAIZE (*ZEA MAYS*) ROOT

Maize Research Institute, Zemun Polje

In our previous electrophysiological studies of electric characteristics of primary root of *Zea mays* L. special emphasis was placed on bioelectrochemical functions of ion transport processes. Root system in plants, including maize, has an extremely important function in transport; this transport function is complex and insufficiently studied. Our attention was attracted by the interdependence of the difference between biopotential and georeaction in primary root of *Zea mays* L. Some results of this phenomenon are listed below:

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— Primary maize root has three physiologically different zones in regard to its transport capacity: first, from root tip to 6 cm; second 6–9 cm; and third, 9–12 cm. The value of the difference of biopotential is determined by the root cap, the first zone. Biopotential difference linearly changes with the change of solution concentration: KCl, NaCl, and  $\text{NH}_4\text{Cl}$ .

— Change of the root position affects the scope of biopotential difference, i.e.  $\psi_K$  reduces by 10% when the root is placed in the inverse position. Biopotential difference also linearly changes with the change of concentration of the investigated solution when the root is in inverse position.

— Correlation between biopotential and geostimulation in primary maize root was found.  $\psi_K$  changes non-linearly with the angle of primary root geostimulation. The highest effect was found for the change of geostimulation angle within the range: 100–155°.

— The results enable a hypothesis to be advanced concerning the character of transport processes in primary maize root under the effect of the gravity force. Tissue of primary maize root performs ion transport with two different intensities and methods: (1) directed ion transport is more intensive and takes place from the tip to the base of the primary root; (2) indirect ion transport is of a reduced intensity and takes place from the basis to the tip of the primary root; (3) directed and indirect transport through the root tissue is regulated by different activity of ion and water channels.

KRAJNČIĆ, B.

### REGULATION OF FLORAL INDUCATION AND DEVELOPMENT OF FLOWERS WITH IAA, EDDHA AND KINETIN

Higher Agronomical School, University of Maribor

As experimental plants, duckweeds (*Lemnaceae*) from Yugoslav area are used. Plants are grown axenically in modified Pirson and Seidel nutrient solution, at 3000 lx illumination, the temperature constant  $28 \pm 1^\circ\text{C}$  day and night. Under given conditions, the experiments gave following results:

In long-day, long-short-day-, and day-neutral- *Lemnaceae* clones,  $50 \mu\text{g}/1$  IAA (= indole-3-acetic acid) under photoinductive conditions, has no significant effect, or an enhancing effect on flowering induction; in higher concentrations ( $100 - 500 \mu\text{g}/1$ ), it is inhibitory and in concentration  $1000 \mu\text{g}/1$ , it completely suppresses the induction. The flowering stimulator EDDHA (=ethylene-diamine-di-o-hydrohyphaenyl-acetic acid), even if added simultaneously with IAA, is unable to annihilate the inhibitory effect of IAA. If, on the other hand, kinetin is added, the inhibitory effect of the IAA is completely blocked. Moreover, the  $50 \mu\text{g}/1$  kinetin and  $50 \mu\text{g}/1$  IAA combination significantly promotes the flowering induction in day-neutral clones of the species *Spirodela polyrrhiza* under long-day or short-day conditions, and in long-short-day clones of the species *Wolffia arrhiza* under photoinductive conditions.

In research of morphogenesis of flowers after the IAA added, in the species *Lemna minor* it has been found:

The IAA in lower concentration has no significant effect on flowering, while in concentration  $100 \mu\text{g}/1$ , it diminishes the percentage of flower primordia, delays the time of their appearance, and promotes elongation of filaments of stamina and pistil-styli.

KRAJNČIČ, B.

## HABITATS AND PHOTOPERIODICAL RESPONSES OF THE LEMNACEAE IN THE AREA OF ISTRIA

Higher Agronomical School, University in Maribor

In the period 1981–84, in the area of Istria, following 26 not yet reported localities of the *Lemnaceae* were discovered:

1) Nine localities of the species *Wolffia arrhiza*, in places: Vižinada, Markovići, near Vižinada, Višnjan, Flengi, Kloštar, Radmani, Ladrovići, Bonaci, Prhati;

2) Seventeen localities of the species *Lamna gibba*, in places: Vižinada, Markovići, Višnjan, Flengi, Kloštar, Radmani, Krušvari, Kukurini, Balabani, Tinjan, Ladrovići, Bonaci, Prhati, Praščari, Klapčiči, and additional two places in the village Šumber.

In the course of field research Yugoslavia in September and October of four subsequent years (1981–84), flowering plants of the species *Wolffia arrhiza* have been found for the first time in water-ponds in places: Višnjan, Kloštar, and Vižinada. In contrast, no flowering plants of the species *W. arrhiza* have been found in the same localities in April, May, June and July, but only flowering plants of the species *L. gibba* in June and July.

The finding mentioned above is important because of two reasons:

a) because it corroborates results which could have only be obtained in lab experiments up to now, showing that the species *Wolffia arrhiza* is a long-short-day plant (Krajnčič and Devide: Ber.Geobot.Inst.ETH, Stiftung Rubel, Zurich, 47, 75–86, 1980),

b) because the localities with flowering plants of the species *Wolffia arrhiza* in places Markovići, Vižinada, and Višnjan, according to data in literature, are the first such places found in Yugoslavia, and except the locality near the Caucasus in the USSR they are the only places of this plant with flowers formed in Europe (Kandeler, in: Hegi, Illustrierte Flora von Mitteleuropa, II., 1,346, P. Parey, Berlin–Hamburg, 1979).

HACIN, J.

## APICAL MORPHOLOGY IN THE HOP (*HUMULUS LUPULUS* L.) DURING FLOWER INITIATION AND FLOWER DEVELOPMENT

Institut za hmeljarstvo in pivovarstvo, Žalec

The morphology of the apical and lateral meristems in the hop plants grown in the field was studied throughout the season using dissecting and SEM technique. One early (=Saxon) and one late (=Challenger) cultivar was chosen for the study and for each cultivar early and late training of the bines was applied.

Individual stages of flower initiation and subsequent flower development on these treatments were identified and approximate time of their occurrence determined. It was shown, that although flower initiation in the early trained Chalanger (=late cv.) took place at approximately the same time as in early trained Saxon (=early cv.) – (between 16 and 23 May), the progress towards flowering was much slower and individual stages of subsequent development occurred about two weeks later when compared to Saxon. Conversely, in the late trained plants, developing flowers in decreasing daylength, initiation and most of the subsequent stages up to flowering occurred in Challenger 4–7 days earlier than in Saxon. Furthermore, the period from initiation to visible flowers in

both late trained cultivars was about 2,5 weeks shorter in comparison with early training. The height of the plants and/or the number of visible nodes appeared to be the determinant factors for flower initiation. Thus, differences in dynamics of flower development on different treatments suggest, that it is the subsequent flower development not the flower initiation which is a response of a cultivar to a daylength. A possible effect of the dynamics of flower development on the number of inflorescences (=cones) produced per plant was also observed.

MANČIĆ, A., VULETIĆ, D.

### ROOTING GREEN CUTTING OF ASPEN UNDER MIST

Institute of Forestry and Wood Industry, Belgrade

Aspen becomes a more and more important species in forest production due to its properties: fast growth, large increment, modest site requirements and others.

For successful establishment of plantations it is necessary to produce vegetatively propagated clonal nursery stock of verified and uniform properties in respect to its height, diameter growth and wood quality, as well as resistance to diseases.

However, aspen is one of hard-to-root species. For production of clonal nursery stock, the most difficult problem is the determination of appropriate method of vegetative propagation.

Since the first attempts on rooting aspen cuttings, carried out by S.Jovanović, 1961. and L.Žufa, 1965. technological progress has brought about new methods of vegetative propagation. One of methods which becomes indispensable in forest tree nursery production is the mist propagation of cuttings.

We have used mist system for rooting of green cuttings of aspen during summer. Percentage of rooted cuttings varied from treatment to treatment, but was never below 10%, while the highest was 50%. Many cuttings formed callus but produced no roots. Although rooting percentage is not sufficient for commercial production, this method is going to be further improved by addition of soil warming cables, different rooting media, growth-promoting substances and other relevant components.

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

### GROWTH INDUCTION OF ADVENTITIOUS ROOTS IN LILAC AND MAGNOLIA SPECIES

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\*\*Department of Forest Genetics and Dendrology,  
Faculty of Forestry, University of Zagreb, Yugoslavia

The propagation of lilacs and magnolias from green cuttings has not been employed in horticultural practice so far, in spite of doubtless advantages of plants produced by this method. In the Department of Botany-Botanical Garden, Faculty of

Science, Zagreb, experiments were carried out in order to develop a simple, fast and efficient method for propagation of lilacs and magnolias from green cuttings. Similar studies were also conducted by Dr. W.D. Christie (1975), by Dr. G. Schmidt (1978) and by Dr. K. Bojarczuk from Institute of Dendrology in Kornik, Poland (1980).

In our experiments the effect of IBA (0.3% w/w-indolylbutric acid) and NAA (0.25% w/w-naphthylacetic acid + 3+ Captan) on the formation of callus and adventitious roots in lilacs (*Syringa vulgaris* L., *Syringa reflexa* Schneid.) and magnolias (*Magnolia stellata* Maxim., *Magnolia liliflora* Desr. 'Nigra') were investigated (1983). At the same time the anatomy and its changes were carefully observed.

From the results it was seen that both growth substances stimulated cambial activity and callus formation. However, adventitious roots were developed only in cuttings with discontinuous sclerenchyma layer. In our experiments as well as in the studies of other authors (Beakbane 1961 Kolečka—Pletikapić 1973, Hartmann and Kester 1975) a very high correlation was observed between rootability and anatomical structure of the primary phloem of the stem. Viewed as a whole, the rooting, according to our investigations for *Syringa vulgaris* and *S. reflexa* was respectively 85.00% and 97.00% in total. From the cuttings treated with IBA we obtained 76.92% of rooted cuttings, while those treated with NAA gave a result of 63.64% of rooted cuttings. The difference between the two substances used (13.28%) in the rooting process proved to be insignificant in the case of *S. vulgaris*. For *S. reflexa* from cuttings treated with IBA we obtained 96.36% of rooted cuttings, while those treated with NAA resulted in 67.69% of rooted cuttings. This difference of 28.67% in the rooting process proved to be statistically significant at the level of 0.01%.

For *Magnolia stellata* and *M. liliflora* 'Nigra'—regarded as a whole, the rooting was respectively 83.00% and 75.00% in total. However, from *M. stellata* cuttings treated with IBA we obtained 70.77% of rooted cuttings, while those treated with NAA gave a result of 61.67% of rooted cuttings. The difference between the two substances used (9.10%) in the rooting process proved to be insignificant.

From *M. liliflora* 'Nigra' cuttings treated with IBA we obtained 84.00% of rooted cuttings, while those treated with NAA yielded in 66.00% of rooted cuttings. This difference of 18.00% in the rooting process proved to be statistically significant at the level of 0.05%.

VELIČKOVIĆ, M., JOVANOVIĆ, M.

#### THE INFLUENCE OF INDOLE-3 BUTYRIC ACID (IBA) ON THE ROOTING OF APPLE CUTTINGS OF VEGETATIVE ROOT-STOCK MM 106

Faculty of Agriculture, Institute of Horticulture, Zemun – Beograd

Indole-3 butyric acid (IBA) had a stimulating influence on the rhizogenetic properties of the MM106 vegetative rootstock cuttings.

At an IBA concentration of 2500 g. a particularly high influence on the rooting of mature cuttings was demonstrated.

With the IBA concentration mentioned the rooting of the cuttings rose up to 94 percent.

The rooted cuttings were categorized according to the number and the length of the roots into 3 qualitative categories: poorly rooted cuttings, medium rooted cuttings

and well rooted cuttings. Correspondingly the number of the cuttings was identified.

The rooting of mature, healthy and according to thickness equalized cuttings took 45 days, under controlled temperature of  $21 \pm 0,1^{\circ}\text{C}$  (warm storage). The substrate for rooting was a homogenized and sterilized mixture of peat, quartz and perlit (50:30:20).

DUBRAVEC, K.

### THE EFFECT OF "CULTAR" ON APPLE LEAVES TRANSPIRATION

Faculty of Agriculture, University of Zagreb

The paper treats the effect of "Cultar" on apple leaves transpiration of Granny Smith and Melrose varieties.

Obtained results suggest the following conclusion:

1. Value of transpiration ( $\text{Tr}$ ) varied in both varieties during the day and vegetation season.

2. The highest mean transpiration value is obtained in July in the phasis of intensive fruit growth and the lowest in September in the phasis of fruit ripening for both varieties on treated as well as on untreated trees.

2. Leaves transpiration intensity of untreated and treated trees was a little bit higher in Granny Smith than in Melrose, what could possibly be a varietal characteristic.

## CELL AND TISSUE CULTURE, *IN VITRO* VEGETATIVE PROPAGATION

VAPA, Lj., and STANKOVIĆ, Ž.

### ISOLATION OF PROTOPLASTS FROM DIFFERENT PLANT SPECIES

Institut of Biology, Faculty of Sciences, Novi Sad

In the available literature numerous methods concerning protoplast isolation from different plant species have been described. In this work procedure for the isolation of protoplasts from the young leaves of wheat, barley, peas, soybean, sunflower and tobacco plants and further cultivation of protoplasts is presented. Protoplasts were isolated using mixture of cellulase and pectinase as suggested by method of Leegood *et al.* (1982) with our slight modifications.

Purity and quality of protoplasts was checked by light and phase contrast microscopes. The yield of isolated intact protoplasts was estimated by their counting using a hemocytometer.

Isolated tobacco protoplasts were further cultivated in liquid and solid MS nutrient medium (Murashige and Skoog, 1962) supplemented with different amounts of auxins and cytokinins. Protoplasts were viable in both nutrient media. Cell divisions as well as spontaneous protoplast fusions were observed. Our future studies will be concentrated on callus induction and plant regeneration from protoplasts.

JELENČIĆ, B., and KOLEVSKA-PLETIKAPIĆ, B.

### CAULOGENESIS IN POPLAR LEAF CULTURE

Faculty of Science, University of Zagreb

Caulogenesis induced *in vitro* by five Leuce-poplar genotypes has been comparatively investigated.

The *in vitro* grown leaves of hybrid species with labels 25 (*P. tremula* x *P. tremuloides*), 53 (*P. alba* x *P. grandidentata*) x (*P. tremula* x *P. tremuloides*), 51 (*P. alba* x *P. grandidentata*) x (*P. alba* x *P. grandidentata*) and 20 (*P. alba*) were used in the study. They were planted on an ACM medium supplemented with 20 mg l<sup>-1</sup> adenine sulfate, 80 mg l<sup>-1</sup> lysine and 100 mg l<sup>-1</sup> myo-inozitol and with 2,3 μM BA and 0,18 μM NAA. The leaves grew on this medium for six weeks, during which time some changes were observed.

Caulogenesis was induced of leaf bases of all the investigated genotypes in the given culture conditions. However, the frequency of induction was very different. The



highest number of shoots per leaf was formed by the genotype with the label 25, a much smaller one by genotypes labelled 53 and 51 and an almost insignificant one by genotype 20. This genotype, on the contrary, formed callus islets either on the leaves or on their borders.

According to this, it would seem that the ability of caulogenesis induction on leaf bases is a characteristic that genotypes produced by the crossing of species *P. tremula* and *P. tremuloides* possess in the highest possible degree. In genotypes which are products of crossing of these two species with the species *P. alba* and *P. grandidentata* this characteristic gradually dies out, while it has almost completely disappeared in the species *P. alba*.

BERLJAK, J., JELASKA, S., PAPEŠ, D., and JUREČIĆ, R.

### REGENERATION AND GENETIC STABILITY OF ADVENTITIOUS SHOOTS IN POTATO CALLUS CULTURE *SOLANUM TUBEROSUM* L. cv. "BINTJE"

Department of Botany, Faculty of Science, University of Zagreb

Regeneration of adventitious buds and shoots in callus tissues has been achieved in explants of tuber tissue culture ( $\phi$  0.6 mm) and internodal segments of juvenile plant cultivated *in vitro*. Callus tissue formation has been obtained in high percentage in both types of explants on the majority of the tested combinations of media. Optimal conditions for adventitious bud regeneration in tuber tissues and stem tissues were different. Optimal regeneration (25%) responsible cultures in tuber callus culture was achieved on agar medium consisting of the MS inorganic and organic constituents (Murashige & Skoog 1962) and 3% sucrose supplemented with ( $\mu$ M): 13.3 BA, 0.05 NAA, 5.7 IAA, 1.4 GA<sub>3</sub> and 1.4 2,4-D. Adventitious buds generated in internodal segment callus on the basal MS medium containing 5% sucrose, 0.8% agar and ( $\mu$ M): 4.4 BA, 5.7 IAA, 28.9 GA<sub>3</sub> either with or without addition of 0.05 mg-l<sup>-1</sup> D-biotine and 0.5 mg-l<sup>-1</sup> folic acid in 27 and 33% cultures respectively. These media, however, were not inducible for tuber tissues.

Obtained results show that higher concentrations of auxin and cytokinin are necessary for the regeneration in tuber callus culture. Photoperiod 16/18 h and temperature +26°C were suitable for shoot induction in the internodal segment culture, whereas short-day conditions (12/12 h; +20°C) were best for the shoot induction in tuber callus cultures. Bud formation occurred after 8–20 weeks of culture. Shoot number per culture was 10–300. Adventitious shoots easily developed into rooted plants after being subcultured on a MS medium lacking in hormones. They were cloned routinely by axillary shoots.

Adventitious shoots differed according to the growth rate, morphological polymorphism and chromosome complement changes. Cytological analysis of 51 regenerated plants (code "14") has revealed that approximately 66% plants had different chromosome number (44–50) compared to the control plants ( $2n=4x=48$ ). Different chromosome number in regenerated plants suggests that chromosome elimination (1–3) or chromosome duplication (1–2) occurred quite frequently in callus tissue. This, however, did not inhibit regeneration.

SIMOVIĆ, N.

**CALLUS INDUCTION ON ALFALFA STEM EXPLANTS (*MEDICAGO SATIVA* L.)**

Faculty of Agriculture, Zemun

10 mm long stem explants with nodes were used to induce callus. Explants were cultured on basal medium (BM) + 5 mg/l 2,4-D + 0,1 mg/l kinetin. After seven days they were transferred to BM +  $10^{-6}$ M IAA +  $10^{-5}$ M kinetin where they stayed for 20 days. Mineral salt solution was prepared according to Gamborg, pH=5,8. Cultures were maintained at room temperature ( $t = 20-25^{\circ}\text{C}$ ) and day light. Samples for histological study were prepared by the paraffin method.

A friable white callus with dark green areas was formed on stem explants. Microscopic analyses have shown the appearance of vacuolated cells and meristemoids groups representing the shoot initials. Multi-cellular structures—embryoids were also observed. As this is not a usual event in callus culture, it is important as an easier way for regeneration of adult alfalfa plants.

RAVNIKAR, M.,\* GOGALA, N.,\*\* ŽEL, J\*

**TISSUE CULTURE OF TOMATO (*LYCOPERSICON ESCULENTUM* Cv. Fl, MI 13)**

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\*\*VDO Biotechnical Faculty, VTO Biology, Ljubljana

Tissue culture technique of tomato (*Lycopersicon esculentum* cv. Fl and MI 13) was introduced and studied in our laboratory. Two methods of antiviral treatment of plants were modified and tested:

– heat treatment of plants in connection with isolation of apical meristems on Omura et al. (1978) medium.

– gradual cultivation of avirous stem callus, for some plant viruses which are resistant to high temperature that already may damage plants.

Both methods were effective for elimination of viruses. During organogenesis it became evident that the method of heat treatment has advantages in a low possibility for genetic defects, since the regeneration starts immediately after the isolation of apical meristem. Organogenesis is easy to obtain, as long as young tissue is formed. A caulogenesis in tip meristem was induced, but organogenesis of old calluses failed. Only green meristemoids were obtained.

For a micropropagation of tomato plants very good results were obtained by initiation of buds and roots on the stem and leaf cuttings.

Ten to fifteen buds on stem cuttings were initiated on Cassell's (1977) medium. Then they were transferred in sterile venniculite with MS medium without hormones.

From the leaves on Kartha et al. (1976) medium, ten to twenty buds were initiated. They made the roots on the same medium.

These two methods proved to be simple and efficient for micropropagation of healthy plants, obtained by tissue culture.

Growth and development of the flower buds was also observed and development of parthenocarpic fruits was initiated.

SREJOVIĆ, V., and NEŠKOVIĆ, M.

**FACTORS AFFECTING FLOWERING OF BUCKWHEAT  
(*FAGOPYRUM ESCULENTUM* MOENCH IN *IN VITRO* CULTURE**

Institute of Biology, Faculty of Science, Kragujevac and  
Institute of Botany and Institute for Biological Research  
"S. Stanković", University of Beograd

Most buckwheat varieties have a high flowering potential; a single plant may bear 1500–2000 flowers. This potential is also evident in cultured buckwheat shoots. Shoots were regenerated in callus tissue culture, derived from cotyledon fragments of the tetraploid variety Pennquad. Optimum medium for bud regeneration (Gamborg B5, supplemented with 3% sucrose, vitamins,  $10^{-5}$ M BAP and  $10^{-6}$ M IAA) permitted the maintenance of organogenic culture for more than 3 years, but the shoots never flowered and rarely produced roots. Flowering in about 75% of shoots may be induced by decreasing the amount of hormones in the medium. Different combinations of auxins and cytokinins (IAA+BAP, 2,4-D+kin, IBA) produced similar responses. In about 50% of shoots flowering was preceded by rooting. The percentage of flowering can be further increased by omitting nitrate from the medium. However, shoots on nitrate-free medium remained short and usually did not produce roots.

KNEŽEVIĆ, T. and NIKOLIĆ, Đ.

**MAIZE IMMATURE EMBRYO AND PROTOPLAST CULTURE**

Maize Research Institute, Zemun Polje

The need for an increase in maize yield demands modifications of the genetic structure of the maize plant. Biotechnology (tissue culture and genetic modification of plants through genetic engineering) together with classical breeding is expected to speed up this process. Therefore, maize tissue culture is of significance as a method which can contribute to the improvement of maize quality and yield.

The number of genotypes which respond by developing embryogenic callus in culture is on the increase. According to the latest data the addition of 12 mM L-proline to the induction medium promotes the appearance of embryogenic callus of many maize genotypes. Although the role of L-proline is still unexplained, its presence in the medium induces the development of a greater number of maize plants from tissue culture. Further cytological studies are necessary in order to confirm the embryogenic character of the callus in culture.

Since, at this point in maize tissue culture, a fine cell suspension (except for Zm suspension) cannot be obtained we tried to obtain protoplast from maize callus tissue. „Embryogenic” calus from 11 different hybrids was used for obtaining protoplasts. Different concentrations of digestive enzymes were used to see which combinations yields the largest number of protoplasts with different time treatments. After enzyme treatment, the suspension is filtered through A 105  $\mu$  mesh and rinsed with protoplast wash three times. After isolating protoplasts, they were grown in osmoticum suspension in micro-chambers over pre-conditioned agar medium (induction medium which was used

for callus growth). A cell wall forms after 3–4 days after which the cells are transferred to fresh suspension medium without osmoticum: the cells have been maintained in suspension for 8 days, however no cell division occurs.

ŠESEK, S., BOROJEVIĆ, K.\* and RADOJEVIĆ, Lj.\*\*

### CALLUS FORMATION AND PLANT REGENERATION IN ANTHER CULTURE OF WHEAT (*TRITICUM AESTIVUM* L.)

Institute of Field and Vegetable Crops, Faculty of Agriculture, Novi Sad,\*

Institute of Biology, Faculty of Sciences, Novi Sad and

\*\*Institute for Biological Research „Siniša Stanković”, Belgrade

Anthers, 0.8–2.0 mm long, with uninuclear microspores, were isolated from 19 genetically diverse wheat genotypes and cultivated in four induction media  $A_1$  –  $A_4$ . After inoculation, the anthers were subjected to a temperature pretreatment, as described by Schaeffer et al. (1979) and cultivated under controlled conditions approximately for 90 days. The induction media contained MS mineral solution (Murashige and Skoog, 1962), 4.5–5% sucrose, 0.7% agar, 10% potato extract and varying concentrations of vitamins, auxins and kinetin.

In the media  $A_2$  and  $A_1$ , the percent of anthers of the genotype „NS–58–97 x Aurora) x Nizija” which formed an androgenous callus were 24.5% and 0.3% respectively, indicating that callus formation was closely connected to the composition of the medium.

The effect of genotype on androgenesis of microspores was even more pronounced. The anthers of some genotypes failed to form calluses, while the percent of callus formation of certain genotypes exceeded 20% in the same medium. In genotype „Veery-4x Neretva”, 43.5% of the anthers formed androgenous calluses in medium  $A_3$ .

Successive cultivation of the calluses in the course of 4 passages in media  $B_1$ ,  $B_2$  and  $B_3$  containing increased concentrations of cytokinins and reduced amount of 2.4–D retarded development of the calluses, even causing necrosis of callus tissue in some genotypes. Only a limited number of genotypes formed rhizogenous calluses. The calluses of the genotype „Veery–4 x Novosadska jara” cultivated for a short interval in the medium  $B_3$ , regenerated four albino plants with haploid chromosome number.

RENGEL, Z.

### INFLUENCE OF BENZYLAMINOPURINE ON INDUCTION OF BARLEY SOMATIC EMBRYOGENESIS

Department of Biology, Faculty of Science,\* Zagreb

Application of cell culture to plant improvement will depend on the regeneration of a large number of genetical uniform plants. Although cereals have not been easy species for *in vitro* culture, a large-scale plant regeneration has recently been achieved through somatic embryogenesis.

\*. Present address: Agricultural Faculty, Zagreb

In the experiments presented here induction of embryogenic callus has been investigated in the somatic cell culture of four winter barley cultivars (*Hordeum vulgare* L.). The explants (about 2 mm long), consisting of scutellum and apical meristem covered with basal parts of the youngest two leaves and coleoptile, were excised from 3-5-day-old seedlings. A yellowish, soft and watery callus tissue (characterised as NE) was induced on explants cultured on the modified MS medium supplemented with different concentrations of 2,4-D or 2,4,5-T (7.8, 19.5, 31.3  $\mu\text{mol/l}$ ) either with or without 2.2 or 4.4  $\mu\text{mol/l}$  of BA respectively.

During the subculturing of NE callus tissue on the media supplemented with the same combinations of growth regulators as in the primary culture or on different ones two processes could be observed: the browning and necrosis of NE callus tissue as well as, the induction of white, opaque, hart and nodular callus tissue (designated as E). Both processes significantly ( $P$  0.01 to 0.05) depended on the addition of BA into medium, especially in combinations with 2,4,5-T.

Total of 832 green shoots and plants developed by precocious germination from embryoids during six subcultures. The best regeneration medium was MS supplemented with 3  $\mu\text{mol/l}$  TIBA.

After a vigorous root system had been established, plants were transferred into soil and their development towards maturity was in progress.

It should be emphasized that cereal embryogenic tissue can be a good source for the isolation of protoplasts from which plants are regenerated. From this point the possibility of manipulation at the level of a single cereal cell offers unthought-of opportunities.

JURETIĆ, B.

### PLANT DEVELOPMENT FROM EMBRYOIDS OF LONG-TERM PUMPKIN CELL CULTURES

Faculty of Science, Zagreb

Somatic embryoids develop either directly from explanted tissue cells (direct somatic embryogenesis) or indirectly, from embryogenic callus cultures after the proliferation of dedifferentiated explanted cells (indirect somatic embryogenesis). Somatic embryoids have been induced in many plant species cultures, but they were rarely capable on normal development and maturation. Pumpkin embryogenic callus lines behave in a similar way: they continually induce embryoids that rarely develop into plants spontaneously. We tested several different treatments which we thought could make it possible to develop plants in a bigger percentage. This experiment used the following lines of pumpkin callus tissue;  $\check{Z}_5$ b (grows with addition of IBA), MSS (with addition of IAA) and  $\check{Z}_5$ b<sub>0</sub> (habituated tissue, grows without auxins). Tissue and individually isolated embryoids in the cotyledone stage were cultivated (3-4 subcultures) on the medium MS with addition of: a) 0 hormones, b) 2.9  $\mu\text{M}$  IAA, c) 5.7  $\mu\text{M}$  IAA, d) 11.4  $\mu\text{M}$  IAA, e) 12  $\mu\text{M}$  TOH, f) 2% active charcoal and g) 3.8  $\mu\text{M}$  ABA. The results show that: a) cell clone influence is present ( $\check{Z}_5$ b line reacted best and gave 52% normally developed plants), b) the biggest percentage of plant development was on the MS medium with addition of 11.4  $\mu\text{M}$  IAA (77%), and on the medium with 2% active charcoal (68%). Double concentration of Na<sub>2</sub>FeEDTA in medium - especially in final stages - reduced the appearance of the vitrified plants and had a favourable effect on chloroplast

development. Induced plants were cloned by axillary bud culture. First electrophoretic analyses of soluble proteins showed that plants corresponded in great proportion. On the basis of these results it may be concluded that pumpkin callus lines have retained their ability to induce embryoids even after 15 years of cultivation and that, after an adequate treatment (changes of medium components), they developed into normal plants.

PETROVIĆ, J. and KIDRIĆ, M.\*

**PHYSIOLOGICAL AND BIOCHEMICAL STUDIES OF ANDROGENESIS  
OF THE HORSECHESTNUT  
(*AESCULUS HIPPOCASTANUM*)**

Institute for Biological Research „Siniša Stanković“, Belgrade, and  
\*Institute of Chemistry, Faculty of Sciences, University of Belgrade

Comparative studies of green and albino embryos originated from anther culture of horsechestnut were performed, as albinism was found to be a constant phenomenon in horsechestnut anther cultures. Electron microscopy revealed clear differences in cell and plastid structures of albino embryos in comparison with green embryos. Biochemical investigations showed that the rate of biosynthesis of total and partially fractionated proteins of albino embryos differed from that of the green embryos, as judged by the extent of incorporation of labelled amino acid mixture. Electrophoretic analyses of proteins of polyacrylamide gels, confirmed these findings. Abundance of individual nucleic acid classes, separated by MAK column chromatography was different in samples isolated from albino embryos and those obtained from green ones. These differences were especially significant in regard to DNA, which was analyzed by determination of  $T_m$  and thermal denaturation in solution. Differences in both  $T_m$  and the shape of derivative thermal denaturation curves the latter being polyphasic suggest different genome organization of albino and green embryos.

ČULAFIĆ, Lj., NEŠKOVIĆ, M.

**REGENERATION OF *RUMEX ACETOSELLA* L. PLANTLETS  
IN VITRO VIA THE INDUCTION OF SOMATIC EMBRYOGENESIS**

Institute of Botany, Faculty of Science and Institute for  
Biological Research „Siniša Stanković“, Beograd

Somatic embryogenesis in many callus tissue cultures can be significantly fostered by sequential changes of hormonal and nutritive components of the medium (Ammirato, 1984). In apical meristem-derived tissue of *R. acetosella*, which has been maintained for micropropagation on solid MS medium, supplemented with vitamins, 2% sucrose,  $10^{-5}$  M BAP and  $10^{-6}$  M IAA, a very high capacity for bud regeneration was shown. Transfer of this tissue to media with higher (4% or 6%) sucrose concentration, changed its differentiation pattern, such that somatic embryos were developed rather than buds. The embryo development through heart and torpedo stages to young plantlets was markedly stimulated by  $GA_3$  (1 or 10 mg  $l^{-1}$ ), added to the medium 14 days after induction.

Liberation of a great number of plantlets from callus culture was obtained by omitting auxin and cytokinin, and transferring to a liquid medium with  $GA_3$ . The plantlets supported very well the transfer to vermiculite and continued growing under greenhouse conditions.

Ammirato, P.V. (1984) Embryogenesis, in Handbook of plant cell culture, Evans, D.A., Sharp, W.R., Ammirato, P.V., Yamada, Y.(Eds), Macmillan, Vol.1., pp.82-123.

BUDIMIR, S., ČULAFIĆ, Lj., VUJIČIĆ, R., NEŠKOVIĆ, M.

### ANATOMY OF EMBRYOGENIC CALLUS TISSUE OF *RUMEX ACETOSELLA* L.

Institute of Botany and Institute for Biological  
Research „Siniša Stanković“, Belgrade

Somatic cells of different plant tissues and organs were induced to somatic embryogenesis *in vitro*, when cultured on media which contain specific hormonal composition.

Anatomical study demonstrated that embryoids develop either directly on vegetative organs, or through formation of intermediary callus tissue which has an embryogenic potential.

When a detached leaf of *Rumex acetosella* was grown on mineral solution MS, 6% sacharose, BAP  $10^{-5}M$  and IAA  $10^{-6}M$ , the cell proliferation and callus tissue was observed on the rim of a basal part of the leaf blade. The callus tissue was embryogenic and 8 days after the induction, embryoids of torpedo stage could be observed. When callus tissue was sectioned, younger stages of embryo development (globular and heart) were found. By means of serial sectioning further embryo development and successive stages were followed.

Further maturation of embryoids and their release from callus was stimulated when the culture was transferred to liquid medium which contained gibberellic acid ( $GA_3$   $10\text{ mg l}^{-1}$ ) instead of cytokinin and auxin. After 30 days, as a result of somatic embryogenesis, formed plantlets were released into the medium.

RADOJEVIĆ, Lj.

### SOMATIC EMBRYOGENESIS IN THE CALLUS TISSUE OF THE CORN "CUDU"

Institute for Biological Research „Siniša Stanković“, Belgrade

According to the data from the available literature, there are only three corn genotypes which can regenerate plants from the callus by somatic embryogenesis. Both genotype and maturity of the embryo are very important factors for the embryogenic callus formation.

The aim of our studies was to determine genotype, maturity of the embryo and composition of the nutrient medium which would be the most suitable for the induction of the embryogenic callus. Embryos of four corn genotypes were isolated on the 21st and 30th day after the pollination and transferred into MS (Murashige & Skoog, 1962)

nutrient medium containing vitamins, sucrose, agar and growth promoting substances (in mg l<sup>-1</sup>: 5 - 10 2,4-D and 0 - 0.5 kinetin). Only in genotype „Cudu”, depending of hormone concentration and embryo maturity, 70-100% of the embryos formed embryogenic callus which regenerated corn plants through somatic embryos. The entire process from the induction of embryogenic callus to regeneration of plants through somatic embryos lasted for about three weeks.

*In vitro* regenerated corn plants „Cudu” were the identical to the donor plants, as judged by the morphology of the root, leaves and flowers.

JELASKA, S.

### REGENERATION IN CULTURE OF INCENCE-CEDAR (*CALOCEDRUS DECURRENS* (TORR. FLORIN)

Faculty of Science, University of Zagreb

Adventitious and axillary bud formation in incence-cedar embryo and seedling cultures was induced in different ways. Non callus-mediated adventitious bud induction was obtained from cotyledon of embryos or 14-day-old germinants on an agar medium WPM (Woody Plant Medium, developed by McCown and Lloyd) in the presence of 7  $\mu$ M bzl<sup>6</sup>Ade.

Flattened cotyledons on agar medium, their connection with an apical meristem and culturing on the induction medium for more than 3 weeks led to a higher percentage of inducible explants and a higher number of buds per culture. In this state regeneration ratio (average number of shoots: seed) was 8:1.

Development of axillary shoots was induced on 6-8 week-old seedling explants and on *in vitro* generated shoots given a 3 h pulse treatment with high-concentration (125  $\mu$ M) bzl<sup>6</sup>Ade, cultured on the WPM medium, supplemented with 0.025  $\mu$ M NAA. Regeneration ratio was 15:1.

Elongation growth was achieved through the subculturing of single shoots on WPM with the addition of a reduced concentration of sucrose (58.4 mM), *myo*-inositol (55  $\mu$ M) and 0.005-0.054  $\mu$ M NAA during 2-3 subcultures, every of 21 days.

The rooting treatment consisted of the culturing of adventitious shoots during 10 days on the GD medium (Gresshoff and Doy 1972) containing 9.8  $\mu$ M IBA with 2.7  $\mu$ M NAA. Shoots were then transferred to a sterile peat-sand-soil (1:1:1) mixture. In culture-room conditions (24°C, 16 h light, under a plastic cover) about 30% of shoots rooted after 6-8 weeks.

VASILJEVIĆ, Lj. and ŠKORIĆ, D.

### USING EMBRYO CULTURE IN SUNFLOWER BREEDING

Institute of Field and Vegetable Crops Novi Sad

With genetically diverse species which may be hybridized, between species and genera, embryos tend to perish due to underdeveloped endosperm.

Embryo culture may successfully be used in sunflower breeding programs to overcome some of the problems encountered.



In this study we set the following objectives: 1) to examine possibilities of obtaining plants of good inbreds and hybrids by embryo culture, and 2) to examine possibilities of producing one extra generation in the course of a growing season.

Three to seven days after pollination, embryos were placed in MS medium (1962) containing 3% sucrose, 0.7% agar, IAA  $1 \text{ mg l}^{-1}$  BAP  $0.5 \text{ mg l}^{-1}$ , and casein hydrolysate.

Having been grown *in vitro* for two weeks, the plants were fit for acclimatization in suitable conditions regarding humidity, temperature, and light. The plants were then transplanted to beds in a greenhouse. The percent of survival was quite satisfactory and plant development and seed forming were normal.

ŽEL, J.,\* GOGOLA, N.,\*\* CAMLOH, M.\*

### FORMATION OF AXILLARY BUDS IN TISSUE CULTURE OF *PINUS SYLVESTRIS* AND THE INFLUENCE OF MYCORRHIZAL FUNGAL METABOLITES ON THEIR DEVELOPMENT

\*Institut za biologijo Univerze v Ljubljani

\*\* VDO za Biologijo Biotehniške fakultete, Ljubljana

The influence of mycorrhiza on the growth and development of scots pine was studied by investigation of the effects of metabolites from mycorrhizal fungus on the tissue culture of *Pinus sylvestris*.

It was found that the best initiation of axillary buds at the seedlings apex takes place on 1/4 Murashige-Skoog medium (modif. by Cheng, 1975). We examined the influence of various hormone concentrations on the initiation of axillary buds in seedlings of different age. The most suitable concentration of BAP was  $10^{-6} \text{ M}$  and  $5 \cdot 10^{-6} \text{ M}$  and under this condition the number of axillary buds varied between 1 to 6. The buds were separated from the mother plant and elongated on the hormone-free medium. Some axillary buds formed spontaneously new axillary buds, what gives a possibility for a continuous micropropagation.

We expect main effects of mycorrhizal fungi on the elongation and rooting of *Pinus sylvestris* in culture. Nevertheless we examined the influence of their metabolites on the initiation of the axillary buds. Fungi were cultivated in a liquid medium (after Shemakhanova, 1960). After a removal of mycelium, the liquid medium with metabolites was added to the medium for the initiation of axillary buds. In some experiments we soaked the apex of seedlings in such liquid medium for various period of time. We found out that there is some but not very pronounced effect on bud formation.

NIKOLIĆ, R.,\* NEŠKOVIĆ, M.,\*\* SPASIĆ, M.\* and MILJIĆ, S.\*

### TISSUE CULTURE AND *IN VITRO* PLANT REGENERATION OF ALFALFA (*MEDICAGO SATIVA* L.)

\*Agricultural Station Zaječar,

\*\*Institute for Biological Research „Siniša Stanković”, Beograd

Alfalfa tissue culture was obtained from the new variety Zaječarska 83, selected for its high yield and abundant seed production. The purpose of the work was to study

the performance of Zaječarska 83 in culture, in order to apply tissue culture methods in further breeding. Explants were isolated from different parts of germinated seeds; callus induction, bud formation and shoot elongation and rooting were obtained by a sequence of media with different hormone content, as described for other alfalfa varieties (Saunders and Bingham, 1972). Zaječarska 83 responds well to the treatments several months after isolation, but its regeneration capacity declines afterwards.

The rooted plantlets were transferred into a greenhouse and then planted in the field. They grew vigorously in natural environment, and developed large and healthy green foliage. Within 5 months, they produced 450–500 seeds per plant. The seeds had high percentage of germination. It is concluded that tissue culture methods are applicable to the new variety Zaječarska 83.

SPASENOSKI, M.

### REGENERATION OF PLANTS FROM APICAL BUDS OF *ARACHIS HYPOGAEA* L. GROWN IN VITRO

Institute of Biology, Faculty of Science, Skopje

Stem tips up to 1–2 mm long, were isolated from apical buds on aseptically grown seedlings, then cultivated on MS (Murashige and Skoog, 1962) mineral solution, 3% sucrose, 0,7% agar and the hormones (0,5 mg l<sup>-1</sup> IAA and 1 mg l<sup>-1</sup> kinetin). After 30 days explants were subcultured on the same medium, where bud multiplication was obtained. In subsequent subcultures the IAA concentration was decreased to 0.1 mg l<sup>-1</sup> which improved branching. The best results in shoot elongation were obtained when shoots were left longer than 30 days on same medium.

Shoots of *Arachis hypogaea* gave 95% rooting when they were cultivated on MS mineral solution with 2 mg l<sup>-1</sup> IAA and 0,1 mg l<sup>-1</sup> kinetin.

The rooted plants were transferred into plastic pots, in a mixture of peat, sand and perlite (1:1:1). During the first few days they had to be kept in a moisture saturated atmosphere, but they gradually became adapted to normal conditions. After one month the plantlets were about 20 cm tall and had well developed leaves and roots.

KUS M.,\* ERŽEN–VODENIK, M.,\* RAVNIKAR, M.,\*\* , GOGALA, N. \*\*\*

### FAST PROPAGATION OF POTATO IN SLOVENIA

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\*\*Institut za biologijo Univerze v Ljubljani

\*\*\*VDO za biologijo Biotehniške fakultete

In agricultural institution KZK Kranj virus free potato plants are grown since 1965, using a method of individual clone selection. In last few years, because of the spreading of new viruses in potato fields (esp. virus Y and M), we began to test and introduce fast micropropagation methods in order to substitute individual clone selection used till now in a routine work.

Our procedure of fast propagation is organized according to the following shedule; uninfected plants and tubers are cultivated in a greenhouse. Initial plantlets are grown

from axillar buds and further propagated by multiple segmentation in the laboratory. Growing plantlets are then transferred into the soil substrate and further cultivated in plants producing tubers.

Original plant material, free of viruses and other pathogens are obtained by cultivation of selected healthy plants in a greenhouse. Their condition is regularly tested, mainly by ELISA – test. In cultivars with a latent infection (e.g. cv. Vesna, infected by M virus), initial material for micropropagation is obtained by a meristem cultivation at the Inst. of Biology, Ljubljana. After a heat treatment of tuber, buds apical meristem tissue is isolated. Young sprouts grown directly from meristem tissue develop roots and are after 30–40 days ready for further propagation. Another possibility is formation of callus tissue culture developing sprouts and roots simultaneously. Because of fast growth of axillar buds from segmented stems of such plantlets we used for further propagation also their apical meristeme. They grew quickly into sprouts with good formation of roots. Plants cultivated this way were tested on virus absence as well.

JUG–DUJAKOVIĆ, M.

### INVESTIGATION OF OPTIMAL CONDITIONS FOR GROWING OF SHOOT TIPS OF CARNATIONS

Institute for Adriatic Crops, Split

The optimal conditions for the carnation shoot tip (0,1–1 mm) development were investigated. The influence of different combinations of concentrations of IAA and KIN, the size of explant, the season, the correlation between the genotype and growing medium were investigated.

Among a few combinations of IAA (1–5 mg/1) and KIN (0,6 and 1 mg/1) concentrations the best growing was achieved with 2 mg/1 IAA and 1 mg/1 KIN. Concentration of IAA of 3 mg/1 and higher stopped the growing of the shoots.

The influence of the size of explants on the growing and development of plants was investigated with cultivar „Scania Tina” on the six different combinations of hormones IAA and KIN. Explants with the size of the 0,1–0,5 mm gave the higher number of vitrified (plants; bigger explants (0,5–1 mm) were better for the establishment of culture.

The influence of genotype and culture medium on the growth and development was investigated on the four different media and with three cultivars („Lena”, „William Sim” and „Sacha”). Both factors were very important, but the higher importance had the genotype.

The season of the year had the influence on the good results of the establishment of the culture, the shoot tips („Pallas”) had better development in December than in June.

The results of this investigation showed that concentration of hormones in the growing medium, the size of explants, the season of the year and the genotype have the important role in the successful establishment of carnation culture.

PEVALEK—KOZLINA, B.

**CLONAL PROPAGATION OF WILD CHERRY  
(*PRUNUS AVIUM* L.) IN VITRO**

Faculty of Science, University of Zagreb

Micropropagation of wild cherry (*Prunus avium* L.) *in vitro* was investigated. Initial explants, issued from the shoot apex or axillary buds, were taken from one-year-old plants (4 genotypes), five-year-old trees (2 genotypes), suckers initiated on roots (3 genotypes) and mature trees (2 genotypes).

Initial explants were successfully disinfected with mercuric chloride. Contamination, percentage was from 4.5 to 27.1%. Using a WPM medium without chlorine ions ( $\text{Cl}^-$ ) with an appropriate balance of cytokinin, auxin and gibberellic acid (6BA 2.2  $\mu\text{M}$ , IBA 2.5  $\mu\text{M}$ ,  $\text{GA}_3$  0.3  $\mu\text{M}$ ) it was possible to induce bud growth. Culture initiation percentage depended on the explant origin and was up to 66.7%. Induction of multiple shoots was obtained (multiplication phase) on the same medium; 3.9 to 6.6 shoots respectively (according to genotypes) were developed from explant during 4 to 5 weeks. Before rooting, shoots can be elongated on the same medium, but with a different concentration of 6BA (0.4  $\mu\text{M}$ ) and  $\text{GA}_3$  (14.5  $\mu\text{M}$ ). This phase can be omitted if the interval between subcultures is prolonged. Roots were initiated in 90% cultures as on the mod. WPM medium with IBA (4.9  $\mu\text{M}$ ) so in sterile mixture of compost, peat and perlite (1:1:1) after a treatment with IBA (246  $\mu\text{M}$  during 4 h). *In vitro* rooted plants successfully adapted to greenhouse conditions.

Regeneration of shoots from callus cultures was obtained on multiplication medium. These shoots were morphologically not different from the initial genotype.

This technique makes it possible to get about 10 million genetically identical plants from one explant with the average rate of multiplication of  $\times 5/1$  month.

SIMOVIĆ, N.

**IN VITRO CLONAL PROPAGATION OF POTATO  
(*SOLANUM TUBEROSUM* L.)**

Faculty of Agriculture, Zemun

For potato clonal propagation *in vitro*, sprout node explants, 5–7 mm long were used. Tubers of five varieties (Desiree, Saskia, Kennebec, Dragačevska and Jelica) were sprouted in dark, at 25°C. Culture medium, without hormones, contained Knop mineral solution. Cultures were maintained at 20°C, photoperiod 12 h light/ 12 h dark at 3000 lux.

In four weeks about 3 cm long and 2 mm thick shoots were formed, with six or seven nodes each carrying a leaflet. Leaflets were simple, only in one case a compounded leaf was observed (Desiree). Several roots were regenerated per each explant, 3–5 cm in length. The roots were growing faster than shoots. Up to now, not a significant difference was observed among the varieties in their reaction to *in vitro* conditions.

The best moment for transfer of plantlets to soil or for further cutting is at their age of four weeks. At older shoots one can observe only the internode growth, node number remaining the same.

Further investigations are concentrated on somaclonal variation.

GOLOŠIN, B. and RADOJEVIĆ, Lj.\*

### **IN VITRO ROOTING OF THE SHOOTS OF THE APPLE ROOTSTOCKS M 26, M 27 AND M 106**

Faculty of Agriculture, University of Novi Sad, Novi Sad, and  
\*Institute for Biological Research „Siniša Stanković”, Belgrade

Adventitious root formation of plant cuttings represents a serious problem especially in woody plant species. Application of tissue culture techniques usually provides higher percent of rooting than the classical methods.

In order to achieve as good rooting as possible, the influence of different IBA concentrations added to the nutrient medium on the rhizogenesis of apple shoots obtained by *in vitro* tissue culture was examined.

Meristems with 1–2 leaf primordia were isolated from the buds after the winter season under sterile conditions using binocular. Three apple rootstocks were used: M 26, M 27 and MM 106. Meristems formed leaf rosette after 6 weeks in both MS (Murashige and Skoog, 1962) and QL (Quoirin and Lepoivre, 1977) nutrient medium containing BAP (0.9, 1.2 or 1.5 mg l<sup>-1</sup>). Branching of the leaf rosettes forming axillary buds was achieved in MS or QL medium containing (in mg l<sup>-1</sup>): BAP 0.9, IBA 0.1 and GA<sub>3</sub> 0.1. Shoots from these cultures were rooted in the same mineral media at half strength of mineral constituents and 1% sucrose. The effects of four IBA concentrations (0.5, 1.0, 2.0 and 50.0 mg l<sup>-1</sup>) on the rooting process were examined. Our results demonstrate that IBA concentration influenced rooting, length and number of roots. The most favourable IBA concentration was 1.0 mg l<sup>-1</sup> for all apple rootstocks studied and the percent of rooting was 78.26%, 54.55% and 75.00% for the apple rootstocks M 26, M 27 and MM 106, respectively.

KOLEVSKA–PLETIKAPIĆ, B.

### **REGENERATION OF BIRCH BY AXILLARY BUD CULTURE**

Faculty of Science, University of Zagreb

It is possible to regenerate plants of *Betula papyrifera* and *B. lutea* by means of axillary bud culture. This simple method of microclonal propagation, used in order to multiply selected birch genotypes of economical importance, could be taken as a basis for the improvement of commercial vegetative propagation.

The initial explants – one-year-old axillary buds – were planted on the induction medium supplemented with the following growth regulators: 2.3 μM BA and 0.18 μM NAA (Ahuja: *Silvae Genet.* 32, 131–135, 1983). The buds first developed and then extended. After five weeks the shoots of *B. papyrifera* were 8–10 cm and those of *B. lutea* 6–8 cm long. At the same time leaves with well developed axillary buds were formed on each explant. Segments with leaves and axillary buds (cuttings) were gradually subcultured every five weeks on the same medium. In this way explants were multiplied at a considerable rate.

The rooting of shoots was possible in both examined species. On the rooting medium, which was the same as the induction one, but contained only 2.5 μM IBA and

no BA and NAA, 4–5 cm long shoots were planted. After one week on this basis the development of adventitious root system was induced. By the end of the fourth week the roots were well developed. In this way whole plants were regenerated *in vitro*.

KOLEVSKA–PLETIKAPIĆ, B.

### MICROCLONAL PROPAGATION OF LEUCE–POPLARS

Faculty of Science, University of Zagreb

Micropropagation is the only alternative of the clonic propagation of elite Leuce-poplars trees.

The possibility of *in vitro* regeneration has been investigated on twenty genotypes of the Leuce-poplars hybrid species, produced by the crossing of *Populus tremula*, *P. tremuloides*, *P. alba* and *P. grandidentata* species. Parent plants used in the crossing were mostly of hybrid origin as well.

One-year-old axillary buds collected before vegetation were planted on an ACM medium enriched with 20 mg l<sup>-1</sup> adenine sulfate, 80 mg l<sup>-1</sup> lysine and 100 mg l<sup>-1</sup> myo-inozitol. The growth regulators added were 2,3 μM BA and 0,18 μM NAA. Buds formed on this medium, with frequently newly induced ones growing on them. This medium was used unchanged in further subcultures where a successive shoot proliferation was induced. The rooting medium differed in that adenine sulfate, lysine and myo-inozitol were left out, and the growth regulator added was only 2,5 μM IBA.

It has been found that – in the given culture conditions – each to the 20 genotypes was able to regenerate whole young plants. There were, however, differences in their morphogenetic potential. They were manifested in different shoot induction frequency in the initial and successive subcultures. The highest frequency of shoot induction has been observed in hybrids produced by the crossing of species *P. tremula* and *P. tremuloides* and their crossings, and the smallest one in hybrids produced by the crossing of species *P. alba* and *P. grandidentata* and their crossings. The shoot induction frequency in hybrid forms produced by the crossing of the *P. tremula* or *P. tremuloides* species with the *P. alba* or *P. grandidentata* ones was higher than in cases when the two latter species were mother plants. The same happened with hybrids produced by the crossing of these two species.

VINTERHALTER, D.

### IN VITRO PROPAGATION OF DRACAENA FRAGRANS

RJ Zavod za voćarstvo i vinogradarstvo RO „PKB–Agroekonomik”

Segments (3–4 mm) of the young, green, stem were used as starting material for micropropagation of *Dracaena fragrans*. Segments cultivated on media with 0,25–0,5 mg/l 2,4–D alone or in combination with 0,2–1,0 mg/l BAP produced callus tissues. Segments cultivated on media with 1 mg/l IBA produced roots.

Callus differentiated spontaneously on the media for callus induction. Differentiation was more pronounced when callus was transferred on media with 1,0–2,0 mg/l BAP and 0,2–2,0 mg/l IBA.

Usually the first sign of callus differentiation was the appearance of pale surface proliferations which soon turned into green structures from which shoot buds or roots emerged. The precise balance of hormones which control callus differentiation was not established but it was observed that BAP stimulated shoot production and IBA rooting.

Micropropagation was further continued by multiplication of shoot buds on media with 1,0–2,0 mg/1 BAP and 0,2–2,0 mg/1 IBA. Shoot buds could be grown successfully and without production of new callus tissue. Multiplication was achieved through adventitious buds which developed on the basal part of the original shoot bud.

Rooting was also achieved on media without growth substances. Rooted plants were planted in peat and they rapidly adapted to outside conditions.

BABIĆ, V.

### THE ESTABLISHMENT OF SHOOT TIPS CULTURE FOR THE ALMOND – PEACH HYBRID GF677 IN VITRO

Institute for Adriatic Groves, Split

Technique for establishment aseptically culture for the hybrid GF677, and proliferation of shoot tips is described.

Shoot tips 1–2 cm were collected from the stock plants grown in a greenhouse under hygienic conditions. Surface sterilization was the same used by BROOM and ZIMMERMAN (1978). 81% sterile explants was obtained in this way.

Explants were cultured in medium containing (mg/1): B<sub>1</sub> 1, B<sub>6</sub> 0,5, nicotinic acid 0,5, glycine 2, BAP 1, sucrose 3%, agar 0,6%, pH 5,7. Different mineral solutions were used: MS, ANDERSON (1978), modified B<sub>5</sub> GAMBORG (1968), WPM McCOWN (1981). The best mineral medium for proliferation of shoot tips was modified B<sub>5</sub> with higher concentration of phosphorus and nitrogen. On this medium each of the explants produced 5 to 7 vigorous shoots after 40 days. Results on the other media were poor, especially on MS. The next experiments will be aimed at establishing the optimal conditions for other phases of incorporation for this hybrid, and for its commercial production.

MEZEI, S., and KOVAČEV, L.

### IN VITRO VEGETATIVE MULTIPLICATION OF SUGARBEET BREEDING LINES AND GENETIC EVALUATION IN F<sub>1</sub> GENERATION

Faculty of Agriculture, Institute of Field and Vegetable Crops, Novi Sad

With selfsterile sugarbeet genotypes, the problems of abortiveness and failures in seed forming occur after a few cycles of inbreeding. Thus, these lines are quickly lost, no matter how useful they may be in breeding programs. Fortunately, it is possible to maintain selfsterile lines by resorting to *in vitro* vegetative multiplication. Furthermore, the method of vegetative propagation facilitates the production of identical plants which is advantageous for breeders when testing homozygosity and combining ability.

In this study research objectives were:

- testing variability of clones in field conditions, after of acclimatization,

– genetic testing of variability in  $F_1$  generation of crosses between identical cms lines and identical fertile lines.

We used a Swedish ms line derived from the hybrid Regina and a domestic inbred line, no. 73.

It was found that the two genotypes had limited and different rates of multiplication, Regina was superior regarding the biological potential for multiplication. Also, the genotypes reacted differently to the acclimatization and growing in field: there occurred a pronounced root branching with the ms line while no. 73 formed relatively normal roots. Phenological observations in field revealed the existence of phenotypic variability in leaf size and position among plants of the same genotype, while the shape of the lamina was not variable. However, these changes are also attributable to the ecological variability. The cytological analysis showed that no changes occurred in the number of chromosomes. In 1985, individual cms plants will be crossed with the fertile line for identity evaluation in  $F_1$  generation.



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